TOS PROTOCOL AND PROCEDURE: PLANT DIVERSITY SAMPLING

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A_DRAFT	10/03/2011	ECO-00280	Draft release
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C	03/18/2014	ECO-01668	Production release, template change, and other changes as detailed in Appendix C (rev C only)
D	11/03/2014	ECO-02341	Migration to new protocol template

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1 OVERVIEW

1.1 Background

The purpose of plant diversity sampling is to describe inter- and intra-annual variation of the presence and abundance of plant species at NEON sites. In addition to providing habitat for local fauna, the flora at each site integrates a variety of biotic and abiotic factors that respond to environmental change.

Plant species diversity will be measured once or twice annually in the field. The plot-based method yields plant species data at multiple scales that will provide an understanding of changes in composition, distribution, abundance, and the presence of non-native plant species. The data will be comparable within and across NEON sites and to other continental vegetation efforts to allow for a comprehensive understanding of the impacts of the drivers of change on the diversity of plant species and the functional role they play in ecological systems.

This document provides detailed guidance for assessing plant diversity in the Distributed Plots in the field, the collection and handling of unknown plant species, and the collection of voucher specimens for training and archiving purposes.

1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. 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.

1.2.1 NEON Science Requirements and Data Products

This protocol fulfills Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (RD[03]).

1.3 Acknowledgments

This protocol is based on a technique for sampling plant species diversity in a multi-scale plot that was created for use in The Carolina Vegetation Survey, the Whittaker, and the Modified-Whittaker plot design.

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.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.001155	NEON Training Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	NEON.DOC.000912	NEON Science Design for Plant Diversity
AD[07]	NEON.DOC.014051	Field Audit Plan
AD[08]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

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.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.001579	Datasheets for TOS Protocol and Procedure: Plant Diversity Sampling
RD[06]	NEON.DOC.014040	TOS Protocol and Procedure: Plant Phenology
RD[07]	NEON.DOC.001025	TOS Protocol and Procedure: Plot Establishment
RD[08]	NEON.DOC.001702	NEON Herbarium Specimen Label and Annotation Generation

2.3 Acronyms

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

2.4 Definitions

N/A

3 METHOD

This document describes the collection of plant diversity information designated to inform the goals and objectives of the National Ecological Observatory Network (NEON). Plant diversity sampling shall occur according to a sample design – a statistically rigorous system that directs the spatial distribution of observations – at plots distributed across NEON sites. Plant species composition or presence and abundance data shall be collected in multi-scale plots, estimates of cover being limited to $1m^2$ subplots that shall be nested in larger plots where plant species composition will be recorded.

Even experienced botanists will not know every species encountered in each plot. Typically it is not cost effective, and sometimes impossible, to spend time identifying a plant in the field. Therefore, instructions for the collection and identification of difficult species are provided. In addition to the unknown species collected for identification, voucher specimens will be collected to provide a record of NEON naming convention, use of authorities, validation, and a means to track taxonomic naming conventions through time.

Voucher specimens of twenty to forty of the common species found in the plots must be collected, pressed, mounted, and stored. Voucher specimens provide a permanent record of the NEON naming convention, use of authorities, validation, and a means to track taxonomic naming conventions through time. The samples must be of archival quality. Specimens should be collected during peak phenology, and must be pressed, dried, and mounted according to herbaria standards such that species identity can be evaluated in the future.

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field technicians **must** follow the protocol and associated SOPs. Use NEON's problem reporting system to resolve any field issues associated with implementing this protocol.

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. 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.

The procedures described in this protocol will be audited according to the Field Audit Plan (AD[07]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (AD[08]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Sampling timing will be determined annually by Field Operations based on criteria for sampling and approved annually by Science.

Complete sampling in approximately a 1-2 month period around peak phenology. Significant delays may change the detectability of species and influence the comparability of sampling bouts. Please check with NEON plant diversity staff scientist with questions and report start and completion dates through approved NEON communication systems.

Plant diversity will be sampled one or two times annually (Appendix E).

4.2 Criteria for Determining Onset and Cessation of Sampling

Sample bouts will be timed to maximize the number of plant species detected at a NEON site. Samples will generally made at peak phenology (when most species are flowering) to facilitate identification of individuals. Many NEON sites will not have a single peak in phenology due to plant adaptations that take advantage of different climate conditions. Sites with more than one peak may require multiple sampling bouts.

The specific timing of sampling bouts will be tied to observations from the NEON phenology measurements (RD[06]), but the timing of sampling has generally been determined to support planning purposes (Appendix E).

4.3 Timing for Laboratory Processing and Analysis

Specimens should not be left in the refrigerator for more than two days. They can be placed in the press, stored in a well-ventilated location, and identified at a later date. Specimens may remain in this state for months. Identification often requires a variety of dichotomous keys, a dissecting microscope, a dissecting kit, and a herbarium with voucher specimens for verification.

4.4 Sampling Timing Contingencies

When unexpected field conditions require deviations from this protocol, the following field implementation guidance must be followed to ensure quality standards are met:

- If the data collection cannot be completed due to safety or logistical reasons, sampling must resume at the plot during the same sampling bout (approximately 45 days) if the plot is to be considered complete. Delay of sampling should be recorded in data about the plot-sampling effort.
- Any changes that the plot undergoes during a particular sampling bout should be noted in the data associated with the plot.
- Deviations associated with the collection of data should not be made from this protocol. The number of people collecting data, the method for defining the plot boundary, and the amount of material collected for the identification of unknown plant species may be altered to meet the needs of Operational constraints.

5 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.

6 PERSONNEL AND EQUIPMENT

6.1 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 1. Equipment list – Materials and supplies required for one crew for the plant sampling procedure

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	50 meter fiberglass tape measure	Establishing plot boundary	1	N
	R	Global Positioning Unit	Finding plot	1	N
	R	Hand lens,20x	Species identification	Many	N
	R	Pruning sheers	Harvesting parts of individuals	1 ea.	N
	R	Weeder	Harvesting parts of individuals	1	N
MX100497	R	Meter stick	Measuring height	1	N
	R	Subplot frame	Delineating $1m^2$ subplot	1	N
	R	Digital camera	Recording photos	1	N
RD[05]	R	PDA or datasheets	Recording data	1	N
	R	Hardware stakes and/or chaining pins	Delineating plot	1	N
	R	Ice packs	Keeping voucher specimens cool/prevent wilting	Many	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	S	Plant press	Species preservation	3-4	N
GN100001	S	Small carabiner and ring binder	Optional unknown carrying rig	1	N
	S	Species list	Facilitation of species that might be found at site	1	N
	S	Dichotomous keys	Species identification	1	N
	S	Cooler	Keeping voucher specimens cool/prevent wilting	5	N
Consumable items					
	R	Field notebook and pencil	Taking notes about unknown plant species	1	N
	R	Pin flags	Marking subplot boundary	3	N
	R	Nylon flagging	Marking subplot boundary	1	N
	R	1-gallon Ziploc plastic bags	Collecting unknown plant species	> 40	N
	R	Adhesive labels	Labeling unknown and voucher specimens	1	N

R/S=Required/Suggested

Table 2. Equipment list – Laboratory processing

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Dissecting microscope	Species identification	1	N
	R	Plant keys	Species identification	No more than 5 references	N
MX100316	R	Standard Plant Press, Wood Frame, 12 in. x 18 in., with Straps	Pressing individual species	2	N
	R	Botany dissection kit	Species identification	1	N
Consumable items					
MX100719	R	Glue Bottle, w/Brush Attached to Lid, Up to 12 oz Capacity	Mounting species	1	N
MX100679	R	Herbarium Mounting Glue	Mounting species	1	N
MX100680	R	Mounting Paper	Mounting species	1	N
306650000	R	Washer Flat .688 ID X 1.5 OD #5/8" SS	Mounting species	Many	N
	R	Herbarium glassine seed envelopes, 2" x 3.25"	Mounting species	1	N
	R	Herbarium labels	Metadata	1	N
	R	Safety pins	Mounting species	1	N
	R	Tape, cloth, white gummed, 0.5", for herbarium mounting	Mounting species	1	N
	R	Wood, 2in x 2in x 8ft furring strip	Mounting species	1	N
	R	Newspaper, tabloid or other appropriate size	Drying species	Many	N

R/S=Required/Suggested

6.2 Training Requirements

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

6.3 Specialized Skills

A minimum of two technicians are required for each plant diversity sampling team. It is mandatory that one technician have experience with the identification of plants – preferably in the habitats found at the site where measures will be made, be able to use a dichotomous key, and have experience identifying plant specimens in the lab with a dissecting microscope and associated tools. At each site this technician must be able to identify most of the species in the field.

