ENSCONET
Seed Collecting Manual
FOR WILD SPECIES

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Contents

Summary of key recommendations

- All collections must be made legally (section 2.1).
- Carefully prepare for the collecting trip (section 2).
- In the absence of better advice, a good start would be to sample five populations from across the taxon's geographical range taking into account ecological variation (sections 3.1 & 3.2).
- Try to collect from at least 50 and preferably 200 plants per population but modify this advice on the basis of local circumstances (section 3.3).
- Collect no more than 20% of the total mature seeds available (section 3.4).
- Try to collect at least 5000 seeds per accession (section 3.4).
- Sample as randomly as possible but for large populations in a uniform landscape, it is often easier to collect in a more systematic way, sampling at regular intervals along a transect (section 3.5).
- Ensure as even a contribution of seeds from maternal genotypes as possible (section 3.5).
- Where the number of plants sampled is less than 20, keep seeds from different plants separate. This will maximise the contribution of the maternal genotypes at regeneration (section 3.5).
- Check for empty or immature seeds prior to collecting, even though the seeds may look outwardly acceptable (sections 4.1 & 4.2).
- Collect seeds into cloth or non-glossy paper bags. Choose bags carefully (section 4.1).
- Place fleshly fruits into plastic bags, keeping the bags open and giving fruits plenty of aeration (section 4.3c).
- Select appropriate harvesting techniques according to the species (section 4.3).
- Seed without data is almost useless and so full information should be recorded about each seed collection (section 5.1 and Appendix 1).
- It is particularly important that the data recorded is as objective as possible and that it will be easy to comprehend several decades from now (section 5.1).
- It is important to record the location of the collection using a map or a Geographical Positioning System (GPS) receiver (section 5.2).
- Collection of herbarium specimens before or during seed harvest allows the verification of identifications made by the collector (section 5.3).
- If transportation back to the seed bank will take several days, it is advisable to dry the seed over silica gel, dried rice or charcoal inside sealed plastic boxes. This is particularly important if the average outside relative humidity (or the seed equilibrium relative humidity as determined by a hygrometer) is greater than 50% (section 6).

In brief:

- The future safety of the plant population is paramount.
- Use commonsense.
- Record what you do.
- Sampling is rarely perfect - so be aware of the genetic variation that is likely to have been captured in your sample.

1 INTRODUCTION

1.1 General

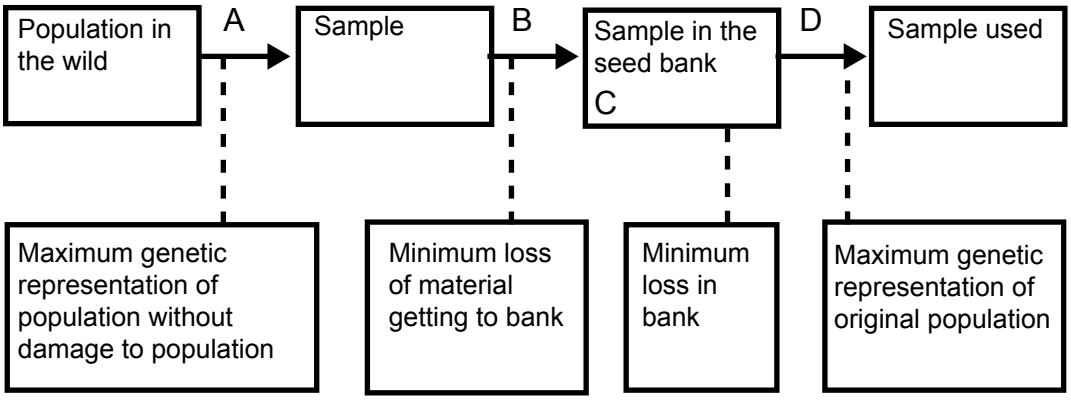
Global biodiversity, including the diversity of wild plants, is of inestimable ecological, economic, and cultural value. There has been a significant loss of global biodiversity during recent decades. Genetic erosion is placing many species at risk of extinction. A key approach to slow this erosion is the conservation of plant species where they grow (*in situ* conservation). However, the conservation of plants away from their natural habitats (*ex situ* conservation) also has an important role in ensuring their survival. This is recognised in the Convention on Biological Diversity (CBD, see http://www.cbd.int), the Global Strategy for Plant Conservation (GSPC, see http://www.cbd.int/gspc/) and the European Strategy for Plant Conservation (ESPC, see http://www.plantaeuropa.org/pe-EPCS-what_it_is.htm). In this manual, we will outline seed collecting techniques for the *ex situ* conservation of European wild plants.

The European Native Seed Conservation Network (ENSCONET, see http://www.ensconet.eu) brings together members from many EU countries involved in the conservation of European native seeds. Most are seed banks working under national or regional organisations such as universities or botanical gardens.

The primary goal of seed collecting by ENSCONET members is the long-term conservation in seed banks of representative samples of the genetic diversity of European seed-bearing plant populations (with priority being given to those that are most threatened). These samples can then be used in research, trialling, reintroduction, reinforcement of weak populations and habitat restoration. Many of these populations are threatened by one of more factors such as land conversion, climate change and air pollution.

The methods included in this collecting manual should be widely applicable (including outside Europe), with adaptation as necessary to local circumstances. Where the biology of the species is well known, the methods may be made more sophisticated. The quality of seed collections depends upon the expertise of the collector, the circumstances at the collection site on the day of collection, and the knowledge available. This guide helps to address the latter. However, ultimately, seed collection is the art of the possible!

Summary of basic stages in seed conservation of a targeted population and associated genetic considerations:



A = Sampling; B = Shipment back to bank; C = Storage; D = Sampling. A & B are addressed by this manual. C & D will be addressed by the ENSCONET curation protocol

This manual builds on a number of other texts such as Falk & Holsinger (1991), Guarino, *et al.* (1995), Hawkes *et al.* (2000) and Smith *et al.* (2003). They also draw on other manuals such as Bacchetta *et al.* (2006) and those of several ENSCONET partners such as RBG Kew's Millennium Seed Bank Project (see http://www.kew.org/msbp/scitech/publications/fieldmanual.pdf and http://www.inia.cl/recursosgeneticos/bancobase/semillasnativas/Documentos/m_sem.pdf).

It is important to note that within wild species there is often significant genetic and physiological heterogeneity. Furthermore, there is rather limited knowledge about their breeding systems and seed maturation. Consequently, collecting seeds of wild species poses a greater challenge than does the collection of rather more uniform crop germplasm for which more has been written.

Please note that much of the terminology used in this document is described in the above publications and in Elsevier's Dictionary of Plant Genetic Resources (1991).

