

PlantPopNet Protocol

The PlantPopNet protocol details data collection for *Plantago lanceolata* demography. The protocol is separated into two parts: **A. Core protocol** - the core data collection, which all sites will have to provide, and **B. Add-ons data collection**, which is optional. Core data collection described in this document will be used in the primary planned publications, while the add-on data will be used in additional publications. Please consider contributing to some of the add-on projects if you have additional time and interest.

A. Core protocol

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The core protocol is easy and can be carried out with confidence by non-demographers. One person can accomplish the core protocol in about 12 hours/year, excluding traveling time. The effort may vary depending on site conditions and vegetation type. The establishment of permanent plots in the first year and the destructive sampling may take up an additional 6-8 hours. Involving a field assistant speed up the work process significantly. Ideally data collection will run for at least 5 years and we are aiming for up to 10 years at a subset of sites. However, just one year of data collection is valuable and two years of data collection will be used to generate the first transition for a basic demographic model. Additional years will be used to examine temporal variation in dynamics and trait values.

Download the four data-sheets ‘*Site description.xls*’, ‘*Individual plant census.xls*’, ‘*Community plots.xls*’, ‘*Destructive sampling.xls*’ from here: www.plantpopnet.com/protocol. Fill out and submit the datasheets to core@plantpopnet.com and buckley@tcd.ie following each annual field census. Please follow the protocol carefully and if in doubt, feel free to contact the coordinator (Prof. Yvonne Buckley, buckley@tcd.ie).

Part 1. Establishment of permanent demography plots (Year 1 only)

This section details site selection, setting up transects and plots, and collection of environmental covariates at the site level. Fill out the “Site description.xls” file and partly the “Individual plant census.xls” file. Column names are marked in bold throughout the text.

Equipment needed (see also Figure 1):

- Bright colored tape to mark transect positions (10 m)
- Pegs/tags/stakes to permanently mark transects and plots (2 for each plot) – must be able to re-find these in subsequent years
- Permanent marker pen for labeling transects/plots
- GPS for recording transect positions, resolution at least 3 m
- Datasheets on computer/tablet or printed on (waterproof) paper provided on PlantPopNet website www.plantpopnet.com/protocol
- Digital camera/phone for taking site/landscape photos

Site selection

Sites can be anywhere in the world where *P. lanceolata* occurs and where there is at least 100 individual plants in 100 m². A periodically updated map of global *P. lanceolata* occurrences and existing PlantPopNet sites, and additional resources to guide the site selection are available to view/download from here: www.plantpopnet.com. We welcome replicate sites in the same geographic region, or replicates within similar environmental conditions; however, if you have to choose between several sites, please get in touch with the coordinator to find out which site would be most valuable for the whole network. The goal is to sample populations from across the whole environmental and/or geographical space of *P. lanceolata*.

The choice of site will need to take into account the following:

- Somewhere you can get to at approximately the same time every year for annual census
- Permission to work there
- Ability to permanently mark plants & plots
- Low risk of vandalism
- Site may or may not be actively managed. If there is a known management regime, (e.g. herbivory, grazing, mowing, mulching, herbicide spraying, fertilization, fire), provide data on the management regime via an initial survey and keep records of management activities in subsequent years (incorporated into “*Site description.xls*” template). Try making sure that the current management at the selected site will not change drastically in the coming years, to make analyses and interpretation less challenging.

Timing of data collection

Data collection should happen at the time of peak flowering of *P. lanceolata* in the chosen system (note this can be quite extended), ideally when some seeds are fully developed so that they can be collected. This means that the time of censuses can vary between sites. For example, for one of the temperate sites in the Northern hemisphere (Ireland), the census takes place in late July/early August. The census needs to happen at approximately the same time every year (within a couple of weeks), in order to capture roughly the same developmental stages of *P. lanceolata*. Try adjusting the timing to yearly changes in weather. Time the census prior to any predictable disturbance (e.g. annual mowing, movement of grazing animals into the area).