It is important that careful attention be paid to the person responsible for identifying plants in the plot. This is important because unknown plant species collected during the effort will be tracked by this identity (measuredBy), the unknown plant name, and the collection number. If multiple botanists are identifying species within a single plot, a single botanist should be responsible for collecting unknown species. Or, botanists can be responsible for all of the species in a 100m^2 subplot where the botanist (measuredBy) can be recorded on the datasheet.

6.4 Estimated Time

A plot should take 1-4 hours for a team of two to complete. The time required will vary depending on a number of factors, such as skill level, species richness at the site, and environmental conditions. The timeframe is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted.

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

1. Assemble nested subplot frames

    - The cover and identity of plant species will be recorded in 1m^2 frames. The frames may be assembled from components prior to the sampling effort or purchased pre-fabricated (Appendix F).

2. Prepare data collection tools

    - Prepare the PDA or datasheets (RD[05]) for collecting data prior to leaving for the field. If the data are collected directly onto an electronic hand-held device, be sure the unit is well charged by keeping it connected to the charger when not in the field. Prior to leaving for the field, confirm that the software is loaded onto the device and the data from prior sampling efforts have been downloaded and deleted. Be prepared to use provided paper datasheets if the device fails (e.g. dunked in a creek, lost, or crashes) or if data is to be collected on paper data sheets.

3. Organize equipment and consumable items

    - Plastic bags will be used to collect unknown plant species. Prior to going to the field or be sure to have an ample number of loose bags and, if desired, assemble a system for collecting unknown species (Appendix G). Adhesive labels will be needed and working permanent markers and pencils.

SOP B Field Sampling

B.1 Plot Establishment

Plant diversity sampling occurs in a square-shaped plot measuring 20m on a side and containing four 100m^2 subplots (Figure 1). Each subplot contains nested subplots: a 1m^2 subplot nested in a 10m^2 subplot in each of two corners. For comparison of data across space and through time, it is important that the dimensions of these plots and subplots be consistent across plots and sites. This protocol assumes that plots will be marked by a center point and four corners. The permanent markers define the corners of the plot and should maintain comparability through time. If this is not the case, plots must be established during each sampling bout according to the Plot Establishment Protocol (RD[07]).

Delineate the sides of the 100m^2 subplot and 10m^2 nested subplot 3.16 m from the nearest permanent marker at the plot corners or center with flags or appropriate markers. Instructions for two methods of delineating plot and subplot boundaries are provided. Instructions in Appendix B.1 assume the plot was established with precise square with exact 20 sides and that the tape can be stretched between corners with no obstacles. Instructions in Appendix B.2 recognize an inevitable lack of absolute precision of the established markers and obstacles that are likely to obstruct the tape when stretched between markers.

The 1m^2 nested subplot will be delineated with a rigid frame anchored at the corner by a permanent plot marker.

The square, multi-scale plot in Figure 1 below will be used to record plant species composition and cover. The plot includes nested subplots at specific location within the plot.

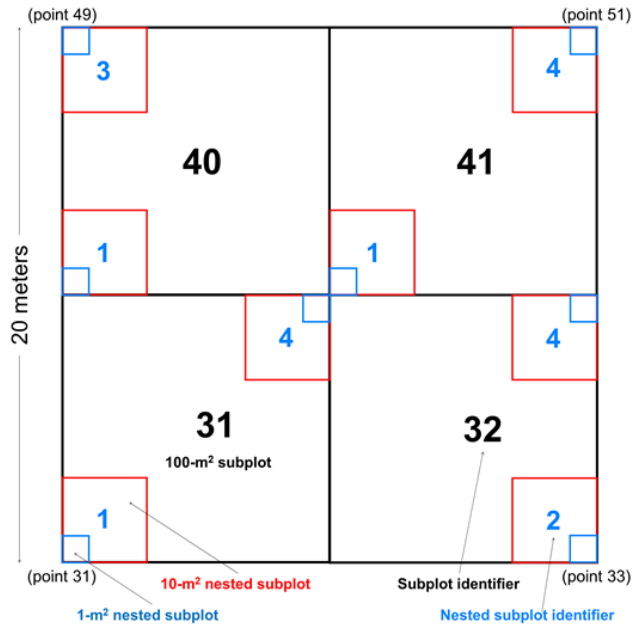


Figure 1. A square, multi-scale plot

B.2 General Collection

Record information such as the botanist (MeasuredBy), date, and start and completion time should be recorded on each datasheet. Time should reflect the time to sample a single 100m^2 subplot which can be aggregated to describe total plot sampling time.

1. 1m^2 Nested Subplot. Measure variables (Table 3) and cover and height of vascular plant species in each 1m^2 nested subplot.
   a. **Place nested subplot frame**. Measure variables (Table 3) and cover and height of vascular plant species in each 1m^2 nested subplot. **Measure and record variables**. Estimate and record the combined cover of abiotic (non-living) elements and non-vascular plant species in each 1m^2 nested subplot. Cover of any one element shall not exceed 100 percent, but the total cover of multiple elements may be greater than 100 percent.

Table 3. Variables other than vascular plant species.

Variable name	Description
Soil	Particles < 5 mm diameter
Rock	Inorganic particles ≥ 5 mm diameter
Wood	Woody organic material ≥ 5 mm diameter lying on the ground
Litter	Organic material lying on the ground
Standing Dead	Desiccated organic material from previous calendar year. Desiccated vascular plant species from the same calendar year that can be identified to genus and species should be included as a species
Water	Standing or flowing water
Lichen	Fungus and cyanobacteria or green algae
Moss	Typically small (1 – 10 cm but up to 50 cm), soft plants of the division Bryoptera
Other non-vascular	Fungus other than lichen
Scat	Animal dung
Overstory	An estimate of the total vegetation greater than or equal to 3 m above the 1-m^2 nested subplot ; should include species not rooted in the nested subplot

   b. **Measure and record plant species data**. Record the species of individuals with stems in the 1m^2 nested subplot, and estimate the combined aerial cover of all individuals less than 3 m tall to the nearest one percent.
      1) Identify vascular plant species. Record the species identity, corresponding standardized Natural Resource Conservation Service (NRCS)/US Department of Agriculture (USDA) PLANTS database code, and cover for each species. If a species cannot be identified in the field, refer to section B.4, Unknown Plant Species. If individuals don't include those parts necessary to identify to species even if individual is collected, enter genus from species list. If species cannot be identified in the field see section B.3, Morphologically Challenging Species, and section B.4, Unknown Plant Species.

2) Estimate cover. The combined cover of all individuals in the nested subplot should be included in the cover estimate.
   a) Familiarize yourself with what particular cover estimates (e.g., 1%, 10%, 15%, etc.) look like and use them as reference sizes. For example, if you know that 1% cover is about the same size as your fist, use your fist as a reference.
   b) Only estimate cover of plants, or portions of plant, with stems that are inside, or partially inside, the subplot frame. Epiphytes not actually rooted on the ground of nested subplot, but that are rooted to trees in the space extending above the nested subplot should be included.
   c) Estimate cover to nearest 1%.
   d) There will often be spatial overlap of plant species. Cover should be recorded as the total aerial coverage for each species; estimates should not exceed 100 percent for a single species, but total subplot cover may be greater than 100%.
   e) Each $1m^2$ nested subplot frame is calibrated in 10cm sections to make cover estimates easier (Figure 2).
   f) Visually group species together into a percent cover.
   g) Fine tune estimate by subtracting out any spaces or gaps.
   h) Enter 't' for estimates of cover <1%.

