PLANT PHENOLOGY PROTOCOLS

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See Configuration Management System for Approval History

Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A_DRAFT	10/03/2011	ECO-00280	Initial draft release
B_DRAFT	01/10/2014	ECO-01138	Updates from 2013. Will be finalized in next rev.

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1 DESCRIPTION

1.1 Purpose

The primary purpose of this document is to provide a change-controlled version of NEON protocols and procedures. This document provides the content for training and field-based materials for NEON staff and contractors. 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.

This document is a detailed description of the field data collection, relevant pre- and post-field tasks, and safety issues as they relate to this procedure and protocol.

1.2 Scope

This document relates the tasks for a specific field sampling or laboratory processing activity and directly associated activities and safety practices. This document does not describe:

- general safety practices
- site-specific safety practices
- general equipment maintenance

It does identify procedure-specific safety hazards and associated safety requirements such as safe handling of small mammals or safe use of required chemicals and reagents. This document describes protocols for taking regular phenological measurements once the phenology transect has been located and individuals tagged. The protocol for beginning of season setup will be provided at a later date.

1.3 Acknowledgements

These protocols are based largely on those defined by the National Phenology Network www.usanpn.org; where pertinent (e.g. phenophase definitions, recommendations for marking plants), descriptive material has been taken directly from their Nature's Notebook monitoring program. The overall sampling framework was developed by the NEON plant phenology working group.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain information that shall be applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

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

2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

RD [01]	NEON.DOC.000008	NEON Acronym List
RD [02]	NEON.DOC.000243	NEON Glossary of Terms
RD [03]	NEON.DOC.000907	Phenology science design document
RD [04]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD [05]	NEON.DOC.014051	Field Audit Plan
RD [06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan
RD [07]	NEON.DOC.001025	Plot Establishment Protocol
RD [08]	NEON.DOC.014042	Plant Productivity Protocol

2.3 Definitions

A **protocol** is a formal summary description of a procedure and its related rationale, and includes information on knowledge and resources needed to implement the procedure. A **procedure** is a set of prescribed actions that must take place to achieve a certain result, and can also be called a method. It differs from a science design in that science designs provide a more complete description of the rationale for selecting specific protocols. It differs from a training manual in that training manuals provide materials in support of skills acquisition in the topic areas including information on how to best train staff rather than detailing only the steps of the procedure.

3 BACKGROUND AND OBJECTIVES

3.1 Background

The NEON plant phenology measurements shall record the seasonal progression of critical biological processes and the timing of ecological events. The NEON phenology measurements shall track sensitive and easily observed indicators of biotic responses to climate variability by recording and monitoring the timing and duration of phenological events in plant communities. Phenology (a branch of science focused on relationships between climate and the seasonal timing of biological phenomena, such as bird migration and blooming dates) is one of the most sensitive and easily observed indicators of biotic response to climate variability. Phenology is affected by forces such as length of growing season, timing and duration of pest infestations and disease outbreaks, water fluxes, nutrient budgets, carbon sequestration, and food availability.

3.2 NEON Science Requirements

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.

3.3 NEON Data Products

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 ([RD04]), NEON.DOC005003.

4 PROTOCOL

The strategy for phenology sampling is divided into two phases over the life of the Observatory. The first phase will focus on the phenology of dominant species and will last for the first three years of operations. Three dominant species will be selected at each site. These three species shall be selected on the basis on a quantitative survey of the relative abundance of plant species in the tower airshed. In sites with no overstory canopy, the three species with the greatest % cover shall be selected. In sites with a distinct overstory, but <50% canopy closure, the single most abundant overstory species shall be selected along with the two most abundant understory species. In sites with >50% canopy closure, the two most abundant overstory species shall be selected, along with the single most abundant understory species. Up to thirty individuals of each species shall be selected and marked for regular phenological observation. In the next phase, a more diverse suite of plants will be monitored at each site ($\leq$ 20 species), with fewer replicates per species (n$\geq$5). The basic sampling protocol, however, will remain the same. For sites where the tower phenocam range of interest does not cover the phenology transect, NEON technicians will select and mark an additional 3 individuals of each dominant species within the phenocam view in order to make explicit linkages between phenocam greenness metrics and in situ phenophase observations, where appropriate individuals should be located and marked outside the

required disturbance buffer zones but within the visible range of interest of the phenocam. Selection of additional individuals to monitor will occur once processed phenocam images become available to define an appropriate region of interest.

At sites with a well-defined, discrete, growing season, the seasonal sampling frequency varies to capture rapid changes during phenological transition periods. The greatest sampling frequency is three times per week during the initial 'green up' period from bud break until 50% adult leaf. Sampling then slows through the middle of the growing season and escalates again to capture fall color change and senescence. At sites that lack a well-defined growing season (e.g. tropical latitudes) sampling will occur less frequently for a longer duration.

NEON plant phenology protocol consists of three procedures which are assumed to begin following plot establishment (see RD[07]):

- Initial selection individuals for phenological monitoring (occurs twice/site for perennial plants, once/season for annual plants)
- Collection of phenology status per monitored individual/patch
- Collection of metadata (location, size) on monitored individuals

Refer to RD [07] for details on phenology transect delineation, placement of permanent markers and steps for annual establishment of the loop.

5 QUALITY ASSURANCE AND CONTROL

The procedures associated with this protocol will be audited according to (RD[05]). 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 (RD[06]).

Ability to identify regionally specific plants on sight is required for the technician who sets up the phenology loop annually and provides instruction and training to seasonal technicians. Once individuals to monitor have been selected and marked and a taxa-specific library of phenophase photos has been developed for each domain, individuals without botany training can conduct surveys provided they are trained by the botany tech and that that individual is available for periodic QA/QC checks in the field and of photos, and to provide guidance.

The QA/QC plan for Plant Phenology monitoring is in development, and all details will ultimately be found in the associated document.

