# 5. SEED TRAITS

The seed traits included in the LEDA Traitbase are seed number per shoot (including seed crop frequency and shedding period), seed weight, seed shape, and seed longevity.

## 5.1. SEED NUMBER PER RAMET

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**Introduction**

Seed number is an important functional trait in understanding regeneration strategies, abundance and dynamics of plant species after for instance disturbance and is often correlated with other traits such as seed size and seedling size (Shipley & Dion 1992). The existence of trade-offs between seed number and seed size for a given reproduction allocation was examined by Leishman (2001). Seed production is a highly variable trait and is very sensitive to, for instance, site conditions, climatic conditions, and predation (Aksoy *et al*. 1998, Salisbury 1942, Harper 1977, Weiner 1988, Lovett Doust & Lovett Doust 1988, Kelly & Sork 2002). The seed production of a species also varies with the different life stages (Harper 1977, Begon 1993). The mean seed number per ramet increases with the ramet biomass (Escarre & Thompson 1991) and also increases within the same species with the plant size (Niklas 1994). Additionally, seed production may exhibit periodic cycles, for example the mast years of some tree species, which may be an endogenous fixed phenomenon (Silvertown & Lovett Doust 1993; see also Seed crop frequency, Chapter 5.1.1).

Seed densities (and as far as known spore densities) are highest in frequently disturbed habitats such as arable fields, and lowest in primary forest (Silvertown & Lovett Doust 1993). In the case of aggressive invaders after disturbance the relationship between seed production and vegetative reproduction depends on the stage of succession (i.e. *Pteridium aquilinum*; Korpelainen 1995). To estimate potential seed production you need the optimal species-specific conditions in the field.

*Trait definition*

Seed number: Is defined as the total seed (or spore) production (filled and unfilled seeds) per ramet/shoot of a species.

In the LEDA Traitbase the seed (or spore) number is measured per shoot, per single stem or per ramet. Note that in the case of clonal plant species the shoot is defined as the ramet (after Kleyer 1995). Weiher *et al*. (1999) defined a ramet as an iteration of the basic form of a plant with obvious connections to other ramets that would clearly unify the parts into one iteration. With this definition we should be able to identify the following examples as individuals:

1. Multiple stemmed shrubs and trees (e.g. *Vaccinium corymbosum, Erica tetralix*) (Fig. 3.15).
2. Ramets of clonal species with stolons (e.g. *Agrostis stolonifera, Carex arenaria, Glechoma hederacea*) or rhizomes (e.g. *Typha latifolia*) (Fig. 3.15).

3. Ramets of clonal species with root sprouting ability (e.g. *Robinia pseudocacia, Prunus spinosa*).
4. Ramets of tussock-forming graminoids (e.g. *Dactylis glomerata*, but also perennial *Juncus* spec. with pectinate-forming tussocks) pose somewhat of a problem in that the tussock acts as a functional unit in terms of holding space (see Eriksson & Jakobsson 1998).

**What and how to collect**

In general five levels (five categories) of seed (spore) reproduction units can be sampled:

1. Per single flower inflorescence (e.g. *Tulipa* with one capsule, but also a single flower of *Clematis*). In some cases for e.g. *Tulipa* the seed number of a single flower is the number of the total plant (species with a single shoot).
2. Per multiple flower inflorescence, per single shoot (e.g. *Viccia cracca*, the umbel of *Daucus carota*, the panicle of a grass species or of the shrub *Sambucus racemosa*).
3. Per multiple flower-stem or per fertile frond (in the case of horsetails per fertile shoot/stem). Species from disturbed habitats have very often multiple-flower stems, for e.g. Compositae, Chenopodiaceae, Umbelliferae or Cruciferae. This category can be also interesting to multiple stem bushes as Ericaceae. This category is defined as the seed number per single stem (branched or unbranched) above ground. A single stem with a root point is defined as one ramet.
4. Per ramet or total individual plant (e.g. a tree or an annual herb).
5. Per square metre. The reproductive capacity of one species per $m^2$ can be calculated as the potential seed number per species to 100 % cover in the vegetation unit. The reproductive capacity of a population is characterized as the number of seeds produced by one species per 1 $m^2$ at its one hundred-percent cover per one season (see Šerá & Šerý 2004).

For the LEDA Traitbase the seed number per ramet or individual (Category 4) was measured and is as such preferred. However, but seed number measurements from published sources obtained per (single/multiple) inflorescence, multiple stems or frond and per square metre obtained will be accepted as optional measurements.

In agreement with the above definitions of 'ramet' and 'individual' different levels of sampling are possible: For non-clonal plants the individual (= the genet) is appropriate; for clonal plants, ramets are probably most appropriate; and in the case of tussock plants, the whole tussock may be most appropriate to be sampled (see Fig. 3.16).

In total a minimum of 10 inflorescences per species should be collected at a sample site, with as a collecting rule, one inflorescence per shoot from a representative randomly selected healthy individual. When multiple inflorescences are present on the sampled shoot, the total number of inflorescences of that shoot should be counted to be able to determine (or estimate) the total seed number per shoot. Note that measurements of seed production should take place under optimal species-specific habitat conditions, for instance, shade tolerant species should not be collected from sunny places.

In case of rare species in Northwest Europe a minimum of 3 inflorescences should be selected per defined search area. Note that for orchids only published data sets will be used in the LEDA Traitbase.

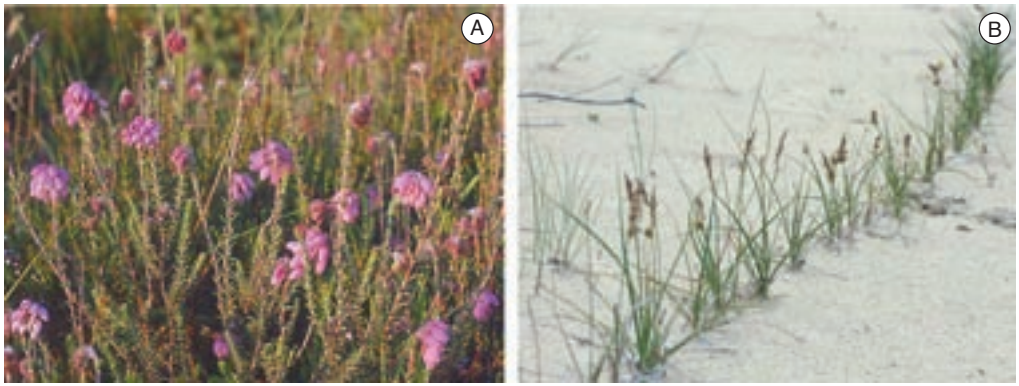


***Figure 3.15.*** *A few examples of seed number one for multiple shoots (Erica tetralix; A), and one for clonal species (Carex arenaria; B).*

*Helper-traits*
For this trait we have to check two obligate helper-traits (Section 3, Chapter 5.1.1):
Seed crop frequency: The frequency of seed production over time.
Seed shedding: The time and duration of seed releasing in the year.
Additional information on the condition of the measured seeds for seed number per ramet can be entered optionally into LEDA in the comment field. For instance information about the viability of the seeds measured, i.e. only viable seeds were measured as the total seed production.

**Storing and processing**
Each collected inflorescence should be put into a separate dry paper bag and stored dry at room temperature. When using a 'seed counting machine' the seeds should be cleaned, e.g. by using a seed-cleaning machine to separate the filled seeds from empty seeds, awns, pappus or adnate structures.

**How to measure**
In general there are several methods used to determine the seed number:

1. Counting method: In this method the whole seed production of the ramet (category 4) or the other sampling units (category 1-3) are counted (most exact method, but time consuming).
2. Counting and extrapolation method: This method is used when only a unit (e.g. one single inflorescence, one multiple inflorescence, a single stem) is collected and counted per ramet. Only the seed number of the collected units is counted (n=5) and the seed production per ramet is subsequently obtained by multiplying the seed number per unit with the total number of units counted per ramet. The five individual replicates could also be used to compare between tussocks. For special clonal species that often produce monostands (i.e. *Agrostis stolonifera*),

all inflorescences in 1 square metre should be collected with a minimum of 3 replicates (see also Shipley & Dion 1992, Šerá & Šerý 2004). For many species it will be easier to count a smaller sampling unit' such as a capsule, panicle or umbel, and to multiply this seed number with the total number of sampling units.