3) Measure height by species.
   a) Record height only for individuals less than 3m to the nearest centimeter.
   b) Measure and record the mean height of the tallest five individuals of each species < 3m in the subplot.
   c) Estimate plant height of epiphytic plant species if possible, otherwise leave height blank.

c. **Photograph $1m^2$ nested subplot**. Prior to removing the $1m^2$ nested subplot frame, take a plane-view picture of the nested $1m^2$ nested subplot such that the subplot frame fills the photograph while standing at the middle of the south edge outside the $1m^2$ nested subplot. If it is not possible to stand at the south edge, move to the west, north, east edge in that order.
   1) Photograph name should include: plot_ID, taxa (Module), subplot, nested subplot, and date in the following format: CPER_001_PlantDiversity_sub31_nestedsub1_20130812

2. **$10m^2$ Nested Subplot**. Record the identity of all species with stems in each $10m^2$ nested subplot.
   a. The subplot will be defined by the tape on the outside edge of the subplot. Other edges and corners will be defined by the flags inserted in the plot establishment phase. If there is question about the presence of a species in a plot, the third tape can be moved to better define the edge of the plot.
3. **$100m^2$ Subplot.** Record the identity of all plant species with stems in each $100m^2$ subplot. It is not necessary to record species already documented in nested subplots in each respective $100m^2$ subplot.

4. Repeat above for each of the four 100m^2 subplots.

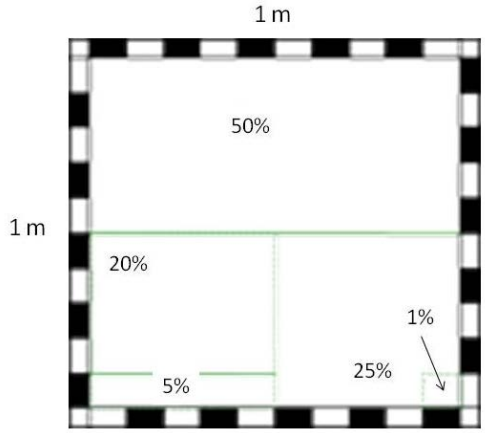


Figure 2. The 1 m^2 subplot is calibrated with black and white marks to make estimates of plant species cover more accurate and repeatable

B.3 Morphologically Challenging Species

- In some cases the morphological parts of an individual necessary for identification may not be present, complicating identification of that individual in the field. Note these cases using the idQ field on the datasheet (Table 4).
- The identity of some species and sub-species will be difficult to separate at some sites. Because comparability of cover estimates must be consistent through time, it will be necessary to identify these groups of species (e.g. genus) and consistently lump them through time and across field sampling efforts. It is difficult to know what this lumping might look like prior to the first field sampling year. A list of grouped species should be developed over the first year of sampling and based on conversations with botanists who work at the site or in the region if possible.

Table 4. Identification qualifier codes (idQ) to designate unknown species or those species with uncertain identification in the field or after identification in the lab.

idqCode	identificationQualifier	Description
UNK	Unknown species	Unknown plant species
CS	cf. species	Roughly equals but "not sure" about the species
AS	aff. species	"Similar to, but is not" the species
CG	cf. genus	Roughly equals but "not sure" about the genus
AG	aff. genus	"Similar to, but is not" the genus
CB	cf. subspecies	Roughly equals but "not sure" about the subspecies
AB	aff. subspecies	"Similar to, but is not" the subspecies
CF	cf. family	Roughly equals but "not sure" about the family
AF	aff. family	"Similar to, but is not" the family
CV	cf. variety	Roughly equals but "not sure" about the variety
AV	aff. Variety	"Similar to, but is not" the variety

B.4 Unknown Plant Species

In the field, perform the following steps for individuals that cannot be identified:

1. Given NEON's long-term monitoring efforts, unknown species should be collected from outside the 40 x 40 m plot. Finding the same unknown species can sometimes take considerable time.
2. Collected unknown specimens should be placed in sealable plastic bags (see Appendix G for optional system for organization and transport of specimens). A cooler with an ice pack may also be used (optional) to prevent wilting of voucher specimens, and may be particularly useful on hot days and/or when there is little shade available.
3. Collect representative parts of the entire individual, including the roots, flowers (if possible), and vegetative growth of grasses and forbs.
    a. A piece of a branch is usually sufficient for trees and shrubs.
    b. If a flower cannot be found, technicians can keep an eye open for an individual in flower for the rest of the sampling effort, but are not expected to return to a particular plot for the exclusive purpose of finding the individual in flower at a later date.
4. Label plant with a unique (to the technician) unknown name, number, description, botanist, date, GPS coordinates, elevation, and plot number (where species was initially found, if appropriate).
5. Enter the unknown name and number in the species column of the datasheet.
6. Enter unk in the idQ column.
7. If collection of an individual is not possible, take photograph(s) of the individual (including flowers and other parts crucial to identification) and record photographic information in notebook. This record is to assist with future identification.
    a. The photograph should be labeled with unknown name, plot_ID, and date as follows: fuzzyfunny_CPER_001_20130812.
8. At the end of the field day, place plastic bags in a refrigerator until they are identified and/or placed in a plant press and dried for identification at a later date. Specimens should not be left in the refrigerator for more than two days. Identification often requires a variety of dichotomous keys, a dissecting microscope, a dissecting kit, and a herbarium with voucher specimens for verification.

B.5 Voucher Specimens

Plant species will be collected at NEON sites for the following purposes: 1) collected: fresh and/or dried samples to be used for identification, 2) to be pressed and incorporated into the domain support facility herbarium for training and quality assurance purposes, and 3) to be preserved as described and mandated by the archive program.

If possible, all parts of a plant should be collected, the roots, stems, flowers, fruits, and seeds as follows:

1. Select specimens in good condition, free of damage from insects and/or disease.
2. If possible, all parts of a plant should be collected, the roots, stems, flowers, fruits, and seeds. Collect at least stems, leaves, and flowers or fruit of herbaceous plants, and twigs, leaves, and flowers or catkins of trees and shrubs.
3. Place all specimens of a single species from one locality into one collection bag.

B.6 Refreshing Sampling Kit

Be sure to have sufficient plastic bags, adhesive labels, and permanent markers for the next field sampling effort.

SOP C Laboratory Processing and Analyses

The lab component of the plant biodiversity sampling includes three parts: plant pressing and drying, identification, and processing of a subset of collected species for voucher specimens. The exact order and need for each step with each specimen will depend on scheduling and time, and the objectives of the particular specimen.

Some species will come from the field and be identified fresh (without pressing), within two days of collection. If the specimen was destroyed during identification or was not intended for vouchering, there is no need to save and press the specimen. Not every unknown plant species must be vouchered and submitted to the archive.

Some species will be collected and the botanist will not have time to identify it within two days, or will not be able to identify the specimen. These specimens should be pressed for identification at a later time, either by the botanist with the help of an herbarium, or sent to an expert.

Other specimens will be collected specifically for vouchering at a NEON herbarium or other archive facility. These specimens should be treated with extra care to preserve diagnostic parts, pressed until dry, and mounted for preservation in a NEON herbarium or transferred to an archive as mandated by collection procedures.

C.1 Sample Processing Timing

Specimens should not be left in the refrigerator for more than two days. They can be placed in the press, stored in a well-ventilated location, and identified at a later date. Specimens may remain in this state for months. Identification often requires a variety of dichotomous keys, a dissecting microscope, a dissecting kit, and a herbarium with voucher specimens for verification.

C.2 Identification of Unknown Species

Ideally, the fresh (not pressed and dried) duplicate specimen that is not to be included in the herbarium should be used for identification. Identification requires basic knowledge of morphological characteristics of different plant families, plant keys, and access to a herbarium (University of Wyoming, University of Florida, etc.), a clean bench space in the lab, a dissecting microscope, and dissecting kit. If there is any doubt, a duplicate specimen should be submitted to a taxonomic expert for identification.

After unknown specimens are identified, update the information in the data sheets, files or database.

C.3 Plant Pressing and Drying

Press plants as soon as possible (and within 2 days of collection) in the lab at a clean and dry bench space. Plants are pressed and dried in standard plant presses and newspaper as follows:

1. Tabloid newspaper is ideal for pressing plants since it is the same size as the plant press and the herbarium paper to which the specimens will be mounted.
2. Within the fold of one sheet of newspaper, arrange plant parts of a single specimen carefully with minimum overlap.
    a. Open some flowers to show both the top and underside to illustrate the arrangement of flower parts.
    b. Squash large fruits on the page or slice them in half.
    c. Place dry, loose seeds or fruits in sealed packets and adhere to mounting paper.
    d. Turn over some leaves or part of a single large leave to show underside.
3. Write the collection number (from the field collection) Domain, site, date, botanist, and initial identification on the inside of the newspaper and any seed packets.
4. Close newspaper and separate individual newspaper folds with blotting paper. Up to three newspaper folds, separated by blotting paper, can be included between cardboard ventilation separators.
5. Place the stack of specimens, blotting paper, and cardboard between the wooden plant press panels 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.
6. Dry specimens in press. Grass and shrub specimens will dry in well ventilated part of the lab. Fleshy or aquatic plant specimens will require a dryer.