The plan will include:

- Hot checks in the field by HQ staff, domain manager or lead plant technician
- Cold checks in the field by an alternate NEON field crew
- Review of photo documentation by alternate field technician

If field conditions are unsafe, stop work, record location along the phenology loop, resume phenology measurements as soon as possible. If sampling must be completed on a different day, begin sampling again from the start point of the transect loop. If sampling must be delayed for several days such that 1 or more sampling bouts are missed, resume as soon as possible; record in the comments that x number or bouts were missed due to…[explain] so that missing bouts may be accounted for in the metadata. Be especially mindful of missed phenophases and determine sampling frequency based on the phenology occurring when monitoring resumes.

When selecting individuals for monitoring, if it is not possible to find individuals that are evenly spaced around the transect it is acceptable to select groups or more closely spaced individuals. If the required number of individuals are not present on the entire transect, select as many as are available, make a note for metadata that only x number of the selected species were available for phenology monitoring. If during the community phenology phase (20 species) a selected species is not present on the transect for monitoring, move to the next species on the list; 50% contingency will be provided for on the site list.

If an individual is lost or killed mid-season and a near-by suitable replacement is not available, a new individual may be selected from another location along the transect; if no suitable replacement is available, make a note for the individual metadata.

At many sites, disturbance is a major factor shaping plant communities. If there is a disturbance at a site that affects most or all of the phenology transect (e.g. fire, unseasonal freeze event), record disturbance for the metadata, drop frequency down to low level, once a week or once every other week depending on severity and monitor for regeneration within the field season.

6 SAFETY

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.

7 PERSONNEL REQUIREMENTS

One field technician must be capable of identifying regionally specific plants by sight or with a dichotomous key. All technicians conducting phenology observations must be able to recognize all applicable phenophases for species being monitored.

If no member of the field crew is able to identify dormant individuals, contracts with a local botanist to identify and mark individuals for phenological sampling may be employed.

8 TRAINING REQUIREMENTS

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

9 FIELD STANDARD OPERATING PROCEDURE

9.1 Sampling Frequency and Timing

9.1.1 Criteria for Determining Sampling Dates

Start of sampling will be determined on a regional, site and potentially species-specific basis based on knowledge of the local flora. MODIS data in Appendix C provide the earliest recorded increase in NDVI derived from available MODIS data. This date provides an estimate of the earliest day of the year when phenology monitoring may begin but should be augmented by observations made at the site (i.e. near the NEON tower) on the species of interest. The swelling of leaf buds and the separation of leaf bud scales should be monitored on the same individual to determine initiation of regular phenology sampling.

At sites with limited or no pronounced dormant season (e.g. tropical, southeast), or >1 growing season/year, sampling will take place throughout much of the year though at a lower frequency such that a similar number of bouts is possible (roughly 52 bouts/year or once a week for sites with no defined growing season)

Some sites, especially in arid environments may be driven by moisture rather than temperature. In this case, sampling dates and frequency may be more episodic but should sampling should have the same goal of maintaining a similar number bouts per year with higher frequency during periods of rapid change.

9.1.2 Sampling Frequency

Sample frequency varies dynamically throughout the growing season. The objective of the plant phenology sampling is to have at least one observation each year ***before*** springtime plant phenological activity begins (variably defined as young leaves, young needles, needles, see column 2 in Table 1a). Intensive sampling (three times a week) occurs in conjunction with the onset of springtime phenological activity and continues through the early spring development. Once >50% of leaf/canopy development has occurred sampling can be reduced to once a week until full canopy has developed. Post 95% canopy development, sampling is further reduced to once every other week to monitor for reproductive phenology. A second intensive stage (sampling twice a week) begins again in the fall to capture leaf senescence/coloring and reduces to once a week through the end of the season.

The varied intensity is intended to strategically use sampling periods in order to monitor phases of rapid phenological change, while minimizing labor/disturbance associated with frequent measurements during times of year of less rapid change (tables 1 and 2). It is recommended that the technicians take phenological measurements on all individuals each time monitoring is performed, however this protocol defines monitoring frequency based on each monitored individuals phenological stage. A census of the full suite of tagged individuals will result in higher quality data.

Table 1. Rule sets for specific growth forms for SPRING phenology sampling at sites with a well-defined growing season

Growth form	Spring Monitor indicator individuals for:	Sample 3x/week until all tagged individuals show:	Sample 1x/week until all tagged individuals show:	Then:	Then:
Pine	Emerging needles	Young needles	No young leaves	Commence every-other week monitoring for cone phenology	End sampling season when no more fresh pollen cones visible
Grass/Rush/ Sedge/Forb	Initial growth	>50% of plant is green or three consecutive bouts of no change	>95% of plant is green	Commence every-other week monitoring for flowering phenology	See fall sampling schedule
Deciduous broadleaf	Breaking leaf buds	>50% of canopy is full with leaves or three consecutive bouts of no change	95% or more of canopy is full with leaves	Commence every-other week monitoring for open flowers	See fall sampling schedule
Deciduous conifer	Breaking needle buds	>50% of canopy is full with needles or three consecutive bouts of no change	95% or more of canopy is full with needles	Commence every-other week monitoring for open pollen cones	See fall sampling schedule
Evergreen conifer	Breaking needle buds	Young needles	No more young needles visible	Commence every-other week monitoring for open pollen cones	No fall intensive sampling; end sampling when all pollen cones are wilted/dried
Broadleaf evergreen	Breaking leaf buds	Young leaves	No more young leaves visible	Commence every-other week monitoring for open flowers	No fall intensive sampling

Table 2. Rule sets for specific growth forms for FALL phenology sampling at sites with a well-defined growing season

Growth form	Fall Monitor indicator individuals for:	Sample 2x/week until all individuals show:	Sample 1x/week until:	Then:
Pine	NA	**NA**	**NA**	**NA**
Grass/Rush/Sedge/Forb	>5% Leaf senescence (e.g percentage of plant that is green <95%)	<95% green leaves	<5% of plant is green	End sampling season
Deciduous broadleaf	One or more colored leaves	One or more colored leaves	<5% of canopy full with green or colored leaves	End sampling season
Deciduous conifer	One or more colored leaves	One or more colored leaves	<5% or canopy full with green or colored needles	End sampling season
Evergreen conifer	NA	NA	NA	NA
Broadleaf evergreen	NA	NA	NA	NA

9.1.3 Sampling Timing Parameters

When sampling at 3x a week frequency, sampling bouts should be approximately 2-3 days apart.