1.2 Important comment – collecting with permission & use of seeds

Seed collecting is a well-defined scientific procedure, widely used for the *ex situ* conservation of plant genetic resources. However, unauthorised, and therefore illegal, seed collection, e.g. by the general public, may damage and threaten the populations of native species (see section 2.1).

The general public also need to be aware that collecting seeds and sowing them elsewhere may introduce 'foreign' genes into a nearby population of that species. This may weaken the local gene pool and affect its future viability. Similarly, be aware that introducing a species to a new area may lead to it becoming a serious pest or to hybridisation with closely related species resulting in loss of genetic integrity of the populations affected. Consequently, plants should only be returned back to the wild with the agreement of the relevant government authorities.

Figure 1 *Linaria alpina* in the Alps.
(© University of Pavia)

2 PLANNING SEED COLLECTING EXPEDITIONS

2.1 Permits and authorisation

All collections must be made **legally**. Anyone interested in seed collecting should:

- Contact an institute responsible for native seed collecting for advice before collecting anything. To find the relevant institute in your country visit the ENSCONET website (http://www.ensconet.eu) or the national CBD focal points (see http://www.cbd.int/information/nfp.shtml). The ENSCONET co-ordinator (details at the ENSCONET website) may be able to advise. Collectors may find it helpful to refer to the FAO International Code of Conduct for Plant Germplasm Collecting and Transfer (http://www.fao.org/ag/agp/agps/PGR/icc/icce.htm).

- Obtain permission from the land-owner/manager of the site/national park authorities and, in the case of protected species, the relevant government authority. Where possible, obtain permission (permits) before setting out. Permission should cover seeds, herbarium specimens etc. of as broad a range of species (including those targeted) as possible (see section 2.2). Permission can take time to obtain. At the end of the trip, remember to provide permit authorities and landowners with a report of the collecting carried out.

- Check that target species are not listed under international agreements or directives that give them special status. Of particular note are:

  - CITES (http://www.cites.org)
  - European Council Regulation (EC) No. 338/97 incl. Annexes (http://www.ec.europa.eu/environment/cites/legis_wildlife_en.htm)
  - The Bern Convention (http://conventions.coe.int/Treaty/EN/Treaties/Html/104.htm)
  - Habitats Directive (http://ec.europa.eu/environment/nature/legislation/habitatsdirective/index_en.htm)
  - The International Treaty on Plant Genetic Resources for Food and Agriculture – Annex1 (http://www.planttreaty.org/)

Contact the national plant health authorities if seeds are to be moved between the EU and other countries.

2.2 Targeting

In most circumstances within Europe, collecting trips will target well recorded populations of species that are the subject of local, national or regional biodiversity action plans (see for instance, National Biodiversity Action Plans such as that for the UK, http://www.ukbap.org.uk/ , and the ENSCONET target plan – under development in May 2009). See section 3.2 and Maxted & Guarino (2003) for advice on selecting the populations to sample. Try and make the most of a trip, however, by including secondary objectives that are less precise and dependant on opportunities that arise. In more isolated locations, you may not revisit the site for many years and should take the opportunity to collect unusual though not targeted material. Requests for collecting permission or permits should therefore cover as broad a range of potential taxa as possible.

2.3 Gathering information on the species to be collected

Look at the local and regional floras, listings, databases and monographs in order to find detailed descriptions and information on how to differentiate between related taxa. *Flora Europaea* (now available on CD) is the primary reference for the European flora. Useful websites include:

- Euro+Med PlantBase (http://www.emplantbase.org/home.html)
- Global Biodiversity Information Facility (http://www.gbif.org)
- EDIT Specimen and observation explorer for taxonomists (http://search.biocase.org/edit/)
- Royal Botanic Gardens, Kew (http://www.kew.org)
- Index Herbariorum (http://sciweb.nybg.org/science2/IndexHerbariorum.asp.)

Herbaria and genetic resources centres are useful sources of information for obtaining precise localities and phenological (flowering / seed maturation time) data. However, the collector should keep in mind that phenological data might need to be verified from a number of sources, especially where data is old. Because many herbarium specimens are collected at flowering, there may be no indication of likely seeding time, but estimates can be made. For example, in the case of NW European herbaceous species, an extra 1.5-2 months is a useful guide for the time between peak flowering and peak seeding (see Appendix 3). However, the collector should be aware of possible deviations in peak flowering or seed maturation periods; 1 or 2 weeks deviation is possible in the Mediterranean due to inter-annual variability in climate. This might be particularly relevant for species with a synchronous and very short seed dispersal period.

Collectors also need to gather information from the above sources on other biological characteristics of the targeted species such as breeding system, including apomixis (see for instance Fryxell (1957) and note that a database is being developed by RBG Kew), ploidy level and seed dispersal strategies (see section 3). Information on the putative diseases or pests that might infect the targeted species is also useful. Collectors should also check that the targeted species are desiccation tolerant and therefore capable of long-term banking. One reference is the Seed Information Database managed by RBG Kew (http://www.kew.org/data/sid/). In general, large, fleshy seeds with thin seed coats from long-lived woody perennials are likely to be recalcitrant. Local experts may also be able to add extra information.

There will be great variation between species as to what is known about their geographical distribution and their known populations. Local botanists and plant ecologists will have detailed knowledge. In the absence of suitable help, there may be a number of types of information. These include species monitoring projects (for rarities), eco-geographic surveys (occasionally available), inventories (national and smaller areas), herbarium specimens (give an historical perspective of the distribution), chorological accounts in botanical journals and distribution maps in floras. Some maps show the species distribution to a very detailed degree and may be available in electronic format for incorporation into geographical information systems (GIS) – see for instance http://www.programanthos.org for Spain. Using soil and climate data, GIS can be used to extrapolate from a species current range to where it might be also be found (see for instance Moat & Smith, 2003).

2.4 Logistics prior to seed-collecting trip

If possible, make a prior visit to the site (or ask a local partner) to locate and determine the extent of the population, confirm its identity when flowers are present and estimate the likely date for seed collecting. It might also be an opportunity to collect a voucher specimen in flower or to tag the individuals to be sampled at the flowering stage. This is particularly useful for rare species, plants that are not easily discernible in the field at the fruiting stage or plants that may be confused with other species at the fruiting stage. Use suitable tagging according to the plant life form and complement with Geographical Position System (GPS) data and draft microsite maps.

If such a pre-trip is not possible, estimate fruiting time either by reference to herbarium data (see above) or by contacting local botanists. In the future, satellite vegetation imagery might prove useful in studying the status of the vegetation (e.g., dry vegetation) and in helping to time seed collecting.