C.4 Sample Preservation Processing Voucher Specimens

Herbarium specimens are catalogued with a standardized label. The label includes the family, genus, species, location description and coordinates, elevation, collector, collection date, and collection number (see RD[08] for label format).

Once specimens are pressed and dry, they must be mounted. Specimen mounting requires skill and patience. All supplies should be of museum quality since the longevity of the specimens is directly related to the substances they contact. Mounting is done at a large and clean bench space in the lab and completed as follows:

1. Glue acid free label to acid free herbarium mounting paper.
2. Leave space on the sheet for seed and fragment packets.
3. Remove any soil clinging to the roots and stems.
4. Use scissors or pruning shears to trim large specimens to fit the sheets.
5. Place a sheet of mounting paper on a cardboard sheet.
6. Arrange the plants on the mounting paper. Avoid placing any material at the edge of the mounting sheet.
7. Hold the specimen down with weights such as plastic-coated lead bars or metal washers until the glue dries.
8. Attach the specimen to the mounting paper with thin ribbons of glue running from the paper across the plant part to the paper. The glue should not cover any parts necessary for identification.
9. Small drops of glue should be applied to the underside of large leaves and flower heads, and multi-stemmed specimens (some grasses) require long glue straps to catch all the stems.
10. When the sheet is finished, dry mounted specimens by separating cardboard supports with wooden blocks.
11. Assign a standard accession number to each prepared sample.
12. Store the samples at -20 degrees C for 48 hours prior to permanent storage in herbarium cabinet.

SOP D Data Entry and Verification

As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

SOP E Sample Shipment

Information included in this SOP conveys science-based packaging, shipping, and handling requirements, not lab-specific or logistical demands. For that information, reference the CLA shipping document on CLA's NEON intranet site.

Shipping details are TBD and will be included in a future revision of this protocol (as of rev D).

E.1 Handling Hazardous Material

N/A

E.2 Supplies/Containers

TBD

E.3 Timelines

TBD

E.4 Conditions

TBD

E.5 Grouping/Splitting Samples

TBD

E.6 Return of Materials or Containers

TBD

E.7 Shipping Inventory

TBD

E.8 Laboratory Contact Information and Shipping/Receipt Days

See the CLA shipping document on CLA's NEON intranet site.

8 REFERENCES

APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 5. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.001579	Datasheets for TOS Protocol and Procedure: Plant Diversity Sampling

These datasheets can be found in Agile or the NEON Document Warehouse.

APPENDIX B QUICK REFERENCES

B.1 Delineating a precise plot with little to obstruct the tape on the perimeter.

The perimeter of the plot and subplots shall be delineated by tape measures and subplot frames as follows (Figure 3):

1. Record date and plot number.
2. Begin in the south-west corner of the plot (point 31), at most sites this permanent marker will be labeled with information about the plot.
3. Anchor a 50 m tape and extend it towards the south-east corner (point 33).
    a. Walk on the south side of the tape to avoid trampling plants on inside the 20 x 20 m plot.
    b. While pulling the tape, insert pin flags into the ground touching the outside edge of the tape at 1 m, 3.16 m, 10 m, 16.84 m, and 19 m.
4. Anchor the tape at the 20 m at the south-east corner of the plot (point 33) and pull it towards the marker at the north-east corner (point 51) of the plot.
    a. Walk on the east side of the tape to avoid trampling plants on inside the 20 x 20 m plot.
    b. While pulling the tape, insert pin flags into the ground touching the outside edge of the tape at 21 m, 23.16 m, 30 m, 36.84 m, and 39 m.
5. Return to the south-west corner (point 31) of the plot.
6. Anchor the second 50 m tape and extend it towards the north-west corner (point 49).
    a. Walk on the west side of the tape to avoid trampling plants on inside the 20 x 20 m plot.
    b. While pulling the tape, insert pin flags into the ground touching the outside edge of the tape at 1 m, 3.16 m, 10 m, 16.84 m, and 19 m.
7. Anchor the tape at the 20 m at the north-west corner (point 49) of the plot and pull it towards the marker at the north-east corner (point 51) of the plot.
    a. Walk on the north side of the tape to avoid trampling plants on inside the 20 x 20 m plot.
    b. While pulling the tape, insert pin flags into the ground touching the outside edge of the tape at, 21 m, 23.16 m, 30 m, 36.84 m, and 39 m.
8. Anchor a third tape at the center of the plot (point 41) and extend it south toward the flag that at 10m.
    a. Insert pin flags into the ground at 1 m and 3.16 m.
9. Return to the center and extend the tape east toward the flag that at 30 m.
    a. Insert pin flags into the ground at 1 m and 3.16 m.
10. Return to the center and extend the tape north toward the flag at 30 m.
    a. Insert pin flags into the ground at 1 m and 3.16 m.
11. Return to the center and extend the tape west toward the flag at 10 m. Leave the tape in this place to facilitate sampling subplot x.
    a. Insert pin flags into the ground at 1 m and 3.16 m.

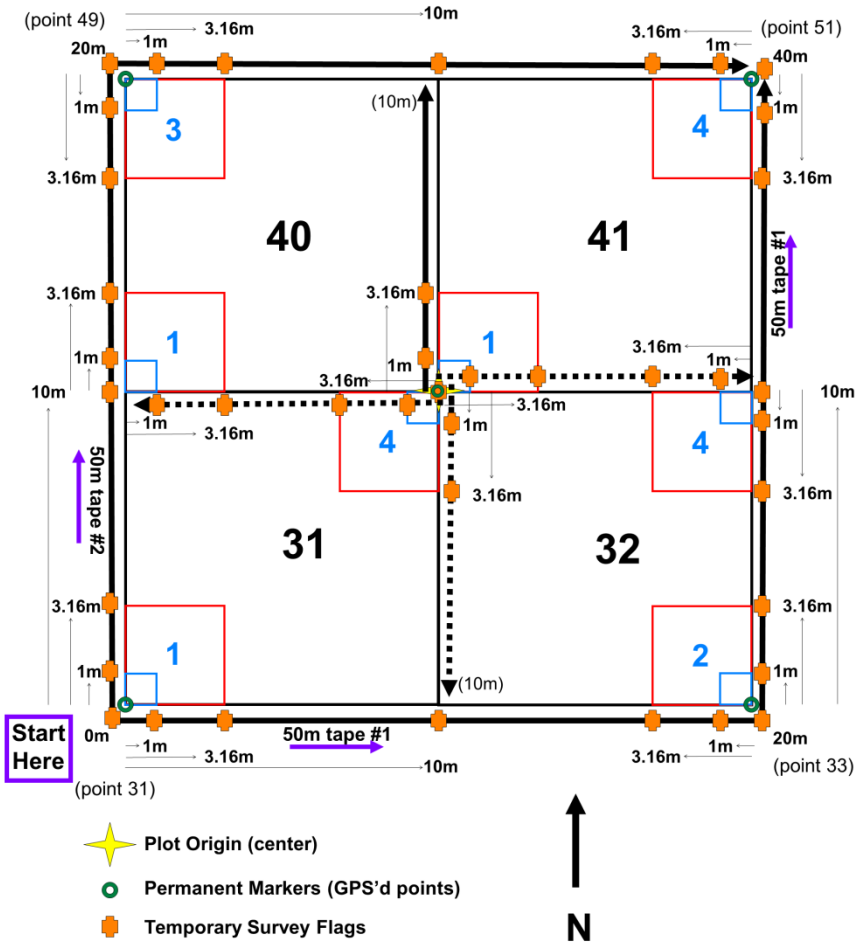


Figure 3. The plot will have some permanent markers and will also require temporary flags that are placed each time the plot is measured.

B.2 Plot delineation with some lack of precision in plot and some obstacles

This method is very similar to the previously described, but it recognizes that deviations in the distance between markers and obstacles in the tape may prevent the measures from working as described in Appendix B.1 (e.g. if the tape must go around a tree between the southwest corner and the south-east corner the tape may not intersect the permanent marker at 20 m). The important difference is that subplots will be established from the nearest permanent marker. The idea is to delineate the plot boundary by connecting the permanent markers with the tape measure. The tape should be kept as close as possible to the ground, be forced through shrubs, and around trees to maintain the straightest line possible between markers. With two people, one person can anchor the tape at the south-west corner and pull the tape towards a person standing at the destination marker, or one person can hold the tape at the south-west corner and a second person can pull the tape towards the target marker. A compass might be helpful for establishing the direction the tape should be pulled.