When sampling at 2x a week frequency, sampling bouts should be every 3-5 days.

When sampling at 1x a week frequency sampling bouts should be 6-9 days apart.

When sampling 1x every other week frequency sampling bouts should be 10-18 days apart.

9.2 Equipment and Materials

Table 3. Field Equipment List

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Binoculars (used when observing phenophases in tall trees)	1	Y	N
	Digital camera capable of producing high quality .jpeg files ≥6MP for QA/QC and taxa-specific image libraries	1	N	N
MX100322	TruPulse 360R Laser Rangefinder for recording location information for selected individuals	1	N	N
	Extra batteries for TruPulse and camera	1-2 each	N	N
	Site specific reference 'photobook'	1	N	N
	Blue aluminium numbered plant tags	10	Y	N
	Hammer	1	Y	N
	Aluminum nails	10	Y	N
	Bailing wire – 1m	1	Y	N
	Wire clippers	1	Y	N
	Tag stakes for marking herbaceous plants at ground level (figure 1)			
	Bright, visible marker for relocation. Suggested: Plant cards (see figure 1)	10	Y	N
	GPS, recreational accuracy	1	N	N
	calibrated 0.25m x 0.25m quadrat frame (sites with patches only)	1	Y	N
	DBH tape (sites with trees only; metadata)	1	Y	N
	10m tape (sites with shrubs only; metadata)	1	Y	N

9.3 Preparation

Prior to each field season, review pre-selected species for each site (Appendix E)

Prior to each data collection bout locate sampling equipment; familiarize yourself with the phenophase definitions and photos as required. Bringing photos with you to the field is advised for all technicians.

Print pre-populated datasheets with individual location, unique ID, species and growth form. Data sheets should be organized sequentially according to location along transect.

9.3.1 Transect Establishment

Plot establishment for plant phenology as described in RD[07] must be completed prior to or concurrently with this procedure.

1) **Navigate** to plot origin (the corner nearest to access point).
2) Use GPS and plot markers to locate the 100 meter secondary marker in the clockwise direction along the transect.
3) **Stretch a 100 meter tape** between each point. Use chaining pins or similar stake at each end to hold the tape in place.
4) **Place a pin flag** every ten (10) meters along the tape. Use pin flags that differ in color from those used to mark other tower plots to provide a visible, reproducible path to walk while monitoring phenophases.
5) **Write the location information** with a permanent maker on each pin flag numbered 0-800 in increments of 10 meters.
6) While marking the transect, **replace** any faded, broken or missing plant cards to facilitate easy location of previously marked individuals/patches.
7) Mark an **access route** to the phenology sampling loop outside of the site-specific "keep-out" area around the NEON tower and soil array. Technicians must use the designated route when accessing the phenology loop to reduce trampling damage within the tower airshed.

9.3.2 Selecting and Marking Individuals

Selection of individuals for phenology monitoring shall occur after annual establishment of the phenology transect.

1) Walk the entire phenology loop to **observe the vegetation patterns** along the transect. This must be done by a technician familiar with local flora. Make notes in the field notebook about how the species chosen for phenology monitoring are spaced (i.e. where there are patches of individuals and where there are gaps) along the transect.
2) Walk the phenology loop transect a second time and **select individuals to monitor** following the generalized criteria for selection:
    a) Individuals/patches must be:
        i) Visible from the transect
        ii) 1< distance to individual <10 meters from the loop
        iii) >10 meters from roads or buildings
        iv) **Represent the average health** for that species at the site. Choose plants that appear to be healthy, undamaged, and free of pests and disease, except in cases where the majority of individuals of that species at a site are affected by the disease (e.g. in a site where most individuals are diseased, fine to select diseased individuals, aim to avoid picking individuals that are not representative of the disease status of the majority of the population)
        v) **Represent a range of size classes**. If more than a single individual is available within the target zone for sampling, aim to include a diversity of sizes (e.g. both canopy emergent and juveniles, for trees), and to sample individuals from alternating sides of the phenology loop at alternating sample points.

vi) Prioritize individuals for monitoring that are **included in productivity sampling** where possible. Only select individuals that may be monitored without excessive trampling of the productivity plots (e.g. plants along the edge which can be accessed/viewed from outside the plots).

vii) Individuals of a single species should be more or less **evenly distributed** around the phenology loop. For Phase I, there should be about 24 meters between evenly spaced individuals of a single species. There will be much more variation in spacing for Phase II species.

viii) **Monitor annual species in patches** in order to avoid confounding individual selection with phenology (individuals cannot be selected until they emerge, which depends on their phenology).

ix) For **biennials avoid choosing the first or the last seedling** to emerge in the spring since they may not be representative of the larger population at the site.

x) **Select collocated groups** of individuals/patches of different species where possible to reduce total sampling time per bout.