Study maps of the area and develop a rough timetable for the collecting trip. If possible, plan the trip by plotting the species distributions plotted by GIS (see section 2.3) with ear-marked populations (see sections 3.1 & 3.2) combined with possible routes, and in isolated regions, accommodation and service stations. It is possible to get detailed climate data and recent weather reports (particularly important when collecting in mountainous areas) from the internet for most places in Europe. Think through contingency plans in the event of an emergency and carry appropriate telephone numbers. Where telephone signal coverage is limited, radio communication may be necessary. Do not collect alone in isolated areas. Local guides can be invaluable in avoiding problems. Before leaving on a collecting trip, give your itinerary to someone who will take appropriate action if regular pre-arranged contact is lost.

A checklist of possible equipment for a trip is included in Appendix 4.

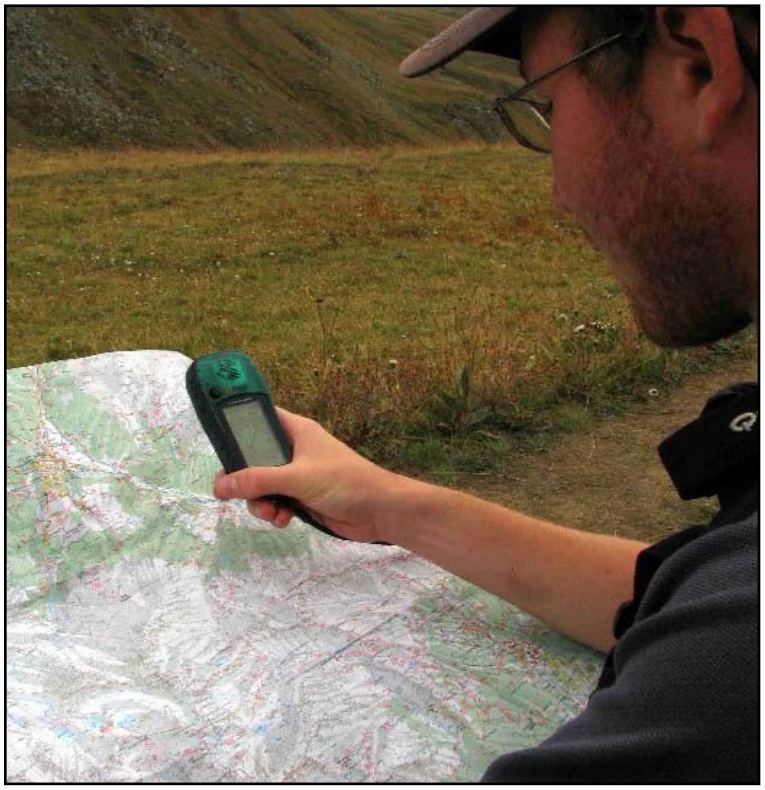


Figure 2 Use of GPS and maps for collection planning.
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3 SAMPLING

Note for simplicity – all the comments on sampling have been brought together in this section. However, in many cases, decisions on the number of populations to sample (see section 3.1) and the selection of the sites may have taken place during planning (see section 2).

3.1 Number of populations to sample

In an ideal world with infinite resources, it would be advisable to collect every population within a taxon's distribution to ensure complete sampling of its genetic variation. In reality, this will not be possible except for the most restricted species where all populations are known. Therefore the decision of how many populations to sample will depend on the resources available, the species concerned and the needs of the collecting programme. In some cases, details such as breeding system, ecological specialisation and detailed distribution may be known and deductions can be made about likely gene flow and the number of populations that should be sampled. For instance, out-crossed wind-pollinated woody perennials have a high proportion of their gene diversity within populations. Consequently, fewer populations may need to be sampled than in the case of selfing annuals where a high proportion of the gene diversity is found between populations (see Hamrick *et al.*, 1991). Similarly, very fragmented distribution of the species leading to isolated populations will indicate that there is likely to be genetic differentiation between these populations. The more information that collectors have to hand (see section 2.3), the better is their decision-taking with regard to sampling. However, collectors are increasingly working against a background of rapid population loss and relatively meagre resources. Therefore, in the absence of better advice, a good start would be to **sample five populations** (see Falk & Holsinger, 1991) from across the taxon's geographical range. Data from four globally rare taxa (Neel & Cummings, 2003) showed that five populations would cover, on average, 67-83% of all alleles. Therefore, except in the case of species with very few populations, targeting of only five populations will not give full coverage. Guerrant *et al.* (2004) recommend as many as 50 populations to achieve fuller coverage while recognising that this may need to be spread out over a long period. In some cases, differences in ecological range (altitude, rock type etc) can be taken into consideration. See section 3.2 for further discussion on selection of populations. Obviously, the fewer populations sampled, the less genetic diversity is likely to be captured. Selection of populations to be collected will follow economic (distance from base and time for collection) as well as eco-geographical criteria.

On arriving at a collecting site, it is important to first have a quick survey of the extent and distribution of the species at the site. The question will arise of where does one population end and another start? Ideally, collectors would want to keep the population samples separate. Merging samples will cause valuable genetic differences to be lost. A great deal of time could be spent worrying about this issue. Therefore, collectors must take a practical approach and be guided by their knowledge of the species and of the underlying population genetics.

In essence, there is no problem in collecting within a population until an obvious barrier to genetic exchange (likely to lead to genetic isolation) is encountered. It would then be advisable to keep samples either side of this barrier separate. The nature of these barriers will depend on the pollen and seed dispersal method of the species. Occasionally, some pollen or seed may travel extreme distances but where this happens the genetic effects within the recipient population are assumed to be swamped by locally-produced pollen and seeds. Therefore, isolation will rarely be absolute; a low probability of exchange will exist. Most dispersal will usually be local. For instance, most seeds disperse less than 100m (Cain *et al.*, 2000). Wind-blown pollen and animal carried pollen can travel much greater distances. As a practical approach, and when there is insufficient

Figure 3 Wild plant populations are rarely as uniform and as well demarcated as this field of *Papaver rhoeas* in the UK. Systematic collecting along a transect could be carried out. Contrast this with the situation in Figure 11. (© RBG Kew)

information on the dispersal of targeted species and its historical and current pattern of distribution (usually the case), the limit of two adjacent populations could be arbitrarily established as the absence of individuals between them over a distance of 10 km. Such an approach has the merit that differences in geographical location between sites 10 km apart will be significantly meaningful to users of the information.

Within populations, there may be genetic differences selected by ecological variation within the habitat. Keeping this ecotypic variation separate (stratified sampling – see section 3.3) may be important in re-introduction programmes. Similarly, it might be wise to keep separate any obvious ploidy types.

If in doubt about population extent or ecotypic variation, it is better to collect more rather than fewer samples as they can be bulked later as the collector gains knowledge about the species. This said, large numbers of small samples create curation problems later on.