After the tape is extended the subplot and 10 m markers can be established by pulling the tape tight from the nearest permanent marker and accounting for trees and other obstacles as needed. A string or equivalent material that measures 3.16 m is likely easier to use for establishing sides of the $10m^2$ subplot. The perimeter of the plot and subplots can be delineated by tape measures and subplot frames as follows (Figure 3):

1. Record date and plot number.
2. Begin in the south-west corner of the plot (point 31), at most sites this permanent marker will be labeled with information about the plot.
3. Anchor a 50 m tape and extend it towards the south-east corner (point 33), walking on the south side of the tape and following a path that creates the straightest possible line towards the marker in the south-east corner.
4. Wrap the tape at the south-east corner/permanent marker (point 33) and extend it to the north-east corner (point 51) at approximately 40 m on the tape.
5. Return to the south-west corner (point 31) and while pulling the tape tight towards the south-east corner (point 33), insert pin flags into the ground touching the outside edge of the tape at 1 m, 3.16 m, 10 m.
6. Proceed to the south-east corner (point 33) and pull the tape tight (either wrapped around the marker or/or with a second person holding) from the south-east corner back towards the south-west corner (point 31) and insert flags at a distance of 1 m and 3.16 m from the south-east corner on the south edge of the plot.
7. With the tape anchored at the south-east corner (point 33), pull it tight towards the north-east corner (point 51) of the plot and insert pin flags at 1 m, 3.16 m, and 10 m from the south-east corner along the east side of the plot.
8. From this 10 m mark on the east edge of the plot, pull the tape tight back towards the south-east corner (point 33) and insert flags at a distance of 1 m and 3.16 m from the 10 m mark towards the south-east corner.
9. Proceed to the north-east corner (point 51) of the plot and pull the tape tight from the north-east corner back towards the south-east corner (point 33) and insert flags at a distance of 1 m and 3.16 m from the north-east corner on the east edge of the plot.
10. Return to the south-west corner (point 31) of the plot. Anchor the second 50 m tape and extend it towards the north-west corner (point 49), walking on the west side of the tape and following a path that creates the straightest possible line towards the marker at the north-west corner (point 49).
11. Wrap the tape at the north-west corner (point 49)/permanent marker and extend it to the north-east corner (point 51) at approximately 40 m on the tape.
12. Return to the south-west corner (point 31) and while pulling the tape tight towards the north-west corner (point 49), insert pin flags into the ground touching the outside edge of the tape at 1 m, 3.16 m, 10 m on the west side of the plot.

13. From this 10 m mark on the west edge of the plot, pull the tape tight towards the north-west corner (point 49) and place flags towards the north-west corner (point 49) at a distance of 1 m and 3.16 m from the 10 m mark on the west edge of the plot.
14. Proceed to the north-west corner (point 49) and pull the tape tight (either wrapped around the marker or/or with a second person holding) from the north-west corner (point 49) back towards the south-west corner (point 31) and insert flags at a distance of 1 m and 3.16 m from the north-west corner (point 49) on the west edge of the plot.
15. With the tape anchored at the north-west corner (point 49), pull it tight towards the north-east corner (point 51) of the plot and insert pin flags at 1 m, 3.16 m, and 10 m along the north side of the plot.
16. Proceed to the north-east corner (point 51) of the plot and pull the tape tight from the north-east corner (point 51) back towards the north-west corner (point 49) and insert flags at a distance of 1 m and 3.16 m from the north-east corner (point 51) on the north edge of the plot.
17. Proceed to the center of the plot (point 41).
18. Extend the third tape from the middle of the plot towards the 10 m mark on the north edge of the plot and while pulling the tape tight from the center, insert flags at a distance of 1 m and 3.16 m from the center.
19. Repeat the previous step in each direction from the plot center.
20. The boundary of the $10m^2$ nested subplots can be defined by tape measures and pin flags. For $10m^2$ nested subplots on the perimeter, a tape can be extended from a previously inserted survey or pin flag that is 3.16m from the corner where subplots are nested. To maintain a square nested subplot, this tape can target a pin flag that is 3.16m from a corner or center on the perimeter of an opposite side of the $100m^2$ subplot (10 m away). Locating and aiming this targeted flag may require the help of a second person in dense vegetation. For example, the edge of the $10m^2$ nested subplot in corner 1 of subplot 31 can be defined by stretching a tape from the flag at 3.16m on the south edge of the subplot toward the flag 3.16 m towards the center of the plot from the west edge. Delineating the boundary of the $10m^2$ nested subplots anchored at the center of the plot requires that the target flag be added 3.16m from the flag at the middle of the a 20m edge of the plot. For example to defining the edge of the $10m^2$ nested subplot in corner 1 of subplot 41 would require a flag 3.16m from the flag that is 10m between point 49 and 51 or the between point 33 and 51.

APPENDIX C CHECKLISTS

N/A

APPENDIX D REMINDERS

N/A

APPENDIX E ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The dates in the table below are based on historic records and are estimates for the start and stop dates of sampling. It is essential that domain staff monitor real-time conditions to determine when to start (and stop) sampling, as described in Section 4 of this protocol.

This table will be completed in a future revision of this document, as data become available.

Table 6. Estimated dates of historical temperature thresholds

Domain	Site	# of Bouts	Approx. Start Date 1	Approx. End Date 1	Approx. Start Date 2	Approx. End Date 2
01	HARV	1	Late June	Early August		
02	SCBI	1	TBD			
03	OSBS	TBD	TBD			
	DSNY	TBD	TBD			
07	ORNL	1	TBD			
08	TALL	TBD	TBD			
09	WOOD	1	June	July		
10	CPER	2	May	Jun	Early August	Late August
	RMNP	1	Late June	Early August		
	STER	1	TBD			
15	ONAQ	1	TBD (May-June?)			

APPENDIX F BUILDING A ONE-METER VEGETATION SAMPLING SUBPLOT FRAME

The assembled frame encloses an area one meter square. For transporting the frame and threading it through vegetation, the frame comes apart into four sides, or bars. The bars are identical, so the assembly instructions are for one bar.

One meter may not seem like much in open space, but hiking with the bars through brush, hauling them around in a crowded rig, or putting them through airport checked baggage can be awkward. Therefore, most of these plans are for "collapsible" versions -- you can fold up the bars to take less space. We also give a non-folding version, and "ultralight" versions.

The collapsible bars fold up to about one third the length. They work on the same principle as the shockcorded poles of dome tents.

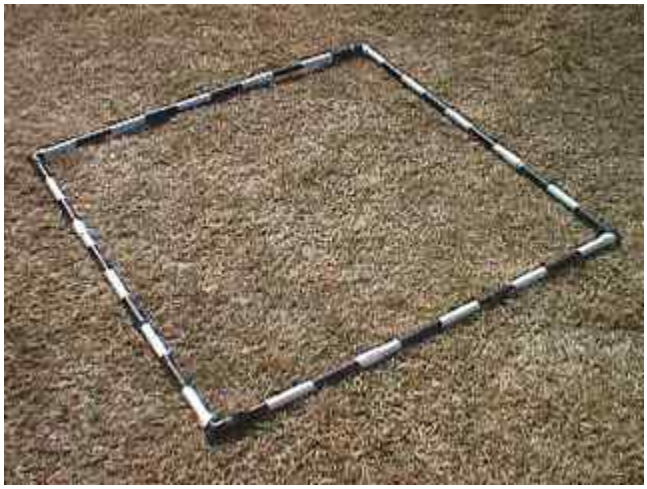


Figure 4. Assembled vegetation sampling subplot frame

Representation of a specific brand name or company does not imply a recommendation by NEON.

FOLDING STURDY PVC FRAME

Materials for one bar are: Three sections of PVC pipe, two straight couplings, one 90 degree elbow, a length of elastic shock cord, and marking material. Here is the parts list for the four bars that make up a complete sampling frame.

These illustrations show "Schedule 40" PVC pipe, which has a wall thickness of about 3 mm (1/8 inch). You can use a lighter weight pipe such a "SDR 13.5", which has a wall thickness of about 1.5 mm (1/16 inch). This will be sufficiently sturdy while saving some weight.