3) **Collect location information** on selected individuals/patches.
   a. **Record the location** along the transect (0-800 m) at which the individual is perpendicular. Use the marked flags and pacing or the TruPulse to determine the location.
   b. Record whether the individual is to the **right or left** of the transect
   c. **Measure the distance** from the transect to the individual using a TruPulse 360R in HD mode. Record the GPS location of the individual/patch.
4) **Mark selected individuals** with a blue aluminum plant tag with a unique number >6000 (for patches, skip to #5).
   a. Trees: Tags may be nailed directly into the trunk of trees at a height of 1.5m.
   b. Shrubs: Use bailing wire (with a wide loop) to attach tag to a prominent branch or main stem. Mark with florescent flagging if necessary for relocation and is permitted by site host.
   c. Perennial grasses and forbs: attach tag to loop at the end of a tag stake, place in the ground at the base of the selected individual, place brightly colored plant card in the same loop with species code and unique ID written in permanent marker. The card will help when attempting to locate the individual for monitoring.
   d. Clonal species (e.g. aspen, staghorn sumac, rhizomatous perennials), try to select individual ramets from different clones.
   e. If a selected individual is already marked for productivity measurement, use a blank blue aluminum tag, punch the number that has already been assigned to that individual into the tag and replace the existing marker. This will help technicians quickly identify individuals selected for phenology monitoring and differentiate them from individuals marked for other measurements.

Note: It is import to ensure that the method of marking chosen does not change the growing conditions of the plant or injure the plant in any way. For example, do not wind wire around a tree branch or trunk where it could cut into the bark and interfere with the tree's growth.

Figure 1. Examples of recommended phenology markers. Photo credit: National phenology network (left), Ben Meadows (center), Forestry Suppliers (right)

5) For small plants that grow in large masses of individual stems, it can be difficult to single out a few individuals to observe over time. Instead **set up a 0.25*0.25 m patch** and report on the phenophases for the patch as a whole as if it were an individual. This method works well for mat forming grasses, clonal species that tend to grow as a groundcover, and very small forbs that tend to grow in clumps of individuals.
   a. **Lay out a 0.25 x 0.25 m quadrat** over the densest or most central portion of a group of plants. **Orient the quadrat** so that one side is parallel with the transect.
   b. **Mark all four corners** of the square with metal tag stakes, PVC, or wooden stakes so that the plot frame may be placed in the same location on subsequent monitoring bouts. *Note : Avoid placing a broad stake next to a small plant that would shade it or cause root damage.*
   c. Attach a blue **unique ID** tag to the lower corner of the transect-side of the patch. Annuals do not need to have a permanent aluminum tag used to mark them.
   d. Place a brightly colored plastic plant card in a tag stake in the same corner of the quadrat as the ID tag, write the species and unique ID on the tag. This should not be repeated in subsequent years.
   e. Annuals occasionally live more than a single season. In order to capture this if it occurs, do not remove plant ID cards until the following year (where allowed).
   f. Record location information for the tagged corner.

6) **Flag individuals**. Small metal tags can be obscured by vegetation making individuals difficult to locate from afar. Add florescent pin flags (plant cards in tag stakes preferred) and/or flagging tape near marked individuals to aid technicians in finding the plant (where permitted by site hosts). Pin flags and flagging do occasionally get eaten or lost, thus pin flags must not be the ONLY method of identification and marking.

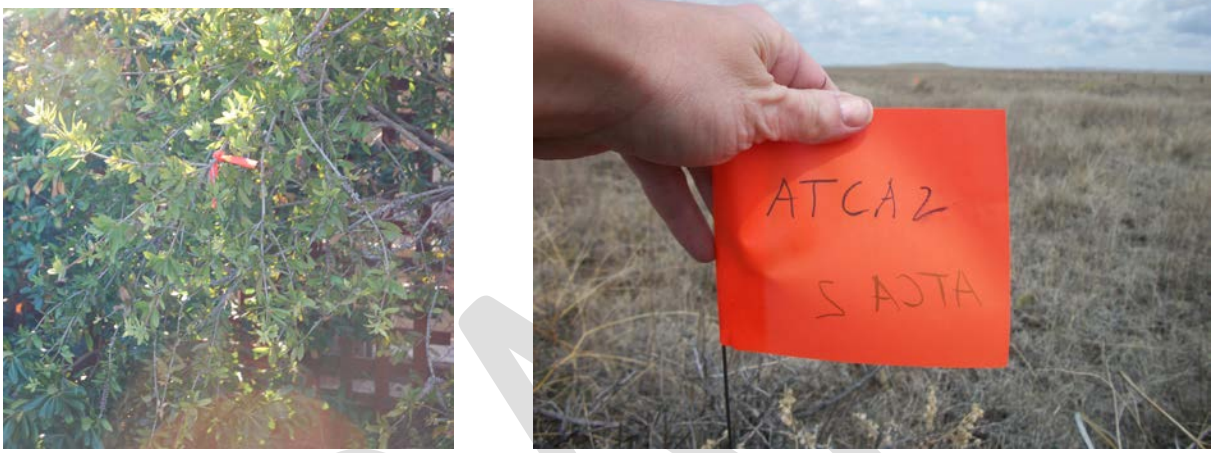

Figure 2. Examples of flagging to increase visibility. Photo credit: National Phenology Network and NEON field crews 2011.

9.4 Data Collection in the Field

1) Locate the corner of the plant phenology transect
2) Collect data from the plant phenology transect systematically, traversing transect in a clockwise direction, stopping at each individual/patch listed on the datasheet. Use a checklist or ordered worksheet of tagged individuals/patches to systematically collect data from each individual/patch along the transect, and to ensure that no individuals are missed.
3) For a given individual/patch, record the following data:
   a) **Date** – MM/DD/YY
   b) Location, Unique ID, Species Code and Growth Form – will be prepopulated
   c) Record Y/N/?/M for each **phenophase** (P1-P6) required for that growth form (see Appendix A)
      i) Yes (Y) – if phenophase *is* occurring
      ii) No (N) – if phenophase *is not* occurring
      iii) Uncertain (?) – if not certain whether the phenophase was occurring
         (1) Take a digital photo of individual to document phenophase, record photo file # on datasheet.
         (2) Review with local expert to identify phenophase and record per data handling procedure.

iv) Missed Phase (M) – if a phase occurred between sampling bouts that was not previously observed and you have reason to believe that it occurred (such as dried flowers on the ground below the plant). Record the start date as last time the plants were sampled and the phenophase had not yet occurred and end date as current date in the comment section of the datasheet. Intensity values for missed phenophases are not recorded.

v) Check for every phenophase for every individual/patch during each sampling bout. Write N/A or cross out the cell on the datasheet if that phenophase is not required for the growth form of the individual to verify that nothing was skipped along the way

vi) If a tagged plant listed on the datasheet cannot be located, record 'lost' for the original plant.