3. Estimation method: This method is used when it is not possible to obtain a 'counted' seed number for the whole plant, which is often the case for e.g. trees, shrubs and liana. For instance in the case of *Clematis vitalba* it is easy to count the seed number per single flower or infructescence, but it is very laborious to count the seed number per ramet. In this case the number of single infructescences is estimated and multiplied with the seed number per single flower to give an estimation of seed production of the whole plant. Note, that this method has the lowest data quality of all mentioned methods.
4. Counting and weighing method: This method is often used by forestry ecologists, agricultural scientists and plant population biologists. This method uses the value of the weight of a counted number of seeds (e.g. 50, 100 seeds per batch, N=5).. By dividing the total seed weight of an individual plant by the weight of a set number of seeds (i.e. 100 seeds), the seed number can be estimated. This method can also be used to calculate seed number of $1 m^2$, but only when the species grows in monoculture (i.e. 100% cover; Šerá & Šerý 2004).
5. Total weight of seed production: To estimate the seed production of trees, forestry scientists often use this method. The seed weight of the total production is used to get an estimation of the total seed number, but without the weighting of a set number of seeds. This method (weighing and estimation) is a low quality modification of method 4 (counting and weighing) because only the total seed weight is known and the seed weight of a set number of seeds is obtained from literature.

To save time it is helpful to use a seed counting machine, note, however, that the seeds have to be cleaned thoroughly before the machine is able to count the seeds. For very small seeds a stereoscope with counting grid ocular should be used. In the case of number of spores LEDA will use only published data (see also Special cases).

*Special cases and sampling methodology*
In several cases extrapolated data are necessary, because the measurement of seed/spore production is more complicated, often with more time exposure:

- Tussock grasses: These produce a lot of tillers with inflorescences - use method 2.
- Mature trees and shrubs ≥ 4 m: For tall trees or shrubs with bigger diaspores another method is used. The seed production is calculated per seed or fruit number lying upon the soil surface under the tree crown. Finely woven gauze is put underneath the tree as a 'seed trap' and all diaspores that fall down are caught in the seed collecting net (e.g. *Fraxinus excelsior*; Gardner 1977). The cover from a crown of the tree is calculated in $m^2$ and the sum of seed production per shoot is extrapolated. Note that some tree species (e.g. *Quercus*) have periodic mast years with varied periodicity. Old individuals of *Quercus robur* produced up to 90,000 acorns per shoot (50,000 acorns in average) in mast years (Jones 1959, Crawley & Long 1995). The estimation of seed production of taller trees (i.e.

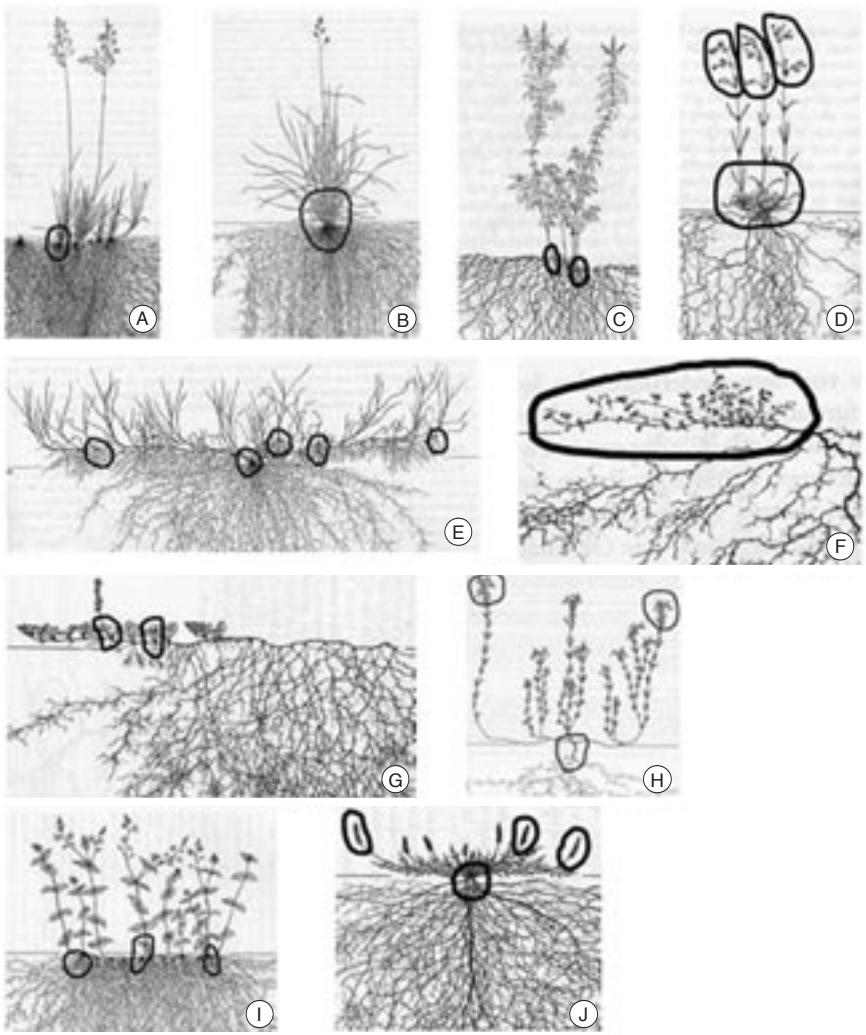


***Figure 3.16.*** *What to collect for seed number per shoot or ramet. Black circles in drawings of different species mark the unit (i.e. individual) to be collected for seed number (the inflorescences marked are to distinguish between seed number per inflorescence and seed number per ramet/shoot). Examples of different shoots are: (a) Agrostis capillaris with loosely short below-ground stem, (b) Dactylis glomerata is very compact ramet group (often with many inflorescences) that is defined as one unit, (c) Mentha longifolia a clonal species with clearly defined shoots, (d) Silene nutans a semi-rosette plant with a branched shoot and one main root, which is defined as one unit with several inflorescences (e) Agrostis stolonifera with large expanded lateral above-ground stems (5 ramets marked) and different root points. (f)Thymus pulegioides a prostrate branched dwarf shrub with one central root, (g) Veronica officinalis a perennial herb with large expanded lateral above-ground stems and different root points, (h) Euphrasia nemorosa a branched annual hemi-parasite with many inflorescence stems and one central root (seasonal ecotypes are typical for this genus), (i) Veronica chamaedrys a perennial herb with a below-ground stem and clearly defined shoots (j) Plantago coronopus a rosette plant with many inflorescences and one central root. (Kutschera & Lichtenegger 1982, 1992, modified by Kunzmann).*

*Populus, Salix*) with wind-dispersed many-seeded capsules is much more difficult. In this case, collect three twigs/branches and count the seeds of ten catkins from each twig, as well as all the catkins of each twig, from which the seed number is obtained by multiplying the two (see Method 2). After that multiply the seed number with the total number of twigs on the tree to get an estimate of the seed production per tree. For each species 3-5 random selected mature trees per defined search area should be counted.

- Mature trees and shrubs < 4 m: The same method as used for the taller trees can be used. There are more effective methods to count trees or shrubs with fruits (e.g. *Sambucus nigra*, Atkinson & Atkinson 2002). For instance by calculating the weight of 10 diaspores of 5-10 different individuals (=bush/tree) per species. For example *Sambucus nigra* will have one seed per fruit, but in the case of *Vaccinium myrtillus* on average 25 seeds per berry can be found (Eriksson & Fröborg 1996). The total harvested fruit crop per bush or tree will be weighed and the seed number estimated per bush or tree by calculating the mean weight of fruits. For each species estimate 5-10 randomly selected mature shrubs or trees per defined search area.
- Spore number of ferns, clubmosses or horsetails: In the case of Pteridophyta (ferns, clubmosses or horsetails) only published data sets will be used. When raw data of spore numbers would be available, the following standardised approach should be followed: Collect 3-5 fertile fronds with spores or other spore-bearing structures, per shoot per defined search area. It is helpful to put the frond with spores on a bigger dry filter paper for some hours. In this time, the frond has died out and the spores are dispersed on paper. To count the spore production of ferns a microscope is needed due to spore size (≤ 30 $\mu$m), and the counting is more difficult as many are produced (e.g. *Pteridium aquilinum* - 300.000.000 spores/single frond; Cody & Crompton 1975). When counting use filter paper with a grid and count the number per $cm^2$ (5 replicates) and than estimate the production for the whole frond. In most cases Pteridophytes have single shoots/stems/ fronds and often form extensive rhizomes or grow as a rosette hemicryptophyte with several leaves/fronds and a short rhizome. The spore number of horsetails (*Equisetum*) is counted by spore number per shoot (sporophyte). In this case it is the same as spore number by inflorescence, because there is only one cone per sterile stem. Normally, horsetails have extensive rhizomes with many shoots. In the same way ferns are estimated (e.g. *Pteridium aquilinum, Polypodium, Cystopteris*) because the fertile frond grows as a single shoot from the larger rhizome. Count the spore number of heterosporous quillwort (genus *Isoëtes*), an underwater rosette hemicryptophyte, as spore number per single stem or shoot. Many ferns are rosette hemicryptophytes with several (sterile and/or fertile) fronds per shoot (short rhizome) (i.e. *Dryopteris, Polystichum, Matteuccia*). So you can distinguish between counting the spore number per fertile frond and the spore number per single stem or shoot.