Item	Qty	Comments	Image (Ctrl-click for larger version)
PVC 90 degree elbows, half inch (nominal)	4	All this PVC is "nominal" half inch. They call it half inch even though the inside diameter of the fittings (and the outside diameter of the pipe) is actually about 21 mm (7/8 inch).	
PVC straight couplings, half inch (nominal)	8		
PVC pipe, half inch (nominal), ten-foot sections	2	This kind of pipe usually comes in ten-foot lengths. You will need two of those to cut the 12 thirteen-inch sections you need.	
Drill and 5/16 inch bit		If you are constructing a frame while on the road, many hardware stores will drill the holes for you.	
Elastic shock cord, 1/4 inch (7 mm) diameter	12 ft		
Black electrical tape (or black paint)	1 roll	For marking calibration bands on the bars.	

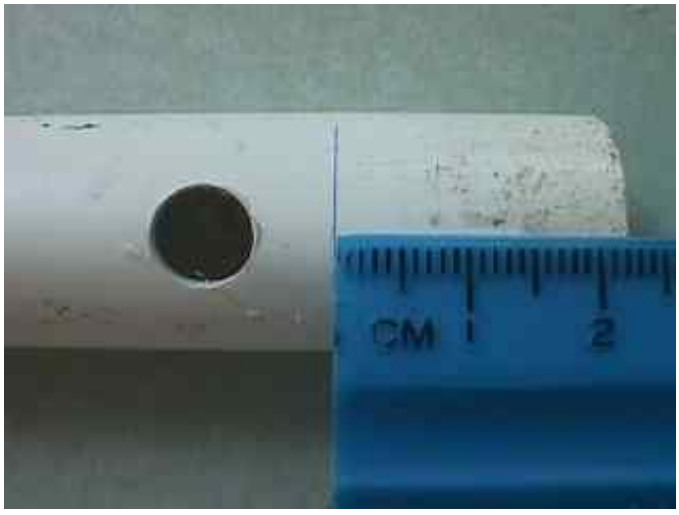
1. Drill a hole in the back of each 90 degree elbow, as shown.

2. Cut 12 PVC sections 33 cm (thirteen inches) long. This size allows for the length added by the connectors, and the length that will slip into them. The finished frame fits almost exactly one square meter.

   - You can cut the PVC pipe any number of ways. If you are on the road, hacksaw blades are cheap and effective. You can wrap one end of the blade with tape to make it easier to grip.
   - There is no need to "bevel" the ends of the pipe sections. Beveling will spoil the stiffness of the finished frame.

3. Drill a hole in the side of three of the PVC sections (one for each bar)

- Drill the hole about 33 mm (1-1/4 inch) from one end. This distance is not critical. It must be at least as far as the length that will slip into an elbow, 22 mm (7/8 inch), but it can be as much as 5 - 8 cm (2 - 3 inches).
- If you are having the holes drilled at the time you buy the pipe, drill them in each end of the two ten-foot sections. Then you can cut the four needed 33 cm (13-inch) sections off of those ends.



4. Push one end of the shockcord through the side hole. Easiest, from outside in, then down the inside of the pipe section.

5. When you have the shockcord through the pipe section and can get enough to work with, thread components together as shown:
   a. Pipe section with side hole
   b. One straight coupling
   c. A pipe section with no side hole
   d. A second straight coupling
   e. Another pipe section with no side hole
   f. A 90 degree elbow

6. Tie a knot close to one end. Pull that end snug to the hole. Bring the PVC pieces together so the shockcord is just about the same length through them. Then pull the shockcord out about a foot so it is stretched.

7. Tie a knot close to the second hole.

8. You can test it at this time to see if the tension feels right. You should be able to pull the PVC sections apart easily and fold up the bar. But when straightened out, the shockcord should hold the pieces tight enough together they don't slip apart by accident. There is no need to glue the PVC sections together.
9. When you are satisfied with the tension, you can tighten up the knot and cut the shockcord off to about an inch (few cm).

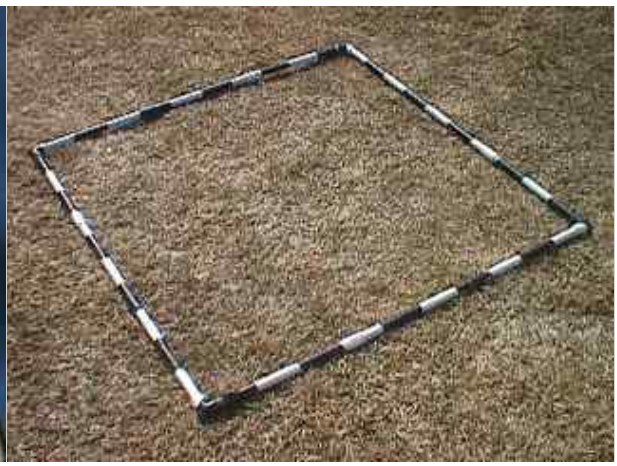
10. Marking the bars in 10 cm bands of alternating color will make it easier to visually estimate percent's of the enclosed 1 square meter area. Electrical tape is a convenient way of marking the bands. Less messy than paint, plus it holds the ends of the shockcord from fraying. If you use the wrapping pattern shown, all the cut ends of the shockcord will be covered.

11. While wrapping the tape, simply skip up and over any couplings you come to.

- Note that tape is free of junctures, so segments can come apart.

12. Make the other three bars the same way. It will be easier to get the shockcord threaded through the bars if you do not cut it first. When you are done, you will have a length of cord left over. You can tie this into a ring and use it like a big rubber band to hold the folded up frame all together in one bundle.

To assemble the frame at a survey site, you push the "empty" end of a bar into the open side of the elbow on another bar. This usually holds together for the minor movements of adjusting the frame position on the ground, but comes apart easily to go around stems and brush.



VARIANT: NON-FOLDING PVC FRAME

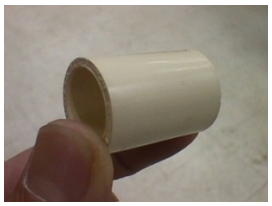
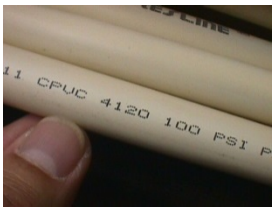
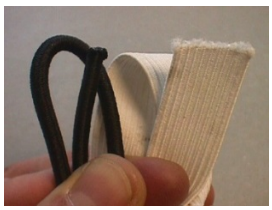
To avoid the complications of the folding junctions, we offer this straight version. Some people prefer this design and use the bars as walking sticks.

Item	Qty	Comments
PVC pipe, half inch (nominal), ten-foot sections	2	
PVC 90 degree elbows, half inch (nominal)	4	All this PVC is "nominal" half inch. They call it half inch even though the inside diameter of the fittings (and the outside diameter of the pipe) is actually about 21 mm (7/8 inch).
PVC glue		
Black electrical tape (or black paint)	1 roll	For marking calibration bands.

1. Cut four sections of pipe, each 39-3/8 inches (about one meter) long. If you are being precise, make them 99.8 cm to allow for length added by the elbows, but this is usually negligible. You will be able to get three pieces out of a ten-foot length of pipe, but you will need another ten-foot section for the fourth bar.
2. Glue one elbow onto one end of each bar. It is necessary to glue them or the frame falls apart too easily in use, and the elbows get lost.
3. Wrap with black tape, or paint bands, to mark the tenths of meters.

VARIANT: LIGHTWEIGHT FOLDING PVC FRAME

This is the same as the regular folding frame except you use (nominal) half inch CPVC pipe and fittings. This type of pipe is usually cream yellow in color, and much thinner and lighter. Even though both these kinds of pipe are called "half inch" the CPVC is about half the size of the "regular" PVC pipe. You can use thinner shock cord or even flat elastic (from a fabric store) in a pinch.

Item	Qty	Comments	Image (Ctrl-click for larger version)
CPVC half inch (nominal), 90 degree elbows	4	All this CPVC is "nominal" half inch. They call it half inch even though the inside diameter of the fittings (and the outside diameter of the pipe) is actually about 16 mm (5/8 inch).	
CPVC half inch (nominal), straight couplings	8		
CPVC half inch (nominal) pipe, ten foot lengths	2	This kind of pipe usually comes in ten-foot lengths. You will need two of those to cut the 12 33-cm (thirteen-inch) sections you need.	
Shock cord, 4 mm (3/16 inch) diameter, or flat elastic 18 mm (3/4 inch) wide.	12 ft		
Drill and 3/16 inch bit		If you are constructing a frame while on the road, many hardware stores will drill the holes for you.	
Black electrical tape	1 roll	For marking calibration bands on the bars.	