Table 4 Phenophases (see Appendix A)

Phenophase #	Phenophase title
P1	Breaking leaf buds
	Breaking needle buds
	Emerging needles
	Initial growth
P2	Increasing Leaf size
	Young Leaves
	Young needles
P3	Canopy
P3	Leaves
	Needles
P4	Open flowers
	Open pollen cones
P5	Colored leaves
	Colored needles
P6	Falling leaves
	Falling needles

4) For each phenophase for which 'Yes' is selected, select an appropriate phenophase **intensity** category (1-6, see figure 4). Growth-form specific phenophases and their descriptions are listed in Appendix A.

Table 5. Intensity class categories

Intensity Classes	1	2	3	4	5	6
#	< 3	3-10	11-100	101-1,000	1,001-10,000	> 10,000
% canopy, flower	< 5	5-24	25-49	50-74	75-94	≥ 95
% leaf size (P2 only)		< 25	25-49	50-74	75-95	≥ 95

5) Take a photograph of at least 3 examples of that phenophase*intensity combination on the encounter in each species. The photographs serve two purposes: (1) to build up site-specific training materials and (2) QA/QC.
6) Record photo file # in the datasheet.
7) If a tagged plant listed on the datasheet cannot be located or has experienced an unrepresentative change in health select and mark a new plant with a new unique ID# in a location near where the original plant was located.
    a) If a long lived species that was healthy becomes diseased or experiences >50% mortality, replace as for missing plants unless the shift in health status is common (representative) among that species at the site (e.g. pest infestation or widespread disease).
    b) If the original plant dies (perennials only), replace as for missing plants.
    c) If the original plant dies (annual plants), data should be recorded as 0% green leaf, phenological monitoring for that individual/patch can end for the season.
8) Once each year record size and location information for each individual/patch selected for phenology monitoring. Record this data at peak season for herbaceous plants and forbs, and at the same time of the year annually for woody species. Record this data during a routine data collection bout. For a given individual, record the following data, using the datasheets provided:
    a) Location Information
        i) Transect location (0-800mm)
        ii) Direction from transect (R/L)
        iii) Unique ID (tag#)
    b) Species code – use USDA plants species codes
    c) Sample form (Patch/Individual)
    d) Canopy Emergence Status (Y/N). Enter Y if individual is part of the overstory, that is, ≥20% of the canopy is exposed to the sky (i.e. visible to AOP over flights). If ≥80% of the canopy is obscured by taller plants, the individual is considered part of understory, enter N in datasheet.
    e) Record biomass/productivity measurements consistent with NEON plant productivity protocols (RD [08]). Use the TruPulse 360R canopy diameter and height measurements where appropriate.
        i) Single stemmed Individuals: Record the diameter at breast height (130cm - DBH), canopy diameter at widest point (1), the diameter perpendicular to widest point (2), the length of an average adult leaf (DBL only - for future estimates of P2 intensity for deciduous trees and shrub) and height. Record canopy and heights in meters, DBH in centimeters.
        ii) Multi stemmed shrubs and herbaceous perennials: Record canopy diameter at widest point (1), the diameter perpendicular to widest point (2), the length of an average adult leaf (DBL only) and height. Record canopy and heights in meters, DBH in centimeters.
        iii) Patches: Record Percent Cover and height (in meters – Remember 12cm = 0.12m). Percent cover follows directly from NEON plant diversity protocols (RD[**]), with the exception that quadrats are 0.25m on a side rather than 1m.

(1) Each frame should be calibrated (painted in 10 cm sections) to make cover estimates easier (refer to figure 4.2 in plant diversity protocol)
(2) Only estimate cover on plants, or portions of plant, that are rooted inside the subplot frame.
(3) Visually group individuals together into a percent cover. Fine tune that estimate by subtracting out any spaces or gaps. There will often be overlap of plants species.
(4) Cover should be recorded as the total aerial coverage of the target species. Estimates should not exceed 100% for the target species.

f) Disease status (H/D – healthy/diseased)
g) Disease type – if known

9.5 Data Handling

1) For data collected on paper datasheets: Transcribe data into phenology Field Data excel file template.
    1. Spreadsheet fields mirror the data sheet, do not change formatting on the provided spreadsheet.
    2. Data should be transcribed following each monitoring bout. Transcription QA shall conform to QA/QC protocols (RD[06]).
2) For data collected using a GPS data dictionary: Transfer data from GPS handheld to a shared folder and then post to the NEON data portal. Ideally this will occur daily at sites where internet access is available.
    1. Open Pathfinder Office software
    2. Open the previously created project.
    3. Check Coordinate System settings.
    4. Connect the Trimble to the computer with the USB cable, and power on the unit.
    5. The Windows Mobile Device Center (Win7 or Vista) should pop-up displaying a connection of the device to Windows.
    6. Within Pathfinder Office on the PC, use the Data Transfer utility to transfer the .tif file to the Trimble:
        a. Go to Utilities > Data Transfer… and in the window that opens, click the “Receive” tab and then click “Add…” and select “Data File” from the list and click Open.
        b. Select all data files relevant to phenology sampling, then click Transfer All.
    7. The .tfw file will be transferred automatically and need not be manually specified. However, the .tfw file must be in the same directory and have the same name as the .tif file.
3) For data collected on the NEON digital data collection device: Download all data according to the protocols developed for data handling. Address any QA/QC concerns.
4) Download photos.
    1. Place all photos in a single folder labeled by date for that specific bout.
    2. Update photo file meta-data with observers’ name.