Setting up transects and plots

Within a site, set up as many 0.5 m wide belt transects as you need to have at least 100 plants altogether. A belt transect will be made up of multiple contiguous 0.5 m × 0.5 m plots (Figure 1a, b). Transect length varies from site to site depending on the population density of *P. lanceolata*, but should not exceed 10 m. Shorter transects may be used if site shape and size dictates, however try limiting the number of transects up to a total of 5. Transect ends must be permanently marked using appropriate markers for any site conditions (e.g. tent pegs, rebar, star pickets, stakes, labeled trees etc.) and georeferenced (record latitude and longitude with a GPS device). In case of multiple transects the ends of each transect should be marked separately. Fill out the **transect_Lat_start**, **transect_Lon_start**, **transect_Lat_stop**, **transect_Lon_stop** columns in the “*Individual plant census.xls*” file for each transect (these are the coordinates of transect ends). Transects run from left to right along the contour of any slope. Leave 2 meters distance between two consecutive transects.

Prepare a site-level map of transect relative positions (similar to the one in Figure 1b) along with a few site and landscape pictures. The map should be a simple sketch similar to Figure

1b, annotated with transect-end GPS coordinates. Label the maps and pictures with site_code – e.g. CH_map; CH_site; CH_landscape.

Once the extent of the transect(s) has been determined, choose the start point randomly along each transect to avoid biasing the starting plot to high or low plant density. Lay down 0.5 m × 0.5 m contiguous plots along the transect(s) (Figure 1b, c). Transects will contain at a minimum 4 plots and can be expanded as necessary, depending on population density, up to 10 m in length. The position of each plot should be permanently marked at the bottom left and upper right corners with a tent peg or similar. Start labeling the plots on each transect from left to right facing up-hill.

Refer to the plots like this:

T1 P1...T1 P20 (Transect 1, P1 up to maximum P20 plots, depending on *P. lanceolata* density)

T2 P1...T2 P20 (Transect 2, if used)

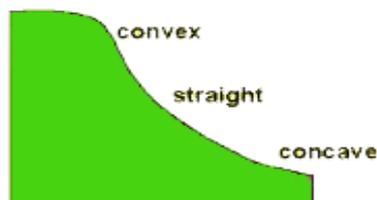
T3 P1...T3 P20 (Transect 3, if used, etc. up to a maximum of 5 transects) (see Figure 1)

Record the name of the site coordinator, site name and site code, country, continent, date of census, site latitude, site longitude, site elevation (meters), number of transects and plots, current management regime, detailed description of management history, co-authors and whom acknowledgment should go in the following columns of the 'Site description.xls' datasheet: **site_coordinator, site_name, site_code, country, continent, date, latitude, longitude, elevation, number_of_transects, number_of_plots, current_management, management_history, co_authors, acknowledgments.**

Site-level environmental covariates

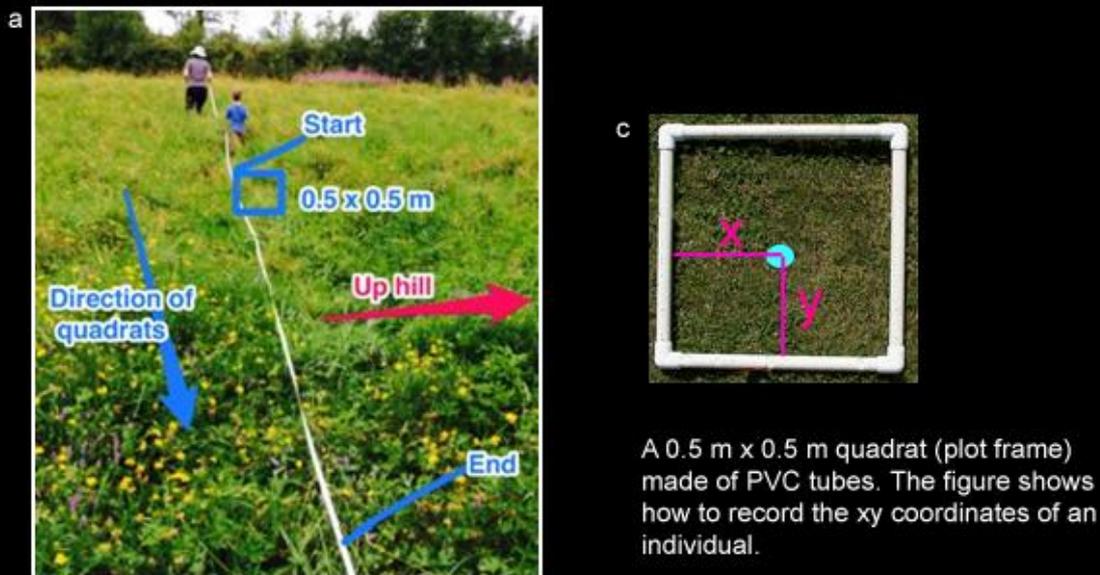
(Fill out the 'Site description.xls' datasheet.)