It should be noted that the methods of seed number counting/estimation mentioned in this section are a choice of species-specific examples. As seed size, seed morphology, and dis-

persal mechanism between plant species are highly various it is essential to accept other species-specific methodologies to estimate the seed number in the most optimal way.

**Minimal requirements**

Seed number per ramet is obtained through measurement and/or estimation and therefore data sets from literature or other sources can not be accepted when the number of replicates and the standard deviation or standard error are not present.

If the methods used in published data are not clear, the seed production of a species can only be assigned to a minimum/maximum range or as a field observation (see General standards). Any data entry of a single observation without information of the habitat counts as mean value. Seed number is a measured (or estimated) trait with many species-specific options for collecting, processing and measuring. Measurements (unpublished data) need to follow the standardised protocol with all described options and other species-specific methods, with mean or the median with the standard deviation or with standard error as an end result. In the case of published and unpublished data LEDA accepts values with unknown number of replication as a single observation, entered as a mean. The lack of information on any of the obligate points mentioned above will result in rejection of the data.

**Data structure**

To collect: 1 inflorescence or flowering unit of 10 different individuals = 10 inflorescences in total per species (per sample site)

Obligate:

- Type of variable: numerical
- Number of individuals per sample (n): 10
- Number of replicates (N): 1
- Unit: seed number/shoot or ramet
- Values: N, minimum, maximum, median, mean, standard deviation, standard error
- Trait specific counting method:
    1. Counting
    2. Counting and extrapolation
    3. Estimation
    4. Counting and weighing
    5. Total weight of seed production
    6. Unknown
- Reproduction unit measured:
    1. Per single flower inflorescence
    2. Per multiple flower inflorescence
    3. Per multiple flower stem
    4. Per shoot or ramet
    5. Per $m^2$
    6. Unknown

- Counted subunit per reproduction unit:
  1. Single inflorescence
  2. Multiple flower inflorescence
  3. Multiple flower stem or single stem
  4. Unknown
- Counted per subunit: number
- Validity range: 0-5.000.000.000

Optional:
- o Seed viability: in %
- o Comment field: Any information of importance to the trait

### 5.1.1. SEED CROP FREQUENCY AND SEED SHEDDING

Additional traits to seed number per ramet

**Introduction**

For the trait seed production two obligate additional features or helper-traits are useful, namely seed crop frequency and seed shedding.

*Trait definition*

Seed crop frequency: Describes the frequency of generative reproduction cycles over time, in other words how often species produce seeds in a certain time period (Silvertown & Lovett Doust 1993).

Seed shedding: Is the time and duration of seed releasing. Note that the duration of seed shedding per individual is in most cases much shorter than duration of seed shedding per population (e.g. *Scilla bifolia* - Kunzmann 1993; *Salix* spec. - Karrenberg *et al*. 2002).

SEED CROP FREQUENCY

The seed crop frequency is of importance for the time in which genets are replaced within a population. The frequency of (annual or interannual) seed production of a species also determines the dispersal in space and time (e.g. to refill the seed bank). Combined with seed number, seed crop frequency could be a weighed measure for annual seed production of a species in a sample area.

The seed crop frequency is one expression of the ratio of time and biomass allocated to growth versus reproduction (Harper 1977). Perennial species allocate biomass in their vegetative growth (e.g. big rosettes, long central roots) often for several years, and die after the only once reproduction at the end of their life time (e.g. *Agave*; Harper 1977).

A special case of interannual seed crop variation is the mast fruiting of perennial plants, especially known from trees and some shrubs. Masting is defined as (synchronous) intermittent seed production of large crops by a population of plants (Koenig & Knops 2000). Herrera *et al*. (1998) criticised the term masting or fruiting mast, because it appeared to be too difficult to classify species as either masting or non-masting species, as well as to define mast years and non-mast years among a masting species in an objective way. However, studies of many long time datasets of trees and shrubs has shown that wind-pollinated and predator-dispersed species have a higher variation of annual seed produc-

tion than biotically pollinated and frugivore-dispersed species (Herrera *et al*. 1998, Kelly & Sork 2002). Kelly & Sork (2002) understand masting as an adaptive reproductive trait overlaid on the direct influence of weather, i.e. trees in cold climatic conditions produce high level of seed crops only at intervals of several years, separated by years with no seed of low levels of seed crops.

Another phenomenon is the within-year seed crop frequency of annuals and perennials. Populations of *Poa annua*, a (pseudo)annual grass, produce multiple variable seed crops within different stages of their life-cycle and also multiple plant generations within a year (Begon *et al*. 1997). Also ramets of the perennial herb *Rumex acetosella* produce flowers and seeds several times within a year (Escarre & Thompson 1991).
Seed crop frequency can be described at two levels, per individual or per population, sometimes with different results for the same species (see Table 3.3). An individual of a biennial species for example reproduces only once in its two-year life-cycle. But an established population of the same biennial species produce seeds yearly, on account of their mixed life stages. As a rule, to understand the reproductive capacity of a species it is more important to focus on the individual level. But to estimate the total seed production of a species per sample area in a year or to understand the (synchronous) mast years of trees or monocarpic perennials, investigations on population level are necessary.

***Table 3.3.*** *Aggregated categories to describe the seed crop frequency per species on the individual or ramet level and population level.*

| Seed crop frequency* | Per individual/ramet | Per population |
|---|---|---|
| 1. More than once year | perennial herbs, some annuals | annuals, perennials |
| 2. Once a year (annually) | annuals, perennials | annuals, biennials, (monocarpic) perennials |
| 3. Once in 2 years (incl. mast fruiting) | biennials, some perennials (trees) | biennials, pluriennials, some perennials (trees) |
| 4. Once in > 2 years (incl. mast fruiting) | monocarpic perennials, some perennials (trees, shrubs, some geophytes) | pluriennials, some perennials (trees, shrubs, some geophytes) |

* Note that the categories including mast fruiting do not distinguish between different levels of masting.

*Categories*
Categories adopted by LEDA are:

Seed crop frequency:
1. More than once year
2. Once a year (annually)
3. Once in 2 years (incl. mast fruiting)
4. Once in > 2 years (incl. mast fruiting)
5. Not applicable
6. Unknown

Mast fruiting years (only for perennial species)
1. Masting
2. Non masting
3. Not applicable (i.e. for annuals)
4. Unknown

**What to collect**

Data on seed crop frequency will be mainly obtained from literature and will be recorded as numbers of frequency within and between annual years. The 'Biological Flora of the British Isles' describes for many plant species, how often seed is set over time. The LEDA Traitbase provides information about the phenomenon 'masting' in two columns. In the seed crop frequency-column information is provided on seed crop frequency within year and between years. The mast years-column gives information about the categorisation from publications; i.e. if masting occurs within a species or not. Note that in the column 'Mast years' no distinguish is made between the different levels of masting.

**Data structure**

Obligate:
- Type of variable: numerical
- Unit: Numbers (in months)
- Value: Minimum period, maximum period
- Trait specific method:
  1. Observation per individual
  2. Observation per population
  3. Unknown
- Masting (production type):
  1. Masting
  2. Non masting
  3. Not applicable (i.e. for annuals)
  4. Unknown
- Seed crop frequency (periodicity):
  1. More than once year
  2. Once a year (annually)
  3. Once in 2 years (incl. mast fruiting)
  4. Once in > 2 years (incl. Mast fruiting)
  5. Not applicable
  6. Unknown

Optional:
- o Comment field: Any information of importance to the trait

SEED SHEDDING

Seed shedding is an additional trait that has close functional relations to seed number, seed crop frequency, seed size, seed dispersal and seed bank longevity. Seed shedding can be described as the process after seed ripening and before seed dispersal, processes that in many plant species overlap in time.

Seed shedding has two dimensions, defined as the time and duration of seed releasing, i.e. which months of the year are the seeds shed (= time) and how long is the time span of seed shedding (= duration; Harper 1977, Bonn & Poschlod 1998). The releasing of seeds in species can occur in a very short time span (i.e. days) up to periods of several months (Harper 1977). The course of seed shedding is influenced by physiological processes and structural organisation of fruit ripening and seed detachment, more so than by direct environmental forces (Harper 1977, Kjellsson 1985).