Finally, there are two additional points to make. It is important to check that the population is wild and not the result of planting or obvious hybridisation. Collectors should also remember that data about the population will be useful for future conservation assessments (see section 5.1).

3.2 Selection of populations (collecting sites)

The main challenge when planning a collecting mission is the selection of the populations to be sampled that maximise the genetic diversity (Neel & Cummings, 2003; Groves, 2003). The genetic diversity of a population depends on inherent factors such as breeding system and on size; it is also conditioned by biotic and abiotic aspects of the environment. The differences in environmental conditions at different geographic locations are likely to impose different selection pressures on the target taxon's populations and thereby promote genetic differentiation between them. Thus, by dividing the territory under consideration into sectors using available eco-geographic data, and assuming that the more distant and environmentally diverse two populations are, the more genetically

diverse they will be, it is possible to obtain a set of sectors in which we consider that populations occurring within the same sector will have similar adaptive traits. Finally, other criteria such as threat can be superimposed on this information. Maxted *et al.* (1995) describe the use of eco-geographical surveys in the selection of collecting sites while the use of genecological zonation is outlined by Dulloo *et al.* (2008). Extensive information can also be found in Bacchetta *et al.* (2008).

The effect of environment on plant species is a factor that should currently be considered in collecting strategies using Geographic Information Systems, GIS (Draper *et al.*, 2003; Draper *et al.*, 2004). GIS is also an important tool to characterise environmental features of the provenance of the samples improving success when the material will be used. The main benefit is the increment in efficiency of collections, cost-reduction of collecting missions, and the increase in the genetic diversity of species sampled.

3.3 Minimum number of plants to be sampled

As a general rule, collectors should aim to sample from as **many individuals as possible, without endangering the population**. Collect at random over as wide an area as possible. If there is an appreciable variation in the habitat, choose a stratified sampling method such that seeds from each ecological type are kept separate (see also sections 3.1 & 3.5).

There is much guidance on the collection of plant genetic resources in the literature. Much of it is derived from work on crop species by Marshall & Brown (1975) who recommend the capture of at least one copy of 95% of the alleles that occur in the target population at frequencies greater than 5%. To achieve this, they estimate that the minimum number of randomly chosen individuals to be sampled should be 30 (out-breeders) or 59 (in-breeders). Because the breeding system may be unknown, the sampling of 50 individuals in a population is recommended as a benchmark figure. The Center for Plant Conservation in the USA recommends the sampling of between 10-50 plants per population (Falk & Holsinger 1991). However, guidance based on capturing single copies of alleles seems more relevant when collecting material for crop breeding than it does in the context of nature conservation. When the material is required for re-introduction, adaptation is likely to be greatest if the frequencies of alleles in the sample closely match those of the population (previously) at the site. When sampling to reflect the frequencies of alleles in a large population, Marshall & Brown (1983) suggest sampling 200 individuals and, for outbreeders, at least five seeds per plant. See also Broadhurst *et al.* (2008) on considerations when collecting for large-scale restoration.

Keep the genetic implications of sample size and breeding system in mind (see Appendix 5). Consider the number of seed that will be collected and the suggested limit to the proportion of the population collected (see section 3.4). Don't overlook the possibility that a population of individuals is in fact one individual joined by rhizomes or stolons, and check for this. If in doubt, make a note in the notes field of the collecting form.

In summary, try to collect from **at least 50** and **preferably 200 plants** but modify this advice on the basis of local circumstances (including very small populations, annual or long-lived perennial, accessibility, time and eventual use). Seed collecting is the 'art of the possible'. Often, it is impractical to obtain a big sample in the field (see suggestion in section 3.5 about keeping individual plant harvests separate). In these circumstances, as long as the sample is curated and used knowing that it may have a limited genetic background, then there is little more that can be reasonably expected.

3.4 Number of seeds per plant and total quantity of seeds in sample

The number of seeds collected from each plant has genetic implications under some circumstances (see section 3.3) and also influences the total quantity of seeds in the sample.

The survival of most plant populations depends on seeds from one year being available in the following year(s). This is most acute in annual species and is least acute in long-lived perennial species. In order to minimise risks to the future survival of plant populations, and particularly in the case of endangered species in small populations, **collect no more than 20% of the total mature seeds available** on the day of collection (see Way, 2003). Also, avoid repeat collections of a species from the same site for two consecutive years unless you reduce the quantity of seed taken to a level well below the 20% limit in each year. Guerrant *et al.* (2004) encourage low levels of sampling spread across several seasons. Obviously, these rules do not apply if the population is about to be destroyed (e.g., by building).

Try to collect enough seeds (see Appendix 6) to maintain a collection without the need for seed multiplication (with its attendant problems of genetic selection, cost and loss). As a general guide, try to collect **at least 5000 seeds per accession**. In the case of very small populations, think long and hard about the desirability of collection. Appendix 7 helps collectors to calculate the number of seeds based on the volume of seed collected. Where small quantities of endangered material are conserved, keep usage in routine viability monitoring to a minimum.

Figure 4 Organising collecting teams is important to ensure even sampling of each population.
(© National Botanic Garden of Belgium & © Institute of Botany, Bulgaria)

3.5 Sampling method

Sample as randomly as possible, noting that being totally random is not that easy. Because of this, for large populations in a uniform landscape, good sampling can be achieved in a more systematic way, sampling at regular intervals along transects. This might take the form of walking, say, three paces in a given direction, sampling and then repeating the exercise; if a team is involved then each member can walk a different transect. Whatever method is used, avoid biased sampling (the selection of individuals on the basis of appearance).

Ensure as even a contribution of seeds from maternal genotypes as possible. Avoid the temptation to concentrate the collection from individuals bearing most seeds as this will bias the genetics of the sample.

Where the number of plants sampled is less than 20, keep seeds from different plants separate. This will maximise the contribution of the maternal genotypes at regeneration. However, note that there will be increased curatorial workload by having many sub-samples of a collection.

Much emphasis is usually made about the spatial aspects of sampling between plants. Consideration should also be given to spatial effects of sampling within plants. There may be significant genetic and maturity differences between seeds from different parts of the inflorescence. For instance, in *Digitalis*, the seeds from the base of the inflorescence relate to earlier flowering and pollination than the ones at the top. In Apiaceae, the outer fruits will have set EARLIER than the inner ones. Sampling from across the seed head will be beneficial provided very immature or very old seeds are avoided (see section 4.2).