- **aspect:** the compass direction a slope faces, using either NA if perfectly flat or one of the 8 cardinal compass points, N, NE, E, SE, S, SW, W, NW
- **slope_angle:** ranges between 0-90°, **slope_position** (transect position on slope (top/middle/bottom), **slope_shape** (whether the slope has concave/convex/straight shape). See figure below for slope shape. To determine slope angle, use a clinometer (<http://en.wikipedia.org/wiki/Inclinometer>) or if using a smartphone, clinometer apps are also available.

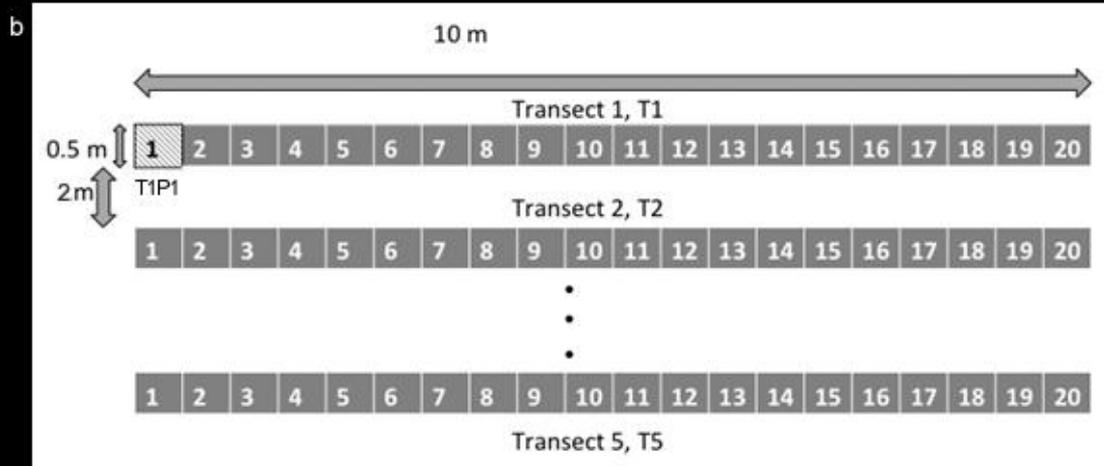


- **standing_water:** whether the site has standing water at any time during the year (yes/no)
- **habitat_type:** concise habitat name; In the European Union, habitat descriptions are available in the Habitat Directive, available here: (http://ec.europa.eu/environment/nature/legislation/habitatsdirective/docs/Int_Manual_EU28.pdf)
- **weather_station:** complete name of the closest weather station and link to URL if available
- **distance_to_weather_station:** distance to the closest weather station (km)

Figure 1. Transect and plot layout



Layout of transects within a site in Coolclogh, Cork, Ireland. Wet hay meadow, semi-natural grassland, mown once per year in August.