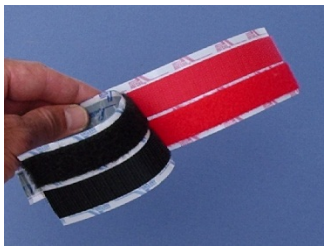
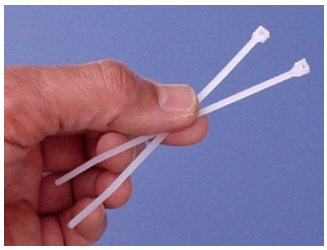
VARIANT: ULTRALIGHT FOLDING TENT-POLE FRAME

This design has proved as serviceable as the sturdy PVC, though at a higher cost in materials. It is a great saving in size and weight: about 0.7 pounds or 11 ounces (300g).

Materials for one bar are: Three tent pole sections, two grommet tips, a length of elastic shock cord, and materials for marking and connection. Here is the parts list for the four bars that make up a complete sampling frame.

You can order starred (*) items from the address given below.

Item	Qty	Comments	Image (Ctrl-click for larger version)
Standard 13-inch tent pole sections, with end insert*	8	The length is 13 inches, not including the insert. The diameter you need for all sections is "three four three", which means 0.343 inches.	
Standard 13-inch tent pole sections, without end insert*	4		
Elastic shock cord, 1/8 inch (3 mm) diameter*	12 ft	This is a standard diameter to fit the tent pole sections. This is sold by the foot.	
Tent pole grommet tips*	8		

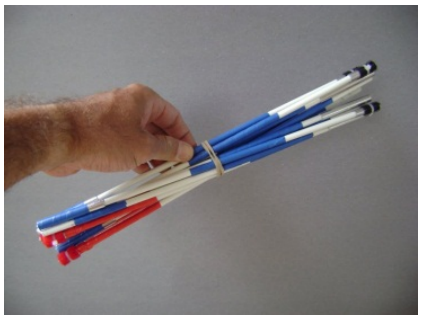
Item	Qty	Comments	Image (Ctrl-click for larger version)
Adhesive backed Velcro, two contrasting colors	4 in	You will only use the hook of one color and the pile of the other color, but the hook and pile are usually sold together. This is typically sold by the half foot, so 6 inches is probably the minimum you can buy. Buy the same length of each color.	
Nylon cable ties.	8	Get the smallest ones available. If possible, match the Velcro colors, 4 of each color.	
PVC "electrical tape", two different colors.	1 roll of each color	For marking the calibration bands on the bars. One color should match one of the Velcro colors, the other should contrast with the bar color (see below).	

* Tent pole sections, and related items, are available from:

Tentpole Technologies, LLC
8212 NE 99th Circle
Vancouver, WA 98662-1300
Phone: 360-260-9527 or 800-266-9527
Fax: 360-260-9937
E-Mail: tentpoles@comcast.net
Website: http://www.polesforyou.com/

Tent pole sections made of fiberglass are becoming available. These are lighter and cheaper.

Assembly is slightly different than shown below, but the principle is the same. The finished frame weighs only about 240g, compared to 300g for aluminum poles.



Before you begin to assemble the tent pole sections, note the following:

- Some of the tent pole sections have an "insert", a narrow extension on the end, which slips into the adjoining section.
- Each bar is made up of two "insert" sections and one "plain" section.
- You should assemble the bar with the two "insert" sections in series, and then the "plain" section. Then, the completed bar will fold up to minimum length. Minimum length is preferred, to carry the equipment in the smallest possible space.

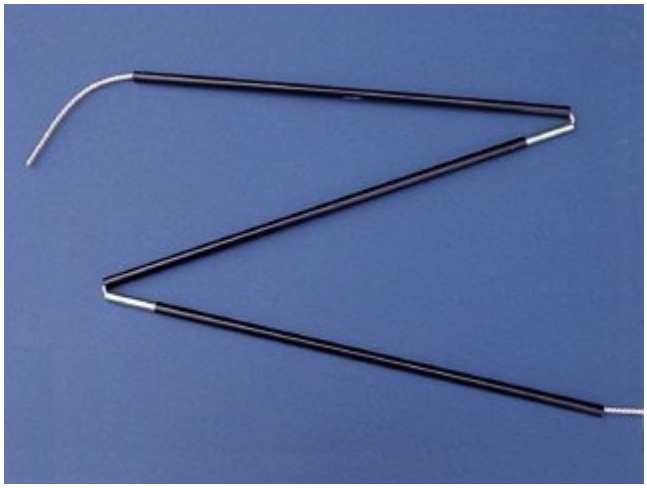
Shortest folding pattern — preferred

Schematic — preferred

Longer — avoid

Schematic —avoid

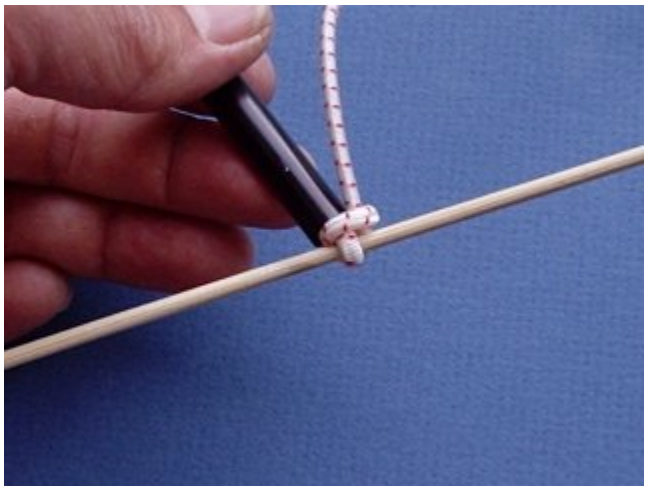
1. Thread the shock cord through the tent pole sections as shown.



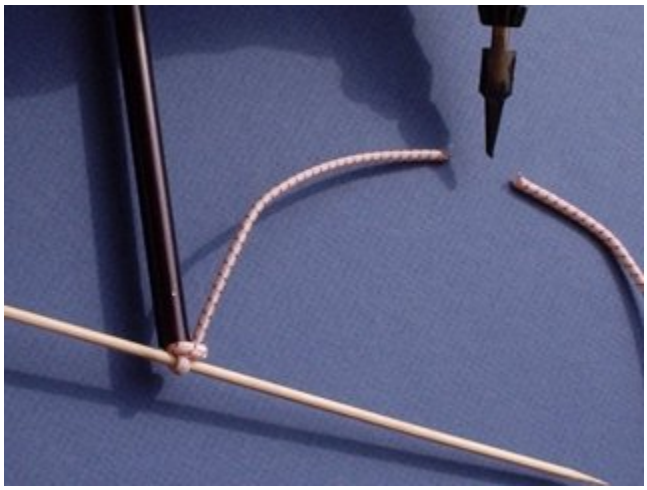
2. Tie the free end of the cord to a grommet tip. A double half-hitch knot is good. Pull the knot tight to make it as small as possible. It will be a close fit inside the tube. Push the grommet tip down into the tube.

3. Pull the other end of the shock cord out about 4 inches (10 cm) beyond slack and mark it to cut.

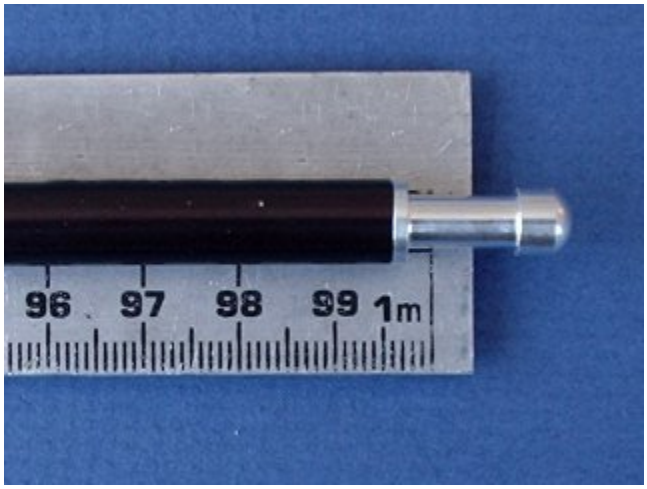
   While working on this end, you can keep the cord from pulling down into the pole section tube by tying a slipknot and putting a small stick through the loop of the knot.