Figure 5
Differential maturation in *Papaver alpinum.*
(© University of Pavia)

Temporal effects may also be very significant. For instance, sampling early or late in the season will bias the genotypes conserved accordingly both with respect to the diversity of alleles and the allelic frequencies. This could have an effect on the adaptation of the sample on re-introduction. If sampling the same population several times in the same season then seed quantities collected on each occasion should be limited such that only 20% of the annual seed production is taken (see section 3.4 above). The variation between samples collected in different seasons might also be important especially in the case of annuals or short-lived species with very dynamic populations. Such repeated samples are probably best kept separate to avoid fresh seed being mixed with aged seed; they can be brought together for use or regeneration. However, collectors may not have the opportunity of repeat visits. Therefore, such bias has to be noted and accepted.

4 SEED COLLECTING TECHNIQUES

4.1 General comments

Check for empty or immature seeds prior to collecting, even though the seeds may look outwardly acceptable. Pull fruits apart, and crush or cut open a small number of seeds (see section 4.2). Families such as Fabaceae often suffer from insect-damaged seeds while Asteraceae and Poaceae regularly have empty seeds (see Appendix 8). If possible, compensate for these losses by extra collecting.

To avoid mixed collections, keep hold of a specimen for reference. If more than one person is collecting, ensure that everyone is clear of what and where they are sampling (to ensure even coverage).

Figure 6 Checking equilibrium relative humidity of a seed sample in the field using a hygrometer. (© RBG Kew)

Collect seeds into well-labelled cloth or non-glossy paper bags. Choose bags carefully. For instance, in the case of collecting dust-like seeds, a paper rather than a cloth bag may be advisable; subsequent cleaning of a cloth bag may be impossible. Similarly, extracting grass seeds with long awns from cloth bags may be a very time consuming process. If collecting in very wet locations, ensure that any paper bags will not quickly disintegrate under the conditions.

Tie cloth bags around the neck, don't just draw them closed. Fold and staple paper bags, checking seams for potential leakage. Double packaging will help avoid losses, although it may slow drying. Take bags or envelopes of different sizes. We recommend the following sizes: 7x 4; 9 x 5; 12 x 9; 19 x 11; 35 x 17; and 50 x 30 cm. Large envelopes are useful, not only for their size when collecting big seed heads but also to allow grouping together of several collections from the same locality. Bags and envelopes should be labelled inside (using tags) and out.

If possible, check the equilibrium relative humidity of the sample in the field (see Probert, 2003 and the MSBP Technical Information Sheet No. 5 at http://www.kew.org/msbp/scitech/publications/05-eRH moisture measurement.pdf). If it is above 50% or the ambient air is too moist (prevailing relative humidity at time of measurement >50%) then active drying using a desiccant is advisable (see section 6). Obviously, the time that the ambient relative humidity measurement is made will be influential. Relative humidity rises at night and drops as the day warms up. Therefore collectors need to bear this in mind when interpreting the readings. Because humidity increases at night, collections should be protected (see section 6).

Avoid plastic bags (and other sealed containers) as sweating and moisture absorption may occur (particularly at night, when it gets cooler), leading to rapid deterioration. However, see note on harvest of fleshy fruits (see section 4.3c).

Be aware that the plant parts that you may touch when collecting may be poisonous. Watch out for irritant hairs and wear gloves as appropriate.

Check clothing and shoes for attached seeds or fruits before leaving a collecting site. Collectors can unwittingly transfer seeds from one population of a species to another. This could lead to undesirable out-crossing in certain narrow endemic species.

Particularly when collecting from rare species at sites close to public areas, try to be as inconspicuous as possible to avoid attracting attention. Similarly, try to avoid heavy trampling around the collecting site that can draw attention to the rare plants.

4.2 Checking for seed maturity

A useful guide to whether the seeds are sufficiently mature is how easily they can be detached from the parent plant. Fruit colour changes may also signal maturity. For instance, many bird-dispersed fruits change to a colour (such as red) that stands out well against green foliage.

Do not collect very immature fruits and seeds. However, it may occasionally be possible (or necessary) to collect slightly immature fruits (still fairly green) and to mature these in the laboratory. Maintain fruits under fairly humid, light conditions until mature, when the seeds should be extracted and dried.

This approach has been found to be particularly useful for species with explosive seed capsules. If in doubt about maturity, examine a few seeds (internally and externally) using a hand lens; soft seed contents might indicate immaturity.

Be aware that with some species (e.g., in Crete, *Juniperus macrocarpa* and certain Campanulaceae) it is possible to encounter fruits or seeds remaining on the parent plant since the previous season.

Figure 7 Dispersal time for *Arnica montana*. (© University of Pavia)

Figure 8 Seed dispersal time for *Viola dubyana*. (© University of Pavia)

4.3 Harvesting

Select appropriate harvesting techniques according to the species:

a. Collect seeds from **dehiscent fruits** (such as siliquas, leguminous pods or capsules) directly into a cloth or paper bag or first into a washing-up-bowl / bucket and remove rough plant debris before pouring the seeds into a bag.

Figure 9 Seed heads such as this one of *Urginea maritima* can be easily emptied into a washing-up bowl or collecting bags. (© Lisbon Botanic Garden)

b. Cut **branched seed heads**, including grass panicles, using secateurs or scissors and place head first into bags. Collect awned grasses into heavy duty paper rather than cloth bags.

This approach works well for many other seed heads including Asteraceae. Collect thorny species e.g. (Onopordum) into heavy duty cloth bags. Rigid plastic bags may also be used provided the seed heads are very dry and are not kept in them for long (see section 6). You may attempt some seed cleaning in the field, but unless space is limiting, or the trip extends over many days, it is probably best to wait until the collection arrives at the seed bank laboratory.

c. Collect larger **fruits** one by one. Place fleshly fruits into plastic bags,keeping the bags open and giving fruits plenty of aeration. To avoid the danger that fleshy fruits might ferment during longer trips, either air-dry the fruits or extract the seeds.