Map of transects and plots. The minimum transect size is four 0.5 m x 0.5 m plot containing at least 100 plants. The first plot is shown using the light gray shading on Transect 1 (T1 P1). The maximum number of transects is five, each one containing 20 contiguous 0.5 x 0.5 m plots to contain at least 100 plants in total in all transects (density of 1 plant per plot). At least 2 meters should be left between transects to minimize effects of trampling on plots.

Part 2. Annual survey of permanent demography plots

This section of the protocol outlines how to mark plants, deal with seedlings and clones, how to measure plants (size, fecundity) and collect community structure data. Data from the community plots will be used as biotic covariates in demographic analyses. Fill out the corresponding columns in the 'Individual plant census.xls' and 'Community plot.xls' datasheets. Column names are marked in bold throughout the text.

Equipment needed (see also Figure 2):

- GPS for checking/finding transect corner positions
 - Differential GPS for recording individual plant positions (optional)
- Bright colored tape to temporarily mark transect positions (2-10 m, depending on transect length)
- Meter ruler and 30 cm transparent ruler for measuring length & width of leaves and inflorescences, resolution to 1 mm
- 1 meter or 2 meters pole painted alternately black and white in 10 cm stripes for vegetation structure measurements (length depends on vegetation height)
- 0.5 m x 0.5 m quadrat (plot frame); The plot frame can be made sticking 4 PVC tubes together with corner tubes, making sure there is exactly 0.5 m x 0.5 m space inside the plot.
- 100-200 plant tags with plastic coated wire or cable ties/zip ties for securing to plants, hair-pins/staples to anchor tag & wire to ground may also be necessary; Numbered tags can be bought e.g. from forestry suppliers or can be made manually (e.g. small linoleum squares numbered with a permanent marker). The tags should not attract attention from humans or other potentially destructive animals (shiny tags are not recommended as they attract birds and rats).
- Datasheets on computer/tablet or printed on (waterproof) paper provided on PlantPopNet website www.plantpopnet.com/protocol
- Pencil/Pen for recording data
- Digital camera/phone for taking plot photos
- A few small paper envelopes for sampling infected individuals
 - Starting the second year, bubble plot of the position/size of marked individuals in every plot (an R code that produces these maps is available on the PlantPopNet website www.plantpopnet.com/protocol, alternatively ask for the maps at core@plantpopnet.com). Optional, but strongly recommended. It helps retrieving plants next year, double checking plant tags and locating plants with missing tags. Print the map out and bring to the field for verification.
 - PAR ceptometer (Photosynthetically Active Radiation) to record light penetrance in the plant community (optional)
 - 1 iButton temperature and humidity data logger for each site (optional; ask the coordinator for supplies)

Marking plants in year 1

(Fill out the '*Individual plant census.xls*' datasheet.)

Permanently mark each plant in every plot until you have at least 100 plants. Complete each plot that you start so that you may end up with >100 individuals (i.e. do not half-survey a plot). You will follow the demographic fates (survival, change in size, clonal propagation and sexual reproduction) of all marked individuals and any new individuals (recruitment) each year. Resurveying the same plots annually will incur losing plants as they die and including new recruits, so subsequent population sizes may be higher or lower than the initial population size.

Tag each individual plant with a unique number. (For subsequent censuses continue to assign unique plant identifiers to new recruits, the sequence of numbers is not important as long as each plant at the site has a unique id). The principle of tagging is that the tagging method is not destructive and it will be persistent i.e., it makes possible retrieving the same plant year after year. Tags should be inert i.e. they won't leach nutrients, toxins or metal oxides into the soil. Follow the tagging method described here as closely as possible:

- Tie a plastic coated wire around the base of the plant so that it forms a loose ring, fixing it to the ground with one or two pins/staples (e.g. hair pin or bobby pin) (Figure 2b-d). Attach a numbered tag to the wire tie, such that each plant gets a different number (**plant_id** column). For mown/grazed sites the anchoring needs to be strong enough to keep the tag on the ground around the base of the plant. If a differential GPS is available, use it to record individual plants (accurate to within 0.2cm!). This is an additional (recommended but optional) measure to help retrieving plants. Mark individuals that have at least 2 leaves.
- Record the x and y co-ordinates of each individual in the plot where the bottom left hand corner of the plot is (0,0) (**x_coord**, **y_coord** columns). Use the meter ruler to measure the distance (in centimeters) from the marked individual to the left-hand- and bottom side of the plot frame (Figure 2c). These positions will be used in models of density dependence and to locate individuals with missing tags.
- Dealing with seedlings. Do not tag the fragile seedlings, as this can be destructive. Seedlings start with cotyledons (first 6 weeks) and have 2-3 leaves after approximately 1 year (Figure 2a). Survey carefully each 0.5 m x 0.5 plot for seedlings (individuals with 1 leaf and individuals in cotyledons stage) and count them (**number_seedlings** column). No marking or measurement is required.
- Dealing with clones. Identifying clones can be challenging. Clones can look very similar to 1-year-old plants and are generally in very close proximity to parent plants (Figure 2b). Treat parent and suspected clone as the same individual (expand one tag to include all clustered, assumed clones – Figure 2e). You may try probing for a connection between the rosettes, to make sure it's a clone and not a new recruit from seed. Record if the individual is suspected to be clonal in the **suspected_clone** column (yes/no).

Individual plant measurements at annual census

(Fill out the '*Individual plant census.xls*' datasheet. Record all measures of plant size in millimeters, to the nearest millimeter.)

- **survival**: whether a previously marked plant is present (survived) or not (died)
- **no_rosettes**: Clones which are clustered together into an "individual" will be treated as one individual but count the number of rosettes. In some habitats, individuals commonly produce side rosettes.
- **rosette_number**: identification number of each rosette in a clone; Record leaf number and size separately for each rosette. The order in which rosettes are measured doesn't matter.
- **no_leaves**: total number leaves in the rosette; only count leaves >5mm long;
- **leaf_length**; **leaf_width**: the length and widest width of the longest leaf (mm) (the transparent ruler is more useful than the meter ruler here)
- **no_fl_stems**: number of flowering stems; *P. lanceolata* produces generally one inflorescence on a long stem per leaf axil (Figure 2a), but the first 5-6 leaf axils may not form inflorescences.
- **fl_stem_height**: height of the flowering stem (mm) (to top of inflorescence i.e., including inflorescence length)
- **inflor_length**: length of the mature inflorescence (mm)
- **inflor_phenology**: inflor_phenology: use one of these names describing the phenology of the inflorescence: "bud" (prior to fully developed flowers), "flowering", "seeds"

developing”, “mature seeds”, “seeds dispersed”, “no seeds” (no signs of seed production in mature inflorescences).

- **Disease:** For each individual note signs of disease (yes / no).
- **Disease comments:** Optional additional details on the infection (see detailed examples of infections on the PlantPopNet website: www.plantpopnet.com/resources). A frequent infection is by powdery mildew fungus, which is visible on host leaves as white-greyish growth (Figure 3). You may also find chlorotic lesions. When an infection is detected, try finding another infected individual outside the plot and place one leaf displaying symptoms into a paper envelope. Sample only one infected individual/site and label the paper envelope with site code and suspected disease name (e.g., CH_powdery_mildew).
- **Herbivory:** For each individual note signs of herbivory (yes / no).
- **Herbivory comments:** Optional additional details on the herbivory (e.g. leaf mine, round feeding hole; see detailed examples on the PlantPopNet website: <http://plantago.plantpopnet.com/resources>), or the percentage of leaf area affected. Take quality pictures of most common symptoms. Label the pictures to include site code (e.g., CH_leaf mine).