***Figure 3.17.*** *The short-term seed-releasing species Scilla bifolia (A), the long-term seed-releaser Carlina vulgaris (B), the ant-dispersed Melica uniflora (C) and the wind-dispersed Luzula multiflora (D) (Photo: see Source list).*

What are advantages (and disadvantages) of a species-specific seed shedding season and duration within a year? For instance dispersal of certain seeds can take advantage of high frequencies of strong winds in autumn, but also of hot summer periods with thermic events or thunderstorms (Kunzmann 2000). Early seed ripening and releasing can also be an advantage to escape higher vegetation, responsible for reducing the wind speed in the vegetation period. The synchronous presentation of ripe fleshy-fruits with bird migration is noted by Bonn & Poschlod (1998).
The duration of seed shedding also differs between the species. For instance fast seed shedding can be a defence against pre-dispersal predation (Harper 1977). On the other hand other species flower and produce seeds nearly all year in Britain (e.g. *Poa annua, Senecio vulgaris*; Harper 1977). Releasing periods in myrmecochorous guilds are restricted to the foraging season of the seed-dispersing ants, for e.g. only 2 days in *Scilla bifolia* (Fig. 3.17A; Kunzmann 1993) or 11 days in *Melica uniflora* (Kjellsson 1985; Fig. 3.17C). One example of long-time seed shedding is *Carlina vulgaris*, a wind-dispersed Compositae, where seed releasing extends from October to April next year, almost continuous (see Fig. 3.17B; Kunzmann 2000).
Kjellsson (1985) distinguished cumulative curve-types of seed fall over time, depending on dispersal type, e.g. a hyperbolic curve in case of ant-dispersed *Melica uniflora* or a linear curve in case of wind-dispersed *Luzula multiflora* (Fig. 3.17D).

**What to collect**
Seed shedding is an interval trait, expressed as the first and the last month of seed shedding. There are three different options to collect records for this trait:

1. Use of (un)published sources (e.g. floras or the Biological Flora of British Isles).
2. Observations during investigations of traits (i.e. seed number, seed size).
   *Note*: Only the season and time space will be noted.
3. Species-specific or interspecific studies of seed shedding and of primary seed fall (e.g. use records of trap experiments to investigate dispersal of diaspores).

*Note:* Sometimes a secondary seed fall of a species in a trap could change the estimated record of seed shedding. It is also important to check the evidence of trap experiments based only on few seeds. For long-time seed shedding species, use only the time span, if in which 95% of the quantitative seed fall was found in traps.

In all three options described above, the first and the last month of seed shedding will be noted. In general each record with an unknown number of replicates will be collected as one single observation. In the case of species-specific studies (option 3) the number of replicates is generally known. For new measurements (option 3) one sample per species (per sample site) is collected consisting of data of 10 individuals, with a preferred number of 30 individuals. A second important obligate qualifier is to know if the species is flowering and fruiting more than once a year. This is interesting for many perennial species, but also for annuals with seasonal ecotypes, for e.g. *Rhinanthus, Euphrasia* (Zopfi 1993a, b, 1997). In the Traitbase there is an optional choice to enter actual measured data on time span of seed shedding in weeks.

**Data structure**

Obligate:
- Type of variable: numerical, interval (ranges)
- Number of individuals per population (n): 10 (preferred n=30)
- Number replicates (N): 1
- Unit duration: Numbers (in months)
- Values: Range from first to last month of seed shedding season. Months:
    1. January
    2. February
    3. March
    4. April
    5. May
    6. June
    7. July
    8. August
    9. September
    10. October
    11. November
    12. December
- Trait specific method:
    1. Observation per individual
    2. Observation per population
    3. Unknown

Optional:
- o Real time span of seed shedding: weeks
- o Comment field: Any information of importance to the trait

# 5.2. SEED WEIGHT & SEED SHAPE

*L. Götzenberger*

**Introduction**

A wide range of seed weights can be found across species, from less then $10^{-6}$ to more than $10^{4}$ g (Harper 1977). Through a better provision of nutrients, large seeds are thought to have a superior chance in establishing as seedlings (Salisbury 1942, Grime *et al*. 1988). This suggestion is supported by several investigations, either from analysing correlations between seed mass and habitat conditions (Hodkinson *et al*. 1998) or from experiments (e.g. Dalling & Hubbell 2002, Leishman & Westoby 1994a, 1994b, Saverimuttu & Westoby 1996, but see Paz *et al*. 1999). Several investigations found seed shape and seed weight to be a predictor of persistence in the soil (Thompson *et al*. 1993, Thompson *et al*. 2001, Cerabolini *et al*. 2003, Peco *et al*. 2003). Alone or in combination with their seed traits, seed shape and seed weight are also thought to be predictors of buoyancy and dispersal by water (Römermann *et al*. 2004).

*Trait definition*

Seed weight: Is the air dried weight of (preferably) 100 germinules or dispersules.

Seed shape: Is the variance of the three dimensions length, width and height, dividing each dimension by length so that length is unity.

**What and how to collect**

The preferred standard for collecting seeds for seed weight and shape is to collect 100 seeds from 10 individual plants. If this is not feasible, e.g. because the sampled species is scarce, at least 25 seeds from 5 different individual plants of a species are collected. Deviation from this minimal requirement is allowed (e.g. 8 seeds of each of 7 individuals), however, when a plant produce less than 5 seeds per individual, all seeds of the individual should be collected. On the other hand it might be necessary to extend the number of seeds per individual when species tend to have very small seeds (e.g. Orchids). All further instructions refer to preferred standard of 10 seeds per individual.

The best time for collection of the seeds is obviously the time of seed maturity. In several cases this point of time might be difficult to define. Clues are attributes such as the colour of the seeds or the capsules containing the seeds, the strength of the attachment of the seeds or capsules to the plant and developmental stages of additional seed structures (e.g. pappus).

Date and location of the collection has to be noted with each population sampled and the sample of each plant is kept separately. Each sample can thus be attached to a corresponding individual and the individuals to the corresponding population.

Although it is quite a subjective decision, average sized seeds of a plant should be collected rather then very small and very big ones (Cornelissen *et al*. 2003). Also one should be careful that no parts of the seeds that belong to the dispersule get lost while collecting.

**Storing and processing**

Because it is necessary to use intact seeds (i.e. with all appendages still attached) for seed weight and seed shape, it is recommended not to use a seed cleaning machine.

The collected seeds should be stored in paper bags, but for smaller seeds closable plastic or glass containers (e.g. film canisters) could be used. Note that the seeds should be dry before closing the containers to prevent the seeds from getting mouldy.

**What and how to measure**

*Seed weight*

There are several ways to measure seed weight that differ in the way what is measured (the seed s. str., the reserve mass, the dispersal unit) and the method for drying the seed prior to weighing (air-dried, oven dried). In LEDA if the seeds are collected in natural populations and can be separated by individual, both dispersules and germinules are measured following the subsequent procedure:

1. Collected seeds are air dried prior to the measurements.
2. Weigh 10 dispersules including appendage(s) of each individual separately. If the number of dispersules differs from 10 note N. Make sure to keep the dispersules separated per individual after weighing!
3. Remove all structures that do not belong to the germinule, i.e. all parts that easily fall off or are likely to decay (Keep in mind that the germinule is the unit that enters the soil). If there are no morphological differences between dispersule and germinule directly proceed to measure length, height and width.
4. Weigh the germinule and still keep the seeds separated per individual for measuring seed shape.
5. Proceed to measure the seed dimensions as described below.
   The measured unit of seed weight is milligrams (mg) and the used scales have to display at least three decimal digits.

*Seed shape*

For each dimension the mean of 5 seeds is noted to calculate the shape index. If possible these 5 seeds are drawn by chance from the same seeds that were used to measure seed weight, each seed from one single individual. The shape ($V_s$) is captured by dividing length, width and height of a seed separately by length and then calculating the variance of the three values with the formula: $V_s = \Sigma\ (x_i - \text{mean}\ (x))^2/n$
with $n = 3$ and $x_1$ = length/length, $x_2$ = height/length and $x_3$ = width/length.
In this way the shape ($V_s$) becomes dimentionless with a minimum value 0 in perfectly spherical seeds and a maximum value of 0.2 in needle- and disc-shaped seeds.
The length of a seed is regarded as the longest dimension, no matter if it is equivalent to the morphological length. For instance the propagules in some Caryophyllaceae are described as wider than long in identification keys but length should be measured on what is here regarded as width, i.e. the longest axis that can be found in the seed.
The width is defined as the widest axis perpendicular to the length axis. Height (sometimes regarded as thickness) is the shortest axis perpendicular to the length axis and perpendicular to the width axis. The unit for all of the three measured dimensions is millimetres (mm).
Further remarks on the measuring of seed length, width and height were made by Otto (2002) when drawing up the BIOLFLOR database. In trigonous seeds (e.g. *Carex* and *Polygonum* species; Fig. 3.18) the widest of the three sides is taken into account for the

width value while the mean of the remaining two sides are considered as height. Width axis and height axis are not perpendicular in such seeds.
Appendages extending from the seed in a direction more or less parallel to the length axis are taken into consideration when measuring the length but the same appendages do not contribute to the width (e.g. perigone of *Scabiosa*) while hairs and spines clearly sticking out are added to the width.
For small seeds a binocular microscope with a measuring ocular is used so that the number of decimal digits can be maximised. Length, width and height can be measured with a (electronic) calliper for bigger seeds, but not with normal rulers because it is not possible to obtain decimal digits. The dimensions are measure in mm, with at least one decimal digit.