Figure 10 *Brachypodium phoenicoides* collecting. (© Lisbon Botanic Garden)

d. Knock or shake fruits from **tall trees**, collecting them onto a canvas or similar piece of fabric laid out under the branches (see also Schmidt, 2000 where climbing techniques are also covered).

e. Adapt the latter method for smaller plants with dehiscent fruits by spreading out a large (e.g., A3 size) piece of paper under each plant. Carefully knock the seeds out onto the paper and pour them into bags.

f. Try to avoid collecting **fruits or seeds that have already fallen** on the ground. Seeds may be old (and have deteriorated badly). The seeds collected under one individual may in fact have come from one nearby (implications to sampling), or the seeds may be from a similar, non-target, species. If you have to collect from the ground, record this on the passport data form to alert seed bank staff to potentially poor germination.

g. Some ENSCONET members have found that the leaf sheaths and rosettes of small **chasmophytes** often retain seeds from other plants, thus acting as 'aerial seed banks'. If such seeds are collected, take great care to ensure that they are from the targeted species.

h. Avoid touching **orchids** fruits by hand (or even gloves) and use a razor to cut the pedicel and drop the fruit directly into a bag. Take extra care in subsequent handling because the seeds are so tiny.

i. Where only small amounts of seed mature at the same time on the seed head or there are explosive fruits, some ENSCONET members have found it useful to **secure small cloth bags over the seed head or fruit** and to return about 1 month later. Alternatively, devise other traps to catch the seed. See also suggestions under section 4.2 about maturing immature fruits. One problem arises with aquatic plants like *Nymphaea*, where fruits sink after dispersal to the bottom of ponds, where they are difficult to locate. Enveloping the flowers with a net secured to the mother plant is a simple way to keep track of the fruits and collect them easily.

j. In some circumstances where plants are not producing seeds at the time of the collecting visit, it may be possible to **remove cuttings or collect whole plants for growing on to seed harvest** under controlled circumstances in a botanic garden (see Chorlton *et al*., 2003). Obviously, this is only recommended where the land-owner has given permission and where there is no likelihood that this will threaten the future survival of the population. It should also be noted that collection of plants (with soil) instead of seed will have plant health implications.

Figure 11 Collecting seed of *Campanula merxmuelleri* using long-handled secateurs on Skyros Island in Aegean Sea.
(© University of Athens)

Figure 12 Collecting seed of *Iberis procumbens* onto a sheet of white paper.
(© Lisbon Botanic Garden)

5 PLANT IDENTIFICATION AND DOCUMENTATION

5.1 Passport data forms

Figure 13 Recording slope aspect.
(© University of Pavia)

Seed without data is almost useless and so full passport information should be recorded about each seed collection. In addition to providing data about the provenance and sampling of the seed collection (see Appendix 1 - mandatory fields are shaded in grey and ENSCONET data schema), it is important to bear in mind that information such as the number of plants found at the site is useful information for long-term monitoring. Data on local uses and obvious threats should also be recorded.

It is particularly important that the data recorded is as objective as possible and that it will be easy to comprehend several decades from now. Many collectors write on paper collecting forms in the field. In time, most data recording is likely to be directly onto hand-held computers or notebooks. This reduces subsequent data handling but it is essential that computerised data is regularly backed-up in the field.

5.2 Recording the location

It is important to record the location of the collection. This can be done using a map or a Geographical Positioning System (GPS) system. Either way the map projection and datum must be recorded i.e. UTM WGS84. It is best to use the projection and datum most commonly used in your country or institute. The European Petroleum Survey Group (EPSG) holds a dataset of parameters for coordinate reference systems. EPSG codes for commonly used projections and datums are listed in the section “Codes for use in Passport Data Form”.

Remember that GPS receivers cannot be used under dense tree covering or at the bottom of deep valleys. Rely on a detailed map under these circumstances. Alternatively, it is possible to pinpoint the collection site to latitude, longitude and altitude using Google Earth (http://earth.google.com/download-earth.html) or Google Maps (http://maps.google.com).

5.3 Specimen identification / verification

Within Europe, it is helpful if a common taxonomy is used for the seed collecting of the continent’s wild flora. Use *Flora Europaea* (now available in electronic format and searchable via http://rbg-web2.rbge.org.uk/FE/fe.html) as far as possible. Only use national, regional or local monographs or floras if the taxonomy has been recently revised and is more up-to-date than *Flora Europaea.*

Figure 14 Checking field identification.
(© Trento Museum)

Collection of herbarium specimens before or during seed harvest allow the verification of identifications made by the collector (or subsequent identifiers) and

establish reference samples that are unambiguous and that permit future verification. Ideally, these specimens should be accompanied by high-quality images of the plant in the field.

Figure 15 Standard herbarium specimen equipment. (© RBG Kew)

These specimens should represent the majority of the identifying characters of the plant and be typical of the plants from which the seed was collected. Details of collecting herbarium specimens are described by Bridson & Forman (1998). If possible, try to collect more than one specimen to allow duplication in other herbaria. Ideally, collect these specimens from separate plants and label them as such, thus providing scientists in the future with information on intra-population variation. In the case of parasitic plants, it may be important to additionally collect a herbarium specimen (and seed) of the host.

Avoid unnecessary damage to populations and especially endangered ones. It may sometimes be difficult to obtain entire specimens. Under these circumstances, simply collect a part of the plant, or use the remains of the specimen used for seed extraction (frequently the case in therophytes) making sure that the material shows sufficient distinct characters for identification, As a last resort, record the reference number of a voucher previously taken from the same population and already stored in a herbarium. Accompany this number with field pictures, of sufficient quality and detail, which can be used to identify the accession.

Herbarium specimens relating to the seed collections must be verified and a record kept (both on the specimen and the seed bank database) of the verifier and their institute.

If the species is naturalised or re-introduced at the collecting site, this should be noted in the notes field of the collecting form.

5.4 Soil samples

It may be helpful to collect a soil sample from near the base of the plant in the case of species that have symbiotic relationships with soil micro-organisms (e.g., orchids, legumes and some trees). This will allow the symbiotic relationship to be re-established when growing out the seeds. In most cases, this is helpful rather than essential. Of course, it is important to label the soil samples with the seed accession identification number.

There are two points to consider when collecting soil samples. Firstly, the movement of soil is strictly controlled by plant health legislation. It is essential to contact the appropriate plant health authorities to check that what you are proposing is not subject to controls. Secondly, if the soil sample is to be conserved for any length of time, then guidance from microbiologists or environmental specimen banks will be needed.

6 CARE OF THE COLLECTIONS AFTER HARVEST

Taking care of the collections after collection and before they are processed for long-term storage is absolutely essential. Significant losses in seed viability may occur if seeds are badly treated. This might be sufficiently serious to translate into the difference between keeping seeds for a decade instead of a century.

Collected samples can be pre-cleaned in the field to evaluate their quantity and to prepare them for transporting. Most cleaning is best left until the seeds are returned to the seed bank where specialist equipment will be available. However, the best technique for pre-cleaning is by using a light plastic washing-up-bowl (easy to carry) where debris can be removed (e.g., by gentle blowing) and the seeds extracted into a paper bag or envelope (see section 4.3a).

It is better to transport seeds and not fruits, except when they cannot be easily separated or time is limited. In the case of fleshy fruits, it might be possible to eliminate the flesh by gently macerating them in water and then air dry or use silica gel. If it is necessary to transport fleshly fruits, place them in plastic bags that are either left open or (where this is impractical) closed with plenty of fresh air. During transportation and until they reach the seed bank, seeds must be stored in permeable containers that allow them to air dry (paper / cloth bags inside larger cloth bags, cardboard boxes or wicker baskets). Avoid the use of plastic bags to hold seeds.