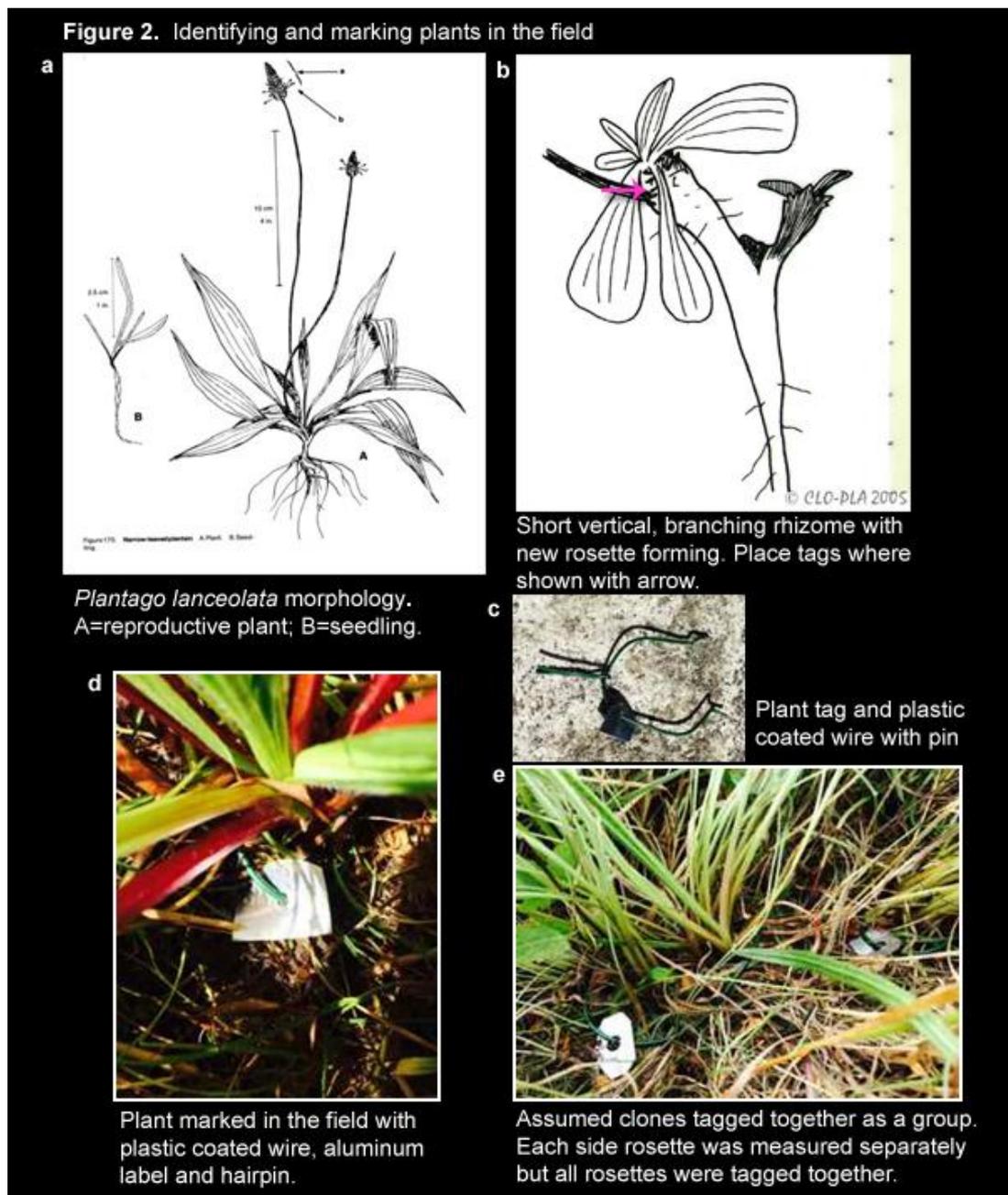


Figure 3. Symptoms of Powdery mildew infection on *P. lanceolata*



Plot-level community and environmental covariates

(Fill out the 'Community plot.xls' datasheet.)