***Figure 3.18.*** *Trigonus seed of Persicaria hydropiper (Photo: see Source list).*

*Special cases*

- When seeds can not be collected in natural habitats, e.g. because the species is very scarce, collection of seed material follows the subsequent priority:
  1. Mature seeds of natural origin from seed lists of botanical gardens.
  2. Mature seeds from wild plants grown in cultivation (seed commerce).
  3. Mature seeds of unknown origin from seed lists of botanical gardens or seed commerce.
- Seeds from botanical gardens and commercial seed companies can often not be assigned to individual plants. In this case all seeds should be weighed together and than be divided by the total number of the seeds to obtain the mean seed weight. This method is also used when collecting seeds in natural habitats and the circumstances prevent the separating of seeds by individuals.
- When deriving data for seed weight and seed shape from published literature some further problems might occur. In most cases no means for different individuals are given and sometimes even no sample size. Also it is possible that only a range is given by a maximum and a minimum value.

**Data structure**

To collect: 10 seeds of 10 different individuals = 100 seeds in total per species (per site)

Obligate:
- Type of variable: numerical, integer, decimal
- Sample size (n): 10
- Number of replicates (seed number per individual; N): 10
- Unit:
  1. Weight: mg
  2. Length, width and height: mm
  3. Shape: unitless
- Values: N, mean, standard variation, standard error
- Validity ranges:
  1. Seed weight = 10-6-104
  2. Seed dimensions (length, width, height) = 0.1-100
  3. Shape = 0.001-0.3
- Diaspore type: see Morphology of dispersal unit (Chapter 5.4)
- Collection date: day/month/year (dd.mm.yy)

Optional:
- o Balance error: mg
- o Comment field: Any information of importance to the trait

## 5.3. SEED LONGEVITY

*R.M. Bekker, J.P. Bakker and K. Thompson*

**Introduction**

Buried viable seed banks are a fundamental aspect of seed plant biology. They play an important role in the conservation and restoration of plant communities (Bakker 1989), and are important predictors of plant response to changing land use and climate (Hodgson & Grime 1990). However, the information on seed/germinule/dispersule/fruit survival in the soil is scattered and for many plant species still unknown. The LEDA project will fill many gaps in this knowledge by means of recent literature compilation (after 1992), field experiments and through the use of correlations with other seed attributes in this database (such as seed weight and shape).

LEDA adopted three types of soil seed banks (Thompson *et al*. 1997, Thompson 1993, Poschlod & Jackel 1993, Thompson 1992, Bakker *et al*. 1991, Bakker 1989), namely:

Transient: Species with seeds that persist in the soil for less than one year, often much less.

Short-term persistent: Species with seeds that persist in the soil for at least one year, but less than five years.

Long-term persistent: Species with seeds that persist in the soil for at least five years.

Formalised classification can be achieved by application of the key to seed bank types (Fig. 3.19). The key applies only to naturally buried seeds and to data of the most common type, that is, an enumeration of seeds in soil sampled on a single occasion. The key uses both direct and indirect evidence of longevity, but gives priority to direct evidence.

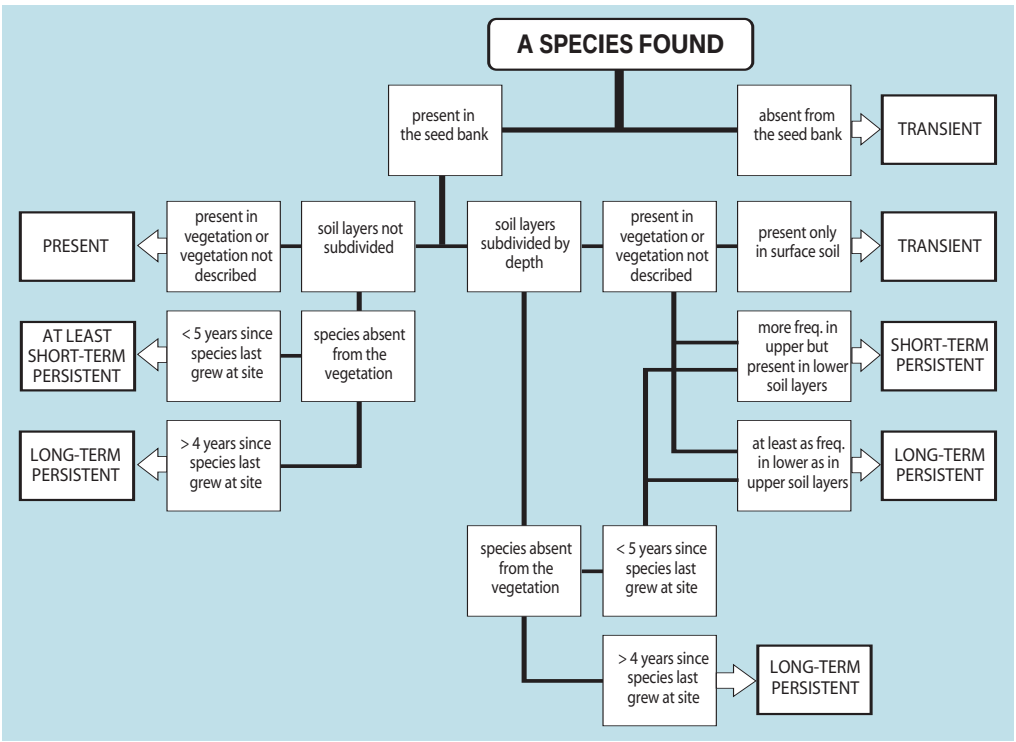


***Figure 3.19.*** *Key to allocate species a seed bank classification (Thompson et al. 1997).*

The key deals with incompletely or inadequately described vegetation, by assuming that all species in the seed bank are also present in the vegetation. Any species in the vegetation but not detected in the seed bank is considered to be transient.
When using seasonal sampling without subdivision by depth, the key is incapable of distinguishing short-term from long-term persistent, and all persistent species will be allocated to the short-term persistent category. The same is true for sampling in frequently disturbed areas such as agricultural fields or urban areas, even when different layers are sampled. Due to this disturbance the depth distribution of the seeds is disturbed and the 'general rule' that deeper buried seeds are older, can not be applied. Only with additional information on management (i.e. when last disturbed) and the vegetation history of the site, the species can be distinguished between short- and long-term persistence. For example, when a species is found in the soil seed bank that is absent from the vegetation for over 4 years, it can be assumed to be long-term persistent. Or when an agricultural field is not ploughed for over four years, it can be assumed that the viable seeds found in the deeper layers are long-term persistent. When information on management and /or the vegetation is absent, the solution is to allocate all persistent species to the short-term persistent category. The guiding principle in dealing with all data sources is to use the data if at all possible, while making the fewest assumptions and using the 'present' category only as a last resort.

*Data collection and admissibility*

In all data sets that have data for one or two buried seeds only will be excluded (possible consequences of contamination or recent dispersal). In order to apply this criterion we have to know exactly how many seeds of each species were actually recovered. The most frequent single reason for rejecting data is an inability to discover this information, usually because it is impossible to work out the actual area sampled. Some classic papers had to be omitted on account of this problem (e.g. Milton 1948).

Other frequent causes of rejection are: burial experiments conducted for too short a period (often measured only in months); data for two or more sites, treatments or taxa pooled; data presented only as frequencies or graphs; species identification poor, e.g. only to genus. For example the classic paper of Van Altena & Minderhoud (1972), describing the established vegetation and seed bank of over 70 meadows, cannot be used since all data were condensed to frequencies.