If transportation back to the seed bank will take several days, it is advisable to dry the seed over silica gel, dried rice or charcoal inside sealed plastic boxes. This is particularly important if the average outside relative humidity (or the seed equilibrium relative humidity as determined by a hygrometer) is greater than 50% (see section 4.2). If using silica gel, it is advisable to use a ratio of about 3:1 (fresh desiccant: seed). The seed should be in close proximity to the desiccant. This said, there should be some air around the seeds. All containers with seeds should be placed inside the vehicle away from the direct sun to avoid high temperature. It should be noted that at night the humidity might rise within the vehicle if the temperature drops significantly. Under these conditions, dried seeds might pick up moisture and should therefore be protected (e.g., by taking them into an air conditioned room if available overnight). If collecting over several days from a single base, leave the collections in a cool dry location (such as an air conditioned hotel room) rather than carrying them around in the vehicle.

Figure 16 Drums of silica gel can be used in the field to dry seed. (© RBG Kew)

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Appendix 1 Passport (Collecting) Data Form

FIELDS IN GREY ARE MANDATORY

Accession ID						**Collection Number** *(same as bag number)*	
Collection date		YYYY	MM	DD			
Main Collector Surname & First Name *(CAPITALS)*						**Institution**	
Other collectors Names and Institutions							
Taxon name							
Vernacular name(s) (+ language)							
Herbarium Voucher	Yes/No Number:	**Number of mature plants found** (tick one)	1..... 2-5..... 5-10..... 10-25.... 25-50.... 50-100.. 100-1000..... 1000+...		**Number of plants sampled** (tick one)	1..... 2-5..... 5-10..... 10-25.... 25-50.... 50-100.. 100-1000..... 1000+...	**Phenology status** *(tick one)*
Soil sample	Yes/No Number:						More flowers than fruits……. More fruits than flowers……. Only fruits…….... Fruits already dispersed…...
Sampling Method *(tick one)*	Random..... Regular..... Transect (linear).... Core of population. Edge of population. Other.....						
Sampling area visited (m x m)					**Seeds / fruits collected from ground?** YES NO Partially		
Photos *(give references)*							
Country					**Primary subdivision**		
Secondary subdivision *(council, municipality…)*							
Locality							
Latitude Y		**Longitude X**		**Units** (tick one)	Degrees Meters	**EPSG Code** (see codes)	
Altitude (m)		**Water depth** (aquatics) (m)				**Altitude Accuracy** (m)	

Geocode provided by collector?	**Geocode Method** *(tick one)*	**Altitude Method** *(tick one)*	**Prevalent Aspect** *(tick one)*	**Slope** *(tick one)*	**Soil texture** *(tick one)*	**Soil pH** *(tick one)*
Yes	GPS	Altimeter	N	Level 0-5%	Gravel	Acidic
No	DGPS	DEM	N-E	Undulating 6-10%	Sand	Alkaline
	Estimate	GPS	E	Rolling 11-20%	Sandy loam	Neutral
	Map	Estimate	S-E	Moderate 21-31%	Loam	
	Google Earth	Map	S	Steep >30%	Clay loam	
			S-W		Clay	
			W		Peat	
			N-W		No soil	

EUNIS Habitat Code *(see codes)*		**Land Use Code** *(see codes)*	**Threats**

Site Notes
(observations or any relevant information)

Associated species
(SPECIFY 3-5 rare or abundant species)

Collecting Notes
(e.g. problems encountered, collecting method, estimate of seed nos., flower colour etc)

Appendix 2 Codes for use in Passport Data Form

I. EPSG (European Petroleum Survey Group) CODES

The EPSG registry (http://www.epsg-registry.org/) holds all codes and descriptive information relating to each one. All EPSG codes can be returned by running a blank search. Codes relating to a particular country can be retrieved from this database using searching by area.

II. EUNIS HABITAT LAND CODES – for Europe

For key and descriptions – see http://eunis.eea.europa.eu/habitats-code.jsp

A: Marine habitats	
A1	Littoral rock and other hard substrata
A2	Littoral sediment
A3	Infralittoral rock and other hard substrata
A4	Circalittoral rock and other hard substrata
A5	Sublittoral sediment
A6	Deep-sea bed
A7	Pelagic water column
A8	Ice-associated marine habitats

B: Coastal habitats	
B1	Coastal Dunes and Sandy Shores
B2	Coastal Shingle
B3	Rock cliffs, ledges and shores, including the supralittoral

C: Inland surface waters	
C1	Surface standing waters
C2	Surface running waters
C3	Littoral zone of inland surface waterbodies

D: Mires, bogs and fens	
D1	Raised and blanket bogs
D2	Valley mires, poor fens and transition mires
D3	Aapa, palsa and polygon mires
D4	Base-rich fens and calcareous spring mires
D5	Sedge and reedbeds, normally without free-standing water
D6	Inland saline and brackish marshes and reedbeds

E: Grasslands and lands dominated by forbs, mosses or lichens	
E1	Dry grasslands
E2	Mesic grasslands
E3	Seasonally wet and wet grasslands
E4	Alpine and subalpine grasslands
E5	Woodland fringes and clearings and tall forb stands
E6	Inland salt steppes
E7	Sparsely wooded grasslands

F: Heathland, scrub and tundra	
F1	Tundra
F2	Arctic, alpine and subalpine scrub
F3	Temperate and mediterranean-montane scrub
F4	Temperate shrub heathland
F5	Maquis, arborescent matorral and thermo-Mediterranean brushes
F6	Garrigue
F7	Spiny Mediterranean heaths (phrygana, hedgehog-heaths and related coastal cliff vegetation)
F8	Thermo-Atlantic xerophytic scrub
F9	Riverine and fen scrubs
FA	Hedgerows
FB	Shrub plantations

G: Woodland, forest and other wooded land	
G1	Broadleaved deciduous woodland
G2	Broadleaved evergreen woodland
G3	Coniferous woodland
G4	Mixed deciduous and coniferous woodland
G5	Lines of trees, small anthropogenic woodlands, recently felled woodland, early-stage woodland and coppice

H: Inland unvegetated or sparsely vegetated habitats	
H1	Terrestrial underground caves, cave systems, passages and waterbodies
H2	Screes
H3	Inland cliffs, rock pavements and outcrops
H4	Snow or ice-dominated habitats
H5	Miscellaneous inland habitats with very sparse or no vegetation
H6	Recent volcanic features

I: Regularly or recently cultivated agricultural, horticultural and domestic habitats	
I1	Arable land and market gardens
I2	Cultivated areas of gardens and parks