Choose randomly four 0.5 m × 0.5 m plots within each demographic transect/s (if there is only four plots, sample them all) and record the following covariates:

- **plot_photo:** Take a downwards facing photo of the plant community plot from above the herbaceous canopy, at a 1.5 m vertical distance. Make sure the boundaries of the 0.5 m × 0.5 m plot are clearly shown and take the photo looking down on the canopy. Label the plot photos to include site, plot, year (e.g. CH_T1P1_2015). This photo will be used to document vegetation cover and revisit community plots for details.
- **camera_type:** Record the make & model of the phone/camera.
- **light_penetrance:** If available, use a PAR ceptometer (Photosynthetically Active Radiation) to record light penetrance at ground level. Otherwise mark 'NA' in the cell.
- **veg_cov; plantago_cov; grass_sedge_cov; forb_cov; legume_cov; moss_lichen_cov; native_vascular_cov; alien_vascular_cov:** percent cover of total vegetation, *P. lanceolata*, grasses and sedges, forbs (Dicotyledonates, including legumes), legumes, mosses and lichens, native vascular plants and non-native vascular plants; If identity of all non-native plants is not known, record the cover of the most conspicuous/known species only or just leave 'NA'.
- **bare_ground_and_stones; litter:** percent cover of bare ground + animal digging + stones and litter respectively; These are indicators of disturbance/lack of disturbance.
- **woody_overstory:** percent cover estimated for woody overstory shading the plot
- **vertical_1; vertical_2:** Measures of vegetation vertical structure. Use the 1 (or 2) m Robel pole painted alternately black and white in 10 cm stripes to record the vegetation vertical structure of the vegetation in each plot at two corners of the community cover plots (2 points per 0.5 m × 0.5 m community plot). Use the meter rule to observe the veg pole at the appropriate height from a distance of 4m from the veg pole. Record the highest band on the pole that is completely obscured by vegetation (e.g. if the pole is fully obscured up to 20cm and only partially obscured thereafter then record "20").
- **iButton:** Record if temperature/humidity data logger is available (yes/no). If not sure how to deploy the iButtons in the field, contact the coordinator.

Part 3. Destructive plant trait samples

(Fill out the '*Destructive samples.xls*' datasheet.)

This part constitutes an “Add-on” project and is optional. However, it is appended to the Core protocol being highly recommended to all participants. Highly committed participants will be sampling during several consecutive years, starting the first year. Sampling the first year is preferred, but if a participant missed the first year, data from subsequent year(s) can be useful. Please contact the coordinator if you missed the first year but still keen on participating.

Equipment needed:

- Plant press with cardboard sheets and porous newspaper or blotter paper for collecting herbarium specimen (optional)
- 15 paper coin envelopes/site for individual seedpods and seed storage
- 1 airtight container (plastic box or large zip-lock bag) to fit the 10 paper coin envelopes with seeds
- 10 large plastic zip-lock bags to temporarily keep moist leaf samples for Specific Leaf Area (SLA) measurement (if not done in the field)
- Cooler/ice-chest/esky etc. for keeping zip-lock bags with fresh plant tissue cool during transport to lab for SLA measurement (if not done in field)
- Portable Leaf Area Meter (if you have this, cooler/ice-chest/esky and 10 large plastic zip-lock bags for leaf samples are not necessary)
- 10 medium size paper bags/site for leaf samples to be sent to coordinator for analysis
- Silica and barcode kit (sent by the coordinator) for leaf sample preservation (10 zip-lock bags half-filled with silica, each will contain one of the medium size paper bags with a leaf sample)
- Datasheet on computer/tablet or printed on (waterproof) paper provided on PlantPopNet website www.plantpopnet.com/resources
- permanent marker for marking zip-lock and paper bags
- pencil/pen for recording data

Many of the subsequent samples will be destructive so it's important not to take these plant samples within the permanent demography plots. Locate 11 plants outside but close to the permanent demography plots in similar vegetation structures, light environments and plant communities. Ensure there is at least 5 meters between each collected plant.