The second most frequent cause of rejection is that no attempt was made to determine the viability of seeds extracted from soil. Much has been written on the relative merits of extraction or germination of seeds from soil samples (e.g. Gross 1990), but germination has the undeniable advantage of guaranteeing that the seeds recovered are alive. Symonides (1978) found that fewer than 10% of seeds of some species extracted from the seed bank were capable of germination. It therefore seems prudent to reject data where no effort was made to determine if seeds extracted from the soil were viable. Germination, staining with tetrazolium, and a firm or white embryo are all accepted as evidence of viability. Some of the problems encountered are a consequence of the inevitable condensation of large amounts of data necessary to meet the demands of journal editors. A further difficulty encountered was inconsistency between methods and results. For every source we attempted to work out if the methods as described could have produced the stated results. To give a simplified and hypothetical example, if a total area of 0.1 $m^2$ of soil was sampled, and the data expressed on a $m^{-2}$ basis, then (a) the minimum possible density was 10, and (b) all densities should be multiples of 10. Surprisingly frequently, calculations of this sort reveal data which could not be obtained from the methods as described. Wherever possible, try to correct methodological problems or abbreviated data by contacting the authors. Following up publications in this way sometimes leads to useful unpublished data. Inevitably, however, some problems remain unresolved and the sources have to be rejected.

**Minimal requirements**

Trial number — Every separate sample, in a particular reference for which separate information is provided, is given a number. Hence the trial number is each separate experiment or site or individual on which different replicate measurements are performed. This can be, for example, samples from separate sites or the same site where on more than one occasion the seed bank was sampled. The trial number is the key identifier between the reference and the data in the database and is obligatory information.

Seed bank method

The method used to sample the seed bank is obligatory information. In the LEDA Traitbase seven categories of seed bank sample method can be chosen:

1. Seeds deliberately buried in a garden plot without subsequent disturbance
2. Seeds deliberately buried in a garden plot with subsequent disturbance
3. Seeds deliberately buried in the field
4. Soil sample from natural vegetation, seeds extracted and germination or viability tested (includes methods involving any reduction of sample volume, other than just discarding part of sample)
5. Soil sample from natural vegetation, seeds germinated without extraction or sample reduction
6. Same as 5, but germination in the field (e.g. first season after sod-cutting or topsoil removal, only individuals with cotyledons)
7. Sequential sampling of natural seed banks on at least 6 occasions per year. (If <6, each sampling date is treated like a separate trail, add details in comments column)
8. Unknown

*Note:* Data from experiments that extract seeds from soil but do not assess germinability or viability should be ignored.

Area unit

The unit in which the data is expressed. The categories for the unit expression are:

1. $inch^2$
2. $m^2$
3. acre
4. $foot^2$
5. $cm^2$
6. hectare (ha)

Area expressed

The units in which the data are actually expressed; usually the actual volume that is sampled per sample core (e.g. 0.2 $m^2$ or 722 $cm^2$) and is expressed as a number with 4 decimals (expressed in the area unit).

n

Number of cores sampled per within one trial (i.e. 10 cores are seen as 10 replicates for that particular trial).

Area sampled

Is the total area that is sampled per trial; For example in a site 60 cores are taken using a core of 8.55 $cm^2$, resulting in a total of 513.179 $cm^2$ soil sampled.

*Note:* If area sampled is not given or cannot be calculated, the data should be ignored.

Sample depth — The total sampling depth in cm, and expressed as a number with 1 decimal.

Number of layers — This is number of separate layers for which data are reported, not necessarily the number actually examined, i.e. the number of separate layers tested for seeds, expressed as integer.

Thickness top layer — Thickness of top layer, including litter (if any) in cm. expressed as a number with 1 decimal. This is the top layer as actually analysed and ideally the top layer should be as close as possible to 5 cm. Therefore it may actually be two layers combined.

Sample month — For seasonal sampling expressed as a number (Month 1-12), with the value 0 if no seasonal information is given. *NOTE:* The months are numbered July-June in Southern hemisphere.

Duration — Length of time (in months) for which germination of buried seeds is continued, expressed as number of months.

Actual density — Seed density expressed as the actual seed numbers found (i.e. as given in the reference). The species where only one or two seeds were found will be omitted as being a possible consequence of contamination or recent dispersal. Expressed as a number with 1 decimal.

Density per $m^2$ — Density (re)calculated into seed number per square metre (i.e. density), using the entries in density, area unit, area expressed and area sampled.
Note that an algorithm needs to calculate this and store this information in the database. This information will be used for output and higher aggregation levels. Seed density per square metre will be expressed in a rounded number (no decimals).

Max longevity — This is the maximum length of time (in years) that the species has survived in the soil. This field is employed when the seeds are definitely known not to have survived any longer, i.e. in burial experiments where the seeds did not survive as long as the experiment (< 1 year is 0). Expressed as the number of years (integer).

Max possible longevity — This field is employed where the conditions above do not apply, i.e. the remaining data from artificial burial experiments, plus all longevity data from naturally buried seeds. Expressed as text, i.e. >2, >5 in years (> + integer).

Seed bank present — Are seeds of species present in soil?
1. Yes
0. No
*Note*: In general avoid data where vegetation is not described, however if it is not known whether a species is present, we assume it is.

Layer distribution
1. At least as frequent in lower soil layers as in upper layers
2. More frequent in upper soil layers but present in lower layers
3. Present only in upper soil layers
4. Unknown
*Note*: This is question 4 in the seed bank key (see figure 3.19) where the upper soil layer refers to top 5cm or the nearest practical approximation. For example: A seed bank is sampled in three layers; 0-2 cm, 2-4 cm and 4-6 cm. In this case the upper layer will be the combination of the first 2 sampled layers (0-2 and 2-4 cm). For the sake of comparison of densities the data of 4-6 cm layer were multiplied by two thus becoming the deeper layer.

Vegetation present — Is species present in vegetation?
1. Yes
0. No

Last occurrence — When was the species last seen in the vegetation?
1. > four years since species last grew at site
0. < five years since species last grew at site, or time since species last grew at site unknown
*Note*: This is question 5 in the seed bank key of figure 3.19.

Seed bank type — Is the conclusion of the key in figure 3.19:
1. transient
2. short-term persistent
3. long-term persistent
4. present (this category represents low quality data and is not included in higher aggregation levels!)

Comments — Space to add treatments, age of a site, differences between trials etc. as a text of maximal 100 characters

**Soil seed bank sampling protocol**

A summarised protocol of seed bank sampling largely follows the points mentioned by Ter Heerdt *et al*. (1996), who used a combined method of concentrating soil samples and germination in the glasshouse.

Depending on the aim of the study one should take the following points into account:

1. Use a preliminary study of the vegetation and soil seed bank to get an impression of the composition of the seed bank and to learn to identify the seedlings as soon as possible. This study should also provide insights into the abundance, distribution and patchiness of the species present. The space needed in the glasshouse can be estimated at this stage. Favourable germination conditions of many species can be derived from the literature (see Hodgson *et al*. 1995, Baskin & Baskin 1998).
2. Deciding whether the species found are persistent or transient is much simpler if at least two layers of soil are sampled separately.
3. To avoid stratification problems, collect soil samples in early spring (see Fig. 3.20a, b). Natural stratification has already taken place in the field.
4. Wash the soil samples with water on a coarse sieve to remove roots, pebbles etc., and on a fine sieve to remove all clay and silt. A mesh size of 0.2 mm will retain seeds of most species.
5. Spread the concentrated sample on a sterilised medium in a layer as thin as possible, and certainly not thicker than 5 mm. Preferably add on top of the medium a thin layer of sterile white sand to mark the border between medium and sample, to be able to sort the remainder of the sample after germination has stopped.
6. If germination is carried out in a glasshouse or open cage, prepare control trays to record contamination by wind-borne seeds (see Fig. 3.20c).
7. Remove emerging seedlings as soon as possible. When germination has stopped, we recommend further disturbance of the sample to enable seeds deeper in the sample to germinate. Keep a careful watch for signs of herbivore activity and take appropriate action if any are seen.
8. Presence of remaining seeds should be checked with a seed separation method followed by hand-sorting (see also 5).
9. Try to give a complete description of the vegetation where the soil samples were taken.
10. Adequate replication is essential in order to be able to perform any statistics on the data. Degree of replication will depend on the density and patchiness of the seed bank. Pooling of small individual cores into larger samples is advocated both for statistical reasons and for ease of handling. Small cores are often easier if the soil is stony.

*Preferred field sampling protocol*

Select ten squares of homogeneous vegetation (at least 2x2 m each, preferably 5x5 m). Remove the litter layer. Sample ten cores to a depth of 10 cm from each square and subdivide each core in a 0-5 cm and 5-10 cm layer (corer 4 cm in diameter). Pool each of ten samples per square per layer. Concentrate samples on a sieve of 0.2 mm and analyse samples according to the seedling emergence method under standard conditions with plenty of light and water. Include control trays to monitor contamination. Sort (part) of the remainder after germination has stopped for ungerminated seed. Provide a vegetation description of each of the ten plots to enable seed bank classification.