3. Confirm uncertain phenophases (i.e. phenophase = ?) with local expert. Update datasheets and note that adjustments data were post-corrected in the lab.
4. Verify phenophase/intensity for any data collected by inexperienced technician (i.e. first 3 bouts). All photos must pass QA/QC procedure before being included in phenology reference library.
5. Add Tags (Species, phenophase, intensity…)to file properties to make them searchable.
6. Do not change file name as this links to datasheet info.

5) Create datasheets for next sampling bout (If using paper data sheets).
   1. Filter spreadsheet to only display results from the current sampling bout.
   2. Copy all rows of data (n=100).
   3. In a new worksheet, create list of the location, unique ID, species and growth form dated for the next scheduled sampling bout.
   4. Paste data from the most recent bout at the end of the list.
   5. Sort all rows by Unique ID and date. This will create a datasheet that will allow the technician to view phenophase data for each individual from the previews sampling bout.

9.6 Equipment Maintenance, Cleaning and Storage

1) Double check that all photos have been downloaded then delete photos from camera.
2) Charge camera batteries.
3) Charge GPS unit.

10 REFERENCES

Denny, E. G., et al. (in revision). "The USA National Phenology Network protocols: Standardized phenology monitoring methodology for plants and animals."

National Phenology Network. "Nature's Notebook." https://www.usanpn.org/natures_notebook.

APPENDIX A PHENOPHASES FOR EACH GROWTH FORM

Summary of Phenophases and intensity measurements

This table is intended as a quick reference for which phenophases to measure for different growth forms and whether intensity should be recorded in terms of absolute number (#) or percentage of individual/patch on which the phenophase is occurring (%). Detailed descriptions of growth form specific phenophase and intensity measurements are provided in following sections.

Growth Form	(P1) Breaking buds / Emerging Needles/ Initial Growth	P1 intensity	(P2) Young Leaves/ Young Needles	P2 intensity	(P3) Full size leaves	P3 intensity	(P4) Open Flowers / Pollen Cones	P4 intensity	(P5) Colored Leaves/ Needles	P5 intensity	(P6) Falling leaves/ Needles	P6 intensity
Deciduous trees and shrubs (DBL)	✓	#	✓	%	✓	%	✓	%	✓	%	✓	-
Broadleaf evergreen trees and shrubs (EBL)	✓	#	✓	#	-	-	✓	%	-	-	-	-
Evergreen Conifer (EC)	✓	#	✓	#	-	-	✓	%	-	-	-	-
Pine	✓	#	✓	#	-	-	✓	%	-	-	-	-
Deciduous Conifer (DC)	✓	#	-	-	✓	%	✓	%	✓	%	✓	-
Forb	✓	-	-	-	✓	-	✓	%	-	-	-	-
Grasses/Rushes/Sedges (GRS)	✓	-	-	-	✓	%	✓	%	-	-	-	-
Cactus	-	-	-	-	-	-	✓	%	-	-	-	-

APPENDIX B DEFINITIONS OF PHENOPHASES

Phenophase #	Phenophase title	Applicable Growth forms	Status Description	Intensity description
P1	Breaking leaf buds	DBL EBL	One or more breaking leaf buds are visible on the plant. A leaf bud is considered "breaking" once a green leaf tip is visible at the end of the bud, but before the first leaf from the bud has unfolded to expose the leaf stalk (petiole) or leaf base.	# class of buds that are breaking
	Breaking needle buds	DC EC	One or more breaking needle buds are visible on the plant. A needle bud is considered "breaking" once a green needle tip is visible at the end of the bud, but before the first needle from the bud has unfolded and spread away at an angle from the developing stem, or from other needles in a bundle.	# class of buds that are breaking
	Emerging needles	Pine	One or more emerging needles or needle bundles (fascicles) are visible on the plant. A needle or needle bundle is considered "emerging" once the green tip is visible along the newly developing stem (candle), but before the needles have begun to unfold and spread away at an angle from others in the bundle.	# class of needles or needle bundles emerging
	Initial growth	Forb GRS	New growth of the plant is visible after a period of no growth (winter or drought), either from above-ground buds with green tips, or new green or white shoots breaking through the soil surface. Growth is considered "initial" on each bud or shoot until the first leaf has fully unfolded. For seedlings, "initial" growth includes the presence of the one or two small, round or elongated leaves (cotyledons) before the first true leaf has unfolded.	NA

Phenophase #	Phenophase title	Applicable Growth forms	Status Description	Intensity description
P2	Increasing Leaf size	DBL	A majority of leaves on the plant have not yet reached their full size and are still growing larger. Do not include new leaves that continue to emerge at the ends of elongating stems throughout the growing season.	% class of full size of most leaves
	Young Leaves	EBL	One or more young, unfolded leaves are visible on the plant. A leaf is considered "young" and "unfolded" once its entire length has emerged from the breaking bud so that the leaf stalk (petiole) or leaf base is visible at its point of attachment to the stem, but before the leaf has reached full size or turned the darker green color or tougher texture of mature leaves on the plant. Do not include fully dried or dead leaves.	# class of young leaves present
	Young needles	EC Pine	One or more young, unfolded needles are visible on the plant. A needle is considered "young" and "unfolded" once it has spread away from the developing stem enough that its point of attachment to the stem is visible, but before it has reached full size or turned the darker green color or tougher texture of mature needles on the plant.	# class of young needles present
P3	Canopy	DBL	One or more live, unfolded leaves are visible on the plant. A leaf is considered "unfolded" once its entire length has emerged from the breaking bud so that the leaf stalk (petiole) or leaf base is visible at its point of attachment to the stem. Do not include fully dried or dead leaves.	% class of the canopy that is full with leaves. Ignore dead branches in the estimate.