- **Herbarium_and_accession_number:** Collect one whole reproductive plant (including rhizome and roots) as a voucher specimen and deposit with a local or regional Herbarium. Provide the Herbarium name and link to URL and accession number (unique identification number of the herbarium sheet deposited) (optional)
- Each of the other **10 plants** sampled will have a unique number, but are not permanently marked. While the plants are still in the ground measure the same **size and reproduction parameters** as for the plants in the permanent demography plots (for details see "Individual plant measurements at annual census" part of this protocol) and record in the datasheet.
- Collect an **additional 5 seedpods**. (15 seed pods in total; please ensure they are in the “mature seeds” development stage.)
- **Seeds:** For each of the 10 plants (+ 5 extra seedpods), individually bag one mature seedpod per plant (Figure 4a). Label the envelope with site_code and plant_id (e.g. CH_1d), bring to the lab and record the following:

- Remove the seeds from the seedpod and take a digital image of the seed sample with a ruler in the image for scale (Figure 4b). Label the picture with site_code and plant_id (e.g. CH_1d).
- **seed_number:** Number of all seeds per seedpod. Count the number of filled, oval, brown seeds, not the flat black seeds.
- **seed_mass:** Air dry the seeds in the lab for at least 24 hours, weigh all seeds counted and record the seed mass (mg if possible).
- Store the air dried seed samples for each individual plant in the labeled paper envelopes, in an airtight container (e.g. plastic box or ziploc bag) at room temperature. Keep seeds for subsequent analysis, permits will be needed to post these to the coordinator at a later date. The coordinator will contact you with respect to permitting issues.

Leaf tissue (samples for genetics, leaf Nitrogen & Phosphorus, Specific Leaf Area, leaf hairiness)

- Sample **2 leaves** (most recent, fully expanded leaves without significant herbivore damage or infection) from each of the 10 plants. Include the petiole with the leaf, removing the leaf from the plant at the base of the rosette where the leaf emerges.
- **specific_leaf_area:** If a portable *Leaf Area Meter* is available, use this in the field to measure leaf area, including the petiole, and then put the leaves into separate labeled paper bags and then in the labeled zip-lock bags half-filled with silica. Label the bags with the site and plant_id (e.g. CH_1d). **If a portable leaf area meter is not available**, just mark "scan" in the spreadsheet column. Take the leaves back to the lab for scanning. To do this, keep each individual leaf separately in a plastic bag labeled with the site and plant_id (e.g. CH_1d) using a permanent marker and place them in an insulated cooler/eskie/chilly bin (depending on what part of the world you are in!). Refrigerate the leaves on return back to the lab until they can be scanned to measure leaf area. Make sure to scan the leaves as soon as possible after returning from the field. Place all 10 leaves on a white background with a ruler for scale and label each leaf visible with plant_id. Scan the leaves using a regular scanner with at least 200 dpi and save the document with site name and .jpeg extension (e.g., CH_SpecificLeafArea.jpg). Record the dry mass of the leaves and calculate SLA as leaf area/dry mass ($\text{mm}^2 \text{mg}^{-1}$). Otherwise, send the dried leaves (bagged with silica) to the coordinator along with the samples for genetic/chemical analysis.
- **genetics_ & leaf_chemistry:** Store each leaf in a paper bag labeled with plant_id (try keeping the leaves entire but folding in two or three is allowed). Put the paper bag in a plastic zip-lock bag half-full with silica and post these to coordinator. The leaves will be used for analyses of population genetics, leaf chemistry and additional traits such as hairiness.



Checklist

By the end of the census, check if the following **photos**, **maps** and **samples** are in place in addition to the filled out **spreadsheets**. Double check if spreadsheets were filled out correctly and completely. Send the documents to the coordinator in one batch as soon as everything is together (use both addresses: core@plantpopnet.com, buckleyy@tcd.ie).

- site map of transects and plots
- site and landscape pictures
- powdery mildew samples
- pictures of common disease and herbivory
- community plot photo
- picture of seeds
- 15 paper envelopes in airtight container with seeds
- 10 plastic zip lock bags with silica and leaf samples in paper bags
- 10 plastic zip lock bags (kept chilled) with leaf samples for SLA (if you don't have a Portable Leaf Area Meter)
- picture of scanned leaves (if you don't have a Portable Leaf Area Meter)