***Figure 3.20.*** *Sample equipment used for soil seed bank sampling (A, B) and seedling emergence of seed bank samples in the greenhouse (C).*

## Data structure

| Data characteristic | Format | Unit | Validity range | Level |
|---|---|---|---|---|
| Trial number | number | - | 1-99 | obligate |
| Method | category (number) | - | | obligate |
| Area unit | category (number) | | | obligate |
| Area expressed | number | $cm^2$, $m^2$... (matching area unit) | | obligate |
| Area sampled | number | $cm^2$, $m^2$... (matching area unit) | | obligate |
| Sample depth | number | cm | 1-100 | obligate |
| Number of layers | number | - | 0-20 | obligate |
| Thickness top layer | number | cm | 0-20 | obligate |
| Sample month | number | - | 0-12 | obligate |
| Duration | number | months | 1-36 | obligate |
| Actual density | number | | $0\text{-}10^6$ | obligate |
| Density/m2 | number | Seeds/$m^2$ | $0\text{-}10^6$ | obligate |
| Max longevity | number | Years | 1-2000 | obligate |
| Max possible longevity | text | Years | | obligate |
| Seed bank present | category (number) | | | obligate |
| Layer distribution | category (number) | | | obligate |
| Vegetation present | category (number) | | | obligate |
| Last occurrence | category (number) | | | obligate |
| Seed bank type | category (number) | | | obligate |

# 5.4. MORPHOLOGY DISPERSAL UNIT

*C. Römermann, O. Tackenberg, L. Götzenberger and P. Poschlod*

**Introduction**

Seed dispersal influences many key aspects of the biology of plants, including spread of invasive species, metapopulation dynamics, and diversity and dynamics in plant communities, but is inherently hard to measure (Cain *et al*. 2000).

The morphology of the dispersal unit can in this perspective be of importance, as the dispersal mode(s) of species can often be recognized by morphological characteristics of fruits and seeds. For example, wings or pappus for wind dispersal, release mechanisms for explosive dispersal, sweet or nutritive fruit pulp for dispersal by frugivorous animals, nutritive nuts for dispersal by granivorous animals, adhesive structures for dispersal in fur, and airy tissues for dispersal by water (Van der Pijl 1982).

Generally there are three main reasons for plants to disperse their seeds, namely to escape potential predators, to escape competition between siblings and to reach sites with suitable conditions for germination and establishment (Fenner 1992). A wide range of morphological adaptations of the structure of the dispersule evolved among seed plants to enable them to disperse efficiently.

*Trait definition*

The trait morphology of dispersal unit consists of the parameters diaspore type and seed structure:

Diaspore type: Indicates if the dispersule (= diaspore) is vegetative or generative and if it is single-seeded or multi-seeded (see below).

Seed structure: For seed structure a coarse categorisation of six morphological structures are followed in the LEDA Traitbase (nutrient rich structures, balloon structures, elongated appendages, flat appendages, other specialisations, no appendages). Per category subcategories allow the classification of seed morphology into a finer scale (see below).

**What to measure**

The morphology of the seeds will be recorded from literature sources and from own observations. There are a great number of publications that feature drawings or photographs from seeds (e.g. Hanf 1990, Young & Young 1992) as well as the bibliography on seed morphology from Jensen (1998). When using these sources for information it is important to make sure that the described seeds are in fact the dispersal units!

As the measurements or observations are only carried out on the dispersule, for each species the dispersules need to be collected in their complete form; with all appendages!. Therefore it is of importance to pay attention to the diaspore structures that might fall of easily (e.g. pappus) when transporting the dispersules. The relationship between 'traditional' morphological and dispersal type classifications is presented in table 3.5.

Note that the dispersules used for the morphology of dispersal unit could afterwards also be used for the collection of other trait data for LEDA (e.g. seed weight, seed shape; see Section 3, Chapter 5.2).

Diaspore type

This parameter indicates the diaspore type to which the morphological structures of the seed type classification refer to. Diaspores can be vegetative or generative, whereas the generative disapores can be either single-seeded (e.g. *Juncus* spec.) or multi-seeded (e.g. *Sanguisorba minor* - 2 seeds per dispersule).

**Categories diaspore type**

Categorisation of diaspore type in the LEDA Traitbase, including sub-categories, comprises:

| | | |
|---|---|---|
| 1. Vegetative dispersule | | Buds, root or shoot fragments i.e. of clonal plants |
| 2. Generative dispersule | a. One-seeded | e.g. *Juncus spec., Trifolium* spec. |
| | b. Multi-seeded | e.g. *Sangisorba minor, Arctium lappa* |
| 3. Germinule | | *Note*: When the germinule is the dispersule, the diaspore type is categorised as generative dispersule! Information about the germinule is only provided for data from which this information on the germinule is already available. |
| 4. Unknown | | |

Seed structure

In LEDA a coarse categorisation of six morphological structures will be used for seed structure (nutrient rich structures, balloon structures, elongated appendages, flat appendages, other specialisations, no appendages). The main categories used within LEDA are adopted from the trait database Bioflor (Klotz *et al*. 2002, see Otto (2002) for descriptions). Per category subcategories allow the classification of seed morphology into a finer scale (see below). Note that in order to classify all appendages a diaspore has, any combination of the seed structure categories is possible. For instance *Geum rivale* does not only have one elongated appendage (thorn) but also many short appendages (hairs). The diaspores of many Poaceae have glumes (open balloon appendages) as well as awns (one long or short, sometimes hooked elongated appendage) and hairs (many short elongated appendages).

*Categories seed structure*

For LEDA the following 6 main seed structure categories are used.

- 1. Nutrient rich appendages or structure

All nutrient rich and often the rich seed/fruit structures that attract potential dispersers belong to this category.

a. Elaisome

Elaisomes are appendages that serve as rewards for ants that carry the seeds away. They can vary greatly in form, in size and in position between species and mostly show a different colour from seeds (for examples see Table 3.4).

b. Aril

An aril is a cup-like structure that partly covers the seeds. It can be fleshy (e.g. *Taxus baccata*) or involucriform (e.g. *Vicia*). Note that it never encloses the whole seed as pulp does (for example see Table 3.4).

c. Pulp

Pulp can be defined as a more or less fleshy structure that encloses one or more seeds. It can be coloured in a stricking way to attract potential animal dispersers (for examples see Table 3.4).

- 2. Balloon structures

Balloon structures are wrapped around the germinule in a more or less balloon-like form, but do not necessarily have to enclose the germinule completely. The structures bind air to the diaspores when they are thrown into water and are derived from numerous morphological structures like calyx and crown (e.g. *Trifolium*), calyx and parts of the stem (e.g. *Agrimonia*) or bracts and prophylls (e.g. glumes of Poaceae, utricle of *Carex*).

a. Open structures

These structures are defined as structures where the air can escape more or less easily (e.g. glumes of Poaceae; for examples see Table 3.4).

b. Closed structures

These structures are more or less closed (aerenchyma) and the air can not escape easily (e.g. utricles of *Carex*; for examples see Table 3.4).

- 3. Flat appendages

Flat appendages comprise all structures that stick out of the more compact part of the dispersule with a flat, thin form, which increases the surface of the main part of the diaspore by at least $1/10^{th}$. Note that if the appendage surrounds the main part of the diaspore it counts twice. These structures are mostly refered to as wings (e.g. *Betula, Acer, Peucedanum*) or as fringes of seeds (e.g. *Ranunculus* spec.).

Note that flat appendages can be confused with elongated appendages (see also below), however for flat appendages the height is considerably shorter than the width and length of the appendage, whereas for elongated appendages the length of the appendage is considerable shorter compared to its width and height.

a. Small flat appendages

Appendages are classified as 'small' if they enlarge the diaspore surface by less than the surface size (for examples see Table 3.4).

b. Large flat appendages

Appendages are classified as 'large' if they double the diaspore surface (related to the main part of the diaspore; for examples see Table 3.4).

- 4. Elongated appendages

Elongated appendages comprise all structures that prominently stick out of the main part of the diaspore, making the seed look longer (i.e. elongated). In order to apply this category, the appendage length must be greater compared to the area where the appendage is attached to the main part of the diaspore. Furthermore the length of the appendage needs to be at least $1/10^{th}$ as long as the diameter of the diaspore. Therefore all structures that do not follow these rules (e.g. papillae) are not considered to be elongated appendages. Note that elongated and flat appendages can be confused (see also above). The main difference is that for elongated appendages the length of the appendage is considerable shorter compared to the other dimensions (width, height) and for flat appendages the height is considerably shorter than the width and length of the appendage.