4. You can cut the cord with a soldering iron, which will melt the ends and prevent any fraying. However, just cutting with scissors works acceptably well.



5. After tying on the other grommet tip and assembling the bar, note that the 1-meter length goes slightly beyond the ends of the pole sections. This 1-meter point is where you want the poles to connect, though a little variation is not critical.

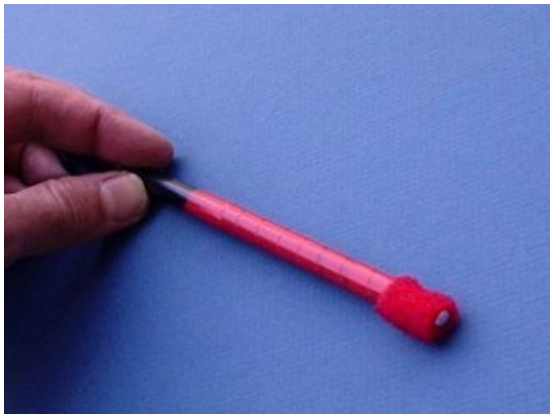


You will use Velcro to "connect" the bars; hook Velcro on one bar end and pile Velcro on the other. The connection does not have to be rigid. In fact it's useful to have the junction flexible, with the ability to easily disconnect and reconnect.

Visual cues help you quickly see which points will stick to each other, so we will make the two ends different colors.

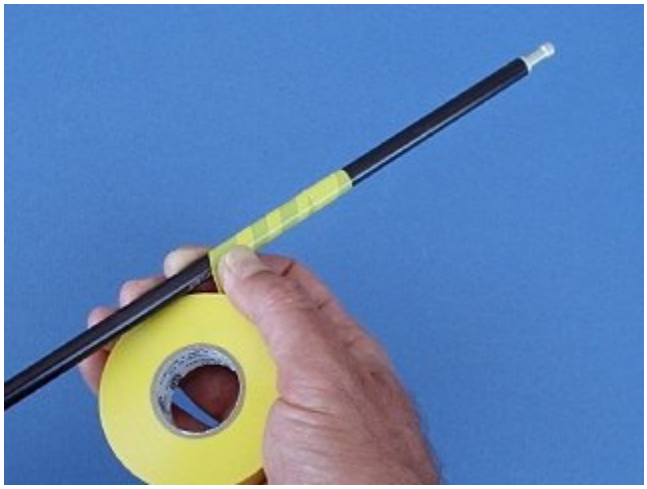
We'll refer to the ends of the bars as the "red end" and the "black end", though of course they can be any contrasting colors. Also, the hook and pile Velcro can be switched from how we describe them here.

6. Cut a strip of red pile Velcro about 10cm long and wrap it around the grommet tip on what will be the red end of a bar. Also, wrap the end of the bar, for 10cm, with tape of the same color.



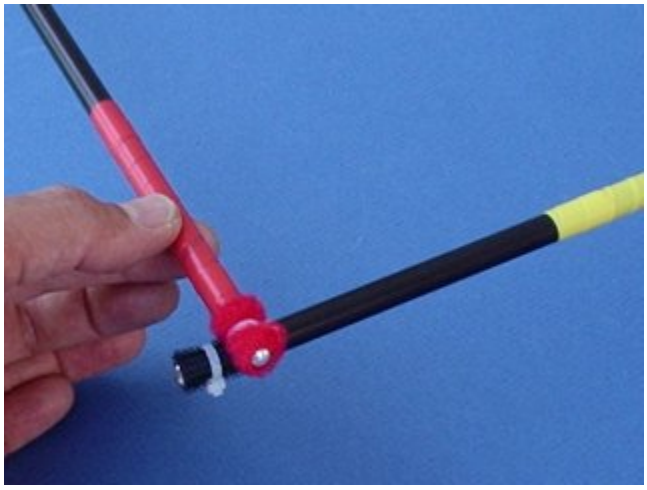
7. The adhesive back of the Velcro makes it easy to apply. However in the field it will tend to come loose with dirt and moisture. So use a cable tie to hold it on securely. You can even hold the Velcro on with a cable tie alone, if you can't get adhesive back Velcro.

8. Wrap the rest of the bar in alternating bands of the contrasting color tape. These 10cm bands make it easy to visually estimate percentages within the enclosed one square meter area. Use the brightest colors you can find, to make the frame visually evident in the field and avoid losing it in the brush.



9. Assemble the black end the same as you did the red end, except use hook Velcro.

10. Assemble the other bars. The red end of one bar will stick to the black end of another bar.



11. The bars will stay together strongly enough to pick up the frame as a piece. In practice, in the field, you will often assemble the frame in place by threading the bars between stems and branches. The ends will stick when you bring them in contact.

12. Folded up, the frame makes a small bundle weighing only 300g (aluminum poles) or 240g (fiberglass poles). You can use a leftover piece of the shock cord to make a band to hold the bundle together. If you buy the poles pre-assembled, a rubber band will serve as well.

APPENDIX G BUILDING A SYSTEM FOR COLLECTING UNKNOWN PLANTS IN THE FIELD

There are many methods for handling plant samples you collect. Here is a "recipe" for equipment that costs little to assemble. Many botanists are familiar with using plastic bags to hold plants; this approach makes everything more hands-free. Many botanists have found this useful.

As with any recipe, you will want to vary it to your own taste.

The basic ingredients are few:

- Large plastic Ziploc bags, available at any grocery store. (Note that depiction of a certain brand name does not imply endorsement of that brand.)
  - Bags *without* the slider work best. The sliders get caught on things
- A miniature "carabiner" key clip
  - You may want to choose a brightly colored mini-biner, to spot easier in the brush.
- A large binder ring (**not** a key ring), available from most any office supply store

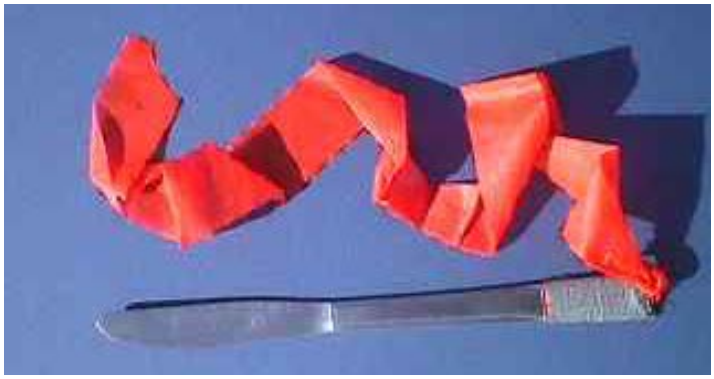
1. Simply punch the binder ring through a corner of each bag.

- You may want to punch through the bottom corners of all bags except one. That way, most openings will hang down. The one bag pointing up serves as a pouch for equipment such as specimen labels, pens, and collecting tools.

2. To complete the setup, close the binder ring and clip the mini-binder through.

A handy tool is a table knife, available for about ten cents at any second hand store. You can use it to both dig and cut. Choose knives constructed in one solid piece.

Mark knives with colored flagging ribbon held on with duct tape. Otherwise, a dropped knife is very hard to see in the brush.



The unit can hang from a belt loop. The slick plastic seldom gets tangled and torn as you walk through brush. When bags do wear out, just replace them.

When you collect a plant, simply swing one of the bags up, drop in the sample, and zip the bag shut.

The one bag with opening at the top stands out as the holder for equipment. (Some people prefer all the bags up. They are concerned specimens will fall out, though this does not happen if the bags are zipped shut.) Some collectors pre-load each bag with a label, ready for the specimen.

Plants don't tend to "cook" in the bags unless left lying directly in the sun. However, this can happen quickly. One case to watch out for is sunlight coming through vehicle windows, even while driving with air conditioning on.

TIPS & TRICKS

You can keep a few un-punched Ziploc bags in the equipment pouch for crispy-dry specimens. Carefully insert the plant, add a squirt of water, blow up the bag like a pillow, and zip it shut. By the time you are ready to press, the dry specimen will have softened enough to flatten without crumbling.

APPENDIX H SITE-SPECIFIC INFORMATION

N/A