Phenophase #	Phenophase title	Applicable Growth forms	Status Description	Intensity description
P3	Leaves	Forb GRS	One or more live, fully unfolded leaves are visible on the plant. For seedlings, consider only true leaves and do not count the one or two small, round or elongated leaves (cotyledons) that are found on the stem almost immediately after the seedling germinates. Do not include fully dried or dead leaves.	Do not report intensity for forbs. GRS - % class of the plant that is green
	Needles	DC	One or more live, unfolded needles are visible on the plant. A needle is considered "unfolded" once it begins to spread away at an angle from the developing stem enough that its point of attachment to the stem is visible, or from other needles in a bundle so that it is no longer pressed flat against them. Do not include fully dried or dead needles.	% class of the canopy that is full with needles. Ignore dead branches in the estimate.
P4	Open flowers	DBL EBL Forb GRS Cactus	One or more open, fresh flowers are visible on the plant. Flowers are considered "open" when the reproductive parts (male stamens or female pistils) are visible between or within unfolded or open flower parts (petals, floral tubes or sepals). Do not include wilted or dried flowers.	% class of all fresh flowers (buds plus unopened plus open) on the plant that are open. For species in which individual flowers are clustered in flower heads, spikes or catkins (inflorescences), estimate the percentage of all individual flowers that are open.
	Open pollen cones	DC EC Pine	One or more open, fresh, male pollen cones (strobili) are visible on the plant. Cones are considered "open" when the scales have spread apart to release pollen. Do not include wilted or dried cones that have already released all of their pollen	% class of all fresh pollen cones (unopened plus open) on the plant that are open. (do not include wilted or dried cones that have already released all of their pollen in this calculation)

Phenophase #	Phenophase title	Applicable Growth forms	Status Description	Intensity description
P5	Colored leaves	DBL	One or more leaves (including any that have recently fallen from the plant) have turned to their late-season colors. Do not include fully dried or dead leaves that remain on the plant.	% class of the canopy that is full with colored leaves
	Colored needles	DC	One or more needles (including any that have recently fallen from the plant) have turned to their late-season colors. Do not include fully dried or dead needles that remain on the plant.	% class of the canopy full with colored needles.
P6	Falling leaves	DBL	One or more leaves are falling or have recently fallen from the plant.	NA
	Falling needles	DC	One or more needles are falling or have recently fallen from the plant.	NA

APPENDIX C DEFINITION OF PHENOPHASE CLASSES

Intensity Classes	1	2	3	4	5	6
#	< 3	3-10	11-100	101-1,000	1,001-10,000	> 10,000
% canopy, flower	< 5	5-24	25-49	50-74	75-94	≥ 95
% leaf size (P2 only)		< 25	25-49	50-74	75-95	≥ 95

Helpful Information

- **Multiple phenophases** may be reported for an individual in a single bout. Evaluate each phenophase independent from the others. For example, for **Breaking buds/ emerging needles / initial growth phenophase (P1)**, each leaf bud, needle bud, or shoot should be judged separately. As long as some buds or shoots on the plant are still breaking or initiating growth and have not yet produced an unfolded leaf or needle, you are seeing 'Breaking leaf/needle buds', 'Emerging needles', or 'Initial growth'. For plants that have more than one bud or shoot, in most cases you will still be seeing 'Breaking leaf/needle buds', 'Emerging needles', or 'Initial growth' in some buds or shoots for many days after you first begin seeing 'Leaves/Needles' or 'Young leaves/needles' from other buds or shoots. It is also possible to see multiple episodes of leaf/needle bud break or initial growth within a season. This might occur after a period of frost, drought, or after a plant is defoliated by insects. However, once ALL the active leaf/needle buds or shoots on the plant have at least one unfolded leaf/needle, you should be reporting that you no longer see 'Breaking leaf/needle buds', 'Emerging needles', or 'Initial growth'.
- If unsure about the intensity class for the **young leaves/needles phenophase (P2)** for deciduous broad leaf species **(DBL),** refer to the metadata from the previous year to see the length of an adult leaf for that species. In the first year of operations this will have to be an educated guess since the data will not yet be available for a quantitative assessment. This measurement is included in order to track the length of the "green-up" period, the amount of time it takes leaves to reach full size, an important aspect of a plant's response to climate change.
- Continue to report seeing **'Leaves/Needles' (P3)** as long as fresh green or colored leaves/needles remain on the plant. Do not include dried, dead leaves or dead, brown needles that remain on the plant, such as occurs with some species throughout the dormant season (e.g. winter or dry season). In some cases, green leaves will remain on the plant in a frozen condition for part or all of the winter. If more than about 5% of the leaves have remained on the plant in this condition, you should continue to report seeing 'Leaves' until they fall off or appear wilted.
- For **grass, sedge and rush species (GSR)** where new growth is from new stems, the plant will probably be 100% green (intensity class 6 for P3) until it begins to turn brown in the late summer or fall. For species where existing stems can turn brown and then re-green, the

intensity for the **leaves phenophase (P3)** may start low at the beginning of the growing season, become higher in the middle of the growing season, and then decline again as the plant turns brown again. In dryland environments where conditions are extreme, it can be particularly difficult to judge what portion of a grass plant is truly dead and what portion has the potential to re-green. If this is the case refer to the reference photobook for phenophase for that species. Take a picture for future reference and discuss with the lead botany technician.