For the subcategories of elongated appendages a more functional classification, instead of a morphological classification, was chosen. This was done because the information about appendage length, appendage number and occurrence of hooks is essential information for other LEDA traits such as attachment capacity or terminal velocity.

a. One short elongated appendage
   The diaspore only has one short elongated appendage sticking out from the main diaspore in a prominent way, but which is shorter than half of the main dimension of the diaspore (for examples see Table 3.4).
b. Two or more short appendages
   Appendages are classified as long if they double the diaspore surface (related to the main part of the diaspore; for examples see Table 3.4).
c. One long elongated appendage
   Appendages are classified as long when the appendage s at least half as long as the main dimension of the diaspore. For instance awns must be at least half as long as the seed length (for examples see Table 3.4).
d. Two or more long elongated appendages
   Diaspores with two or more elongated appendages, both measuring at least half as long as the main diaspore diameter (for examples see Table 3.4).

Additional: Hooked
   Can be indicated separately when the appendages in subcategories 4a-4d are hooked (for examples see Table 3.4). Note that in LEDA hooked is used as a functional term, therefore all bristles that are directed downwards and act as hooks are included!

- 5. No appendages

In this category only diaspores without any obvious appendages are included, which includes all diaspores that or not assigned to categories 1 to 4.

a. Diaspores with structured surface
   All diaspores with structured surfaces are included in this subcategory (e.g. *Sanguisorba minor, Petrorhagia prolifera*). For small seeded species the structure might not be apparent and therefore seed should be magnified to examine the surface structure (e.g. with magnifying glass; for examples see Table 3.4).
b. Diaspores with smooth surfaces
   All diaspores without any obvious structured surface are included in this category (e.g. *Juncus* spec.). For small seeded species the seed should be magnified to examine the surface structure (e.g. with magnifying glass), as the structure might not be apparent (for examples see Table 3.4).

- 6. Other specialisations

This category is for all diaspores that have appendages which do not fit in one of the above mentioned categories. Note that when applying this category the specialisation needs to be described in the comment field!

***Table 3.4.*** *Summary of the seed structure categories with belonging sub-categories and examples.*

| Main category | Sub-category | Example | Photo |
|---|---|---|---|
| 1 Nutrient containing structures | a. Elaiosome | *Viola hirta*; (photo: *Carex ornithopoda*) |  |
| | b. Aril | *Taxus baccata* | |
| | c. Pulp | *Prunus* spec. | |
| 2 Balloon structures | a. Open structures | Glumes from Poaceae (photo: *Arrhenaterum elatius*) |  |
| | b. Closed structures | Utricles of *Carex* spec. (photo: *Carex alba*) |  |
| 3 Flat appendages | a. Small appendages | *Ranunculus acris*, *Leucanthemum vulgare* (photo: *L. vulgare*) |  |
| | b. Large appendages | *Acer* spec., *Fraxinus, Angelica* spp. (photo: *Oxyria digyna*) |  |
| 4 Elongated appendages | a. One short appendage | Photo: *Ranunculus repens* |  |
| | b. Two or more short appendages | Short hairs (e.g. *Bromus arvensis, Geum urbanum*) (photo: *B. secalinus*) |  |
| | c. One long appendage | Awns, thorns (photo: *Geum urbanum*) |  |
| | d. Two or more long appendages | Long hairs (e.g. *Epilobium* spec.) or long pappus (*Centaurea* spec., *Hieracium pilosella*). (photo: *Epilobium tetragonum*) |  |
| Additional info: | Hooked structures | For seeds of *Bidens* spec., *Agrimonia eupatoria* (photo: *Bidens cernua*) |  |
| 5 No appendages | a. Coarse surface | *Silene vulgaris, Sanguisorba minor* (photo: S. *minor*) |  |
| | b. Smooth surface | *Juncus compressus, Lotus corniculatus, Erica tetralix* (photo: *L. corniculatus*) |  |
| 6 Other specialisations | | | |
| 7 Unknown | | | |

***Table 3.5.*** *Relationship between 'traditional' morphological and dispersal type classifications into the LEDA functional categorisation of the trait morphology of the dispersal unit.*

| Traditional terms[1] | Main category used in LEDA | Sub-categories used in LEDA (several possibilities per diaspore; partly combined) |
|---|---|---|
| Awns | Elongated appendages | One short appendage (can be hooked)<br>One long appendage (can be hooked)<br>Two or more short appendages (can be hooked)<br>Two or more long appendages (can be hooked) |
| Hooks | Elongated appendages | One short appendage, hooked<br>One long appendage, hooked<br>Two or more short appendages, hooked<br>Two or more long appendages, hooked |
| Hairs | Elongated appendages | Two or more short appendages (can be hooked)<br>Two or more long appendages (can be hooked) |
| Pappus | Elongated appendages | One long appendage and two or more long appendages (e.g. stalked pappus of *Taraxacum*)<br>Many long appendages (e.g. *Hieracium* spec.)<br>Many short appendages |
| Plumed | Elongated appendages | Two or more short appendages<br>Two or more long appendages |
| Prickles | Elongated appendages | One short appendage (can be hooked)<br>One long appendage (can be hooked)<br>Two or more short appendages (can be hooked)<br>Two or more long appendages (can be hooked) |
| Thorns | Elongated appendages | One short appendage (can be hooked)<br>One long appendage (can be hooked)<br>Two or more short appendages (can be hooked)<br>Two or more long appendages (can be hooked) |
| Wings | Flat appendages | Large flat appendages (e.g. *Acer* spec.)<br>Small flat appendages (e.g. *Leucanthemum vulgare*) |
| Balloons | Balloon structures | Closed structure (e.g. *Utriculus* of *Carex* spec.)<br>Open structure (e.g. glumes of many Poaceae) |
| Acanthochoreous diaspores | Elongated appendages | One short appendage, hooked<br>One long appendage, hooked<br>Two or more short appendages, hooked<br>Two or more long appendages, hooked |
| Cystometeorochoreous diaspores | Balloon structures | Closed structures |
| Elaiosomochorous diaspores | Nutrient containing structures | Elaiosome |
| Lophocorous diaspores | Elongated appendages | Two or more short appendages<br>Two or more long appendages |
| Pogonochorous diaspores | Elongated appendages | Two or more short appendages<br>Two or more long appendages |
| Ptero(meteoro)chorous diaspores | Flat appendages | Large appendages (e.g. *Acer* spec.)<br>Small appendages (e.g. *Leucanthemum vulgare*) |
| Sarcochorous diaspores | Nutrient containing structures | Aril<br>Pulp |
| Stomatochorous diaspores | Nutrient containing structures | Elaiosome |
| Trichometeorochorous diaspores | Elongated appendages | Two or more short appendages<br>Two or more long appendages |

[1] Traditional terms from: Luftensteiner (1982), Van der Pijl (1982), Müller-Schneider (1986), Bonn & Poschlod (1998).

*Special cases*

- In some species, the intra-individual variation, often occurring within the same infrutescence, is large and different types (or morphs) of seeds or fruits can be defined. This variation is associated with heteromorphism, which is an example of phenotypic variation as it refers to within-individual variation. Therefore, seed heteromorphism can be defined as the production of different types of seeds by one single individual (Imbert 2002). For instance, the variation of achene shape in *Calendula* sp. is a well-known example of heterocarpy, where three or four achene morphs can be present (Heyn *et al*. 1974). However, plant species commonly show intra-individual variation in seed size, either mass or length.

**Data structure**

To collect: Minimum of 5 seeds per species.

Obligate:
- Type of variable: nominal
- Sample size (n): 5
- Number of replicates (seeds per individual, N): -
- Unit: Categories
- Diaspore type categories:
  1. Vegetative dispersule
  2. Generative dispersule
     2a. One-seeded 2b. Multi-seeded
  3. Germinule
  4. Unknown
- Seed structure categories:
  1. Nutrient containing structures
     a. Elaiosome b. Aril c. Pulp
  2. Balloon structures
     a. Open structures b. Closed structures
  3. Flat appendages
     a. Small appendages b. Large appendages
  4. Elongated appendages
     a. One short appendage
     b. Two or more short appendages
     c. One long appendage
     d. Two or more long appendages
     Additional: Hooked structures
  5. No appendages
     a. Coarse surface b. Smooth surface
  6. Other specialisations
  7. Unknown

Optional:
- o Collection date: day/month/year (dd.mm.yy)
- o Comment field: Any information of importance to the trait