- When estimating intensity class for **Open Flowers (P4)** on plants with inflorescences, the percentage of individual flowers open on a single inflorescence (flower heads, spikes or catkins), will often be the same for all inflorescences on the plant. If this is the case, you can choose a single inflorescence, estimate the percentage of open flowers on it, and use that value to represent the entire plant. For larger plants, it is generally a good idea to check a few inflorescences (for example, one towards the bottom of the plant, one in the middle and one towards the top), and average the percentage of open flowers on each of these inflorescences to represent the entire plant.
- To estimate the percentage of the canopy that is full with **colored leaves/needles (P5)**, consider only the colored portions of leaves and needles that are left on the plant and do not include the green portions. For instance, if the plant canopy is 100% full with leaves but about half of them are green and half are colored, you would report that 100% of the canopy is full with leaves, and 50% of the canopy is full with colored leaves. If it is windy the next day, and half of the colored leaves fall off (but none of the green leaves fall off), you would now report that 75% of the canopy is full with leaves and 25% of the canopy is full with colored leaves. As the days go on, more of the leaves change color and some fall off, and you might eventually find that only half of the leaves remain on the plant and there is no green left in them. At this point you would report that 50% of the canopy is full with leaves and 50% of the canopy is full with colored leaves. Note that the percentage of the canopy full with leaves or needles (green plus colored) should steadily decline from 100% to 0% as leaves or needles fall off. However, the percentage of the canopy full with colored leaves or needles may go up and down during this time of leaf/needle fall.
- There are no intensity options for **Falling Leaves (P6)** because the percentage of leaves or needles that have fallen from a deciduous plant can be calculated from the percentage of leaves or needles that remains on the plant. This is already captured in the value you reported for percentage of the canopy is full with leaves/needles for the 'Leaves/needles' phenophase (P3).

APPENDIX D DATA SHEETS

Selection of Individuals

Recorded By: ______________________________ Page __of__

Locality: ______________________________ eventDate (yyyy/mm/dd ______________

Transect m	direction from transect (R/L)	90° distance (m)	catalogNumber (tag #)	taxonID	idQ	Growth Form	associatedMedia #

Spring/Summer Sampling

RecordedBy:

Locality:

Current Bout date Date (yyyy/mm/dd):

Phenophase Occurance Codes: Y=yes; N=no; M=missed; ?=un

Remarks:

* if P5 or P6 occuring, fi out Fall datashee

Previous Bout Date(yyyy/mm/dd)	Transect m	Dir from Tr. (R/L)	90° distance (m)	catalog Number (tag #)	taxonID	Growth Form	P1	P1 intensity	P2	P2 intensity	P3	P3 intensity	P4	P4 intensity	P5 & P6*	associa dMedia

Fall Sampling

Plant Phenology Datasheet P5-P6

RecordedBy: _____ Current Bout Date (yyyy/mm/dd): _____ Remarks: _____

Locality: _____ Phenophase Occurance Codes: Y=yes; N=no; M=missed; ?=uncer

eventDate (yyyy/mm/dd)	Transect	Direction from transect (R/L)	90° distance (m)	catalog Number (tag #)	taxonID	Growth Form	(P5) Colored Leaves/ Needles	P5 intensity	(P6) Fallen leaves/ Needles	Associated Media #	

Phenology Metadata

Annual_Data

RecordedBy: _____

locality: _____ eventDate (yyyy/mm/dd): _____

i= required measurement on individuals only; p=required measurement on 0.25 x 0.25m p

Transect m	**Dir. From Tr. (R/L)**	**catalog Number (tag #)**	**TaxonID**	**Growth Form**	**Patch / Individual**	**Canopy Emergent** (Y/N)	**Status** (Live/Dead / Lost) [i, p]	**DBH** (cm)[i]	**canopy diameter 1** (m) [i]	**Canopy diameter 2** (m) [i]	**% cover** [p]	**height** (m [i, p])	**Adult leaf length** (cm, DB only)

Indicator Individual Phenology

Indicator phenology

RecordedBy: _____ Page ___of ____

locality: _____

* monitor indicator individual near NEON tower while conducting routine tower check during dormant season. Report first sign of bud swell to lead botany tech

eventDate (yyyy/mm/dd)	taxonID	Bud swell occurring? (Y/N)	eventDate (yyyy/mm/dd)	taxonID	Bud swell occurring? (Y/N)

APPENDIX E SITE SPECIFIC INFORMATION

When to Sample

To view NDVI/EVI graphs for your sites go to the 'create subset' link a the ORNL website (http://daac.ornl.gov/MODIS/). The dates in this table were generated from the 'MODIS Phenology time series' report for an area 2 km on a side centered on the lat/long of the tower location at each site.

Date of earliest and latest greenness increase and latest minimum greenness date as estimated by MODIS averaged EVI values from 2001-2009. These values are provided as a rough guide for when phenology monitoring may begin (between earliest and latest greenness increase date) and end (latest return to minimum greenness date). This information should be augmented by on-the-ground phenology observations made by a tower technician on indicator individuals using the provided datasheet.

Domain	Site	Earliest greenness increase (DOY)	Year	Latest greenness increase (DOY)	Year	latest onset of Min greeness (DOY)
1	HARV	100	2002	121	2003	310
3	DSNY	40	2001	105	2005	340
3	OSBS	55	2006	110	2007	355
10	CPER	62	2006	110	2009	320
10	STER	70	2005, 2007	150	2002	310
10	RMNP	110	2002	135	2009	280

What to sample

List of species selected for Phase I (dominants) phenology monitoring

Domain	Site	Phase I Sp1	Phase I Sp2	Phase I Sp3
1	HARV	*Acer rubrum*	*Quercus rubra*	TBD
3	DSNY	TBD	TBD	TBD
3	OSBS	TBD	TBD	TBD
10	CPER	TBD	TBD	TBD
10	STER	TBD	TBD	TBD
10	RMNP	TBD	TBD	TBD