J: Constructed, industrial and other artificial habitats	
J1	Buildings of cities, towns and villages
J2	Low density buildings
J3	Extractive industrial sites
J4	Transport networks and other constructed hard-surfaced areas
J5	Highly artificial man-made waters and associated structures
J6	Waste deposits

X: Habitat complexes

B. LAND USE CODES

L1: Agriculture	
L1.1	Pasture
L1.2	Fallow
L1.3	Crop
L1.4	Grassland
L1.5	Forest
L1.6	Woodland
L1.7	Protected enclosure

L2: Commercial	
L2.1	Draining
L2.2	Dumping
L2.3	Aquaculture
L2.4	Quarrying / Mining
L2.5	Industrial
L2.6	Peat cutting

L3: Grazing	
L3.1	Light
L3.2	Moderate
L3.3	Severe

L4: Leisure	
L4.1	Walking
L4.2	Fishing
L4.3	Hunting
L4.4	Golf
L4.5	Sports pitch
L4.6	Camping
L4.7	Horse riding
L4.8	Cycling

Appendix 3 Examples of time between flowering and fruiting in European species

Species	Average Flower Month (1 = January)	Average Fruit Month (1 = January)	Difference in months	Flora and ref.
Woody species				
Acer campestre	5.5	9.5	4	British Isles ref [1]
Arbutus unedo	11	11	12	Mediterranean ref[2]
Betula nana	5	7	2	British Isles ref [1]
Buxus sempervirens	4.5	9	4.5	British Isles ref [1]
Calicotome villosa	3.5	7	3.5	Mediterranean ref[2]
Cistus albidus	4	7	3	Mediterranean ref[2]
Cistus ladanifer	4	7	3	Mediterranean ref[2]
Cistus monspeliensis	4	7	3	Mediterranean ref[2]
Cistus salvifolius	4	7	3	Mediterranean ref[2]
Coronilla valentina	4	6.5	2.5	Mediterranean ref[2]
Daphne mezereum	3	8.5	5.5	British Isles ref [1]
Hippophae rhamnoides	3.5	9	5.5	British Isles ref [1]
Ilex aquifolium	6.5	12	5.5	British Isles ref [1]
Juniperus communis	5.5	9.5	4	British Isles ref [1]
Phillyrea angustifolia	3	11	8	Mediterranean ref[2]
Phillyrea latifolia	3	11	8	Mediterranean ref[2]
Pistacia lentiscus	3	11.5	8.5	Mediterranean ref[2]
Ribes alpinum	4.5	7	2.5	British Isles ref [1]
Salix alba	4.5	7	2.5	British Isles ref [1]
Salix arbuscula	5.5	6	0.5	British Isles ref [1]
Salix cinerea	3.5	5.5	2	British Isles ref [1]
Salix lanata	6	7	1	British Isles ref [1]
Salix lapponum	6	7.5	1.5	British Isles ref [1]
Salix myrsinifolia	4.5	5.5	1	British Isles ref [1]
Salix myrsinites	5.5	6.5	1	British Isles ref [1]
Salix pentandra	5.5	6.5	1	British Isles ref [1]
Salix purpurea	3.5	5	1.5	British Isles ref [1]
Salix reticulata	6.5	7.5	1	British Isles ref [1]
Salix triandra	4.5	6	1.5	British Isles ref [1]
Salix viminalis	3	4.5	1.5	British Isles ref [1]
Sorbus anglica	5	9	4	British Isles ref [1]
Sorbus devoniensis	5.5	9	3.5	British Isles ref [1]
Sorbus minima	5.5	9	3.5	British Isles ref [1]
Sorbus rupicola	5.5	9	3.5	British Isles ref [1]
Sorbus subcuneata	5.5	9	3.5	British Isles ref [1]
Sorbus torminalis	5.5	9	3.5	British Isles ref [1]
Sorbus vexans	5	9	4	British Isles ref [1]
Ulmus glabra	2.5	5.5	3	British Isles ref [1]
Ulmus minor	2.5	5.5	3	British Isles ref [1]
Average for woody species			**3.5**	

	Average Flower Month (1 = January)	Average Fruit Month (1 = January)	Difference in months	Flora and ref.
Non-woody species				
Arum italicum	4.5	8.5	4	British Isles ref [1]
Atriplex littoralis	7.5	8.5	1	British Isles ref [1]
Atriplex portulacoides	8	9.5	1.5	British Isles ref [1]
Blysmus compressus	6.5	8.5	2	British Isles ref [1]
Carex acutiformis	6.5	7.5	1	British Isles ref [1]
Carex appropinquata	5.5	6.5	1	British Isles ref [1]
Carex atrofusca	7	9	2	British Isles ref [1]
Carex caryophyllea	4.5	6.5	2	British Isles ref [1]
Carex curta	7.5	8.5	1	British Isles ref [1]
Carex diandra	5.5	6.5	1	British Isles ref [1]
Carex distans	5.5	6.5	1	British Isles ref [1]
Carex disticha	6.5	7.5	1	British Isles ref [1]
Carex divisa	5.5	7.5	2	British Isles ref [1]
Carex echinata	5.5	6.5	1	British Isles ref [1]
Carex extensa	6.5	7.5	1	British Isles ref [1]
Carex filiformis	5.5	6.5	1	British Isles ref [1]
Carex flava	6	7	1	British Isles ref [1]
Carex hirta	5.5	6.5	1	British Isles ref [1]
Carex hostiana	6	7	1	British Isles ref [1]
Carex humilis	4	6	2	British Isles ref [1]
Carex lachenalii	6.5	7.5	1	British Isles ref [1]
Carex laevigata	6	7.5	1.5	British Isles ref [1]
Carex limosa	5.5	7.5	2	British Isles ref [1]
Carex magellanica	5.5	6.5	1	British Isles ref [1]
Carex maritima	6	7	1	British Isles ref [1]
Carex microglochin	7.5	8.5	1	British Isles ref [1]
Carex ornithopoda	5	6.5	1.5	British Isles ref [1]
Carex panicea	5.5	6.5	1	British Isles ref [1]
Carex paniculata	5.5	7	1.5	British Isles ref [1]
Carex pendula	5.5	6.5	1	British Isles ref [1]
Carex punctata	6.5	7.5	1	British Isles ref [1]
Carex rariflora	6	7	1	British Isles ref [1]
Carex rostrata	6.5	7.5	1	British Isles ref [1]
Carex rupestris	6.5	7.5	1	British Isles ref [1]
Carex strigosa	5.5	8.5	3	British Isles ref [1]
Carex sylvatica	6	8	2	British Isles ref [1]
Carex vaginata	7	8.5	1.5	British Isles ref [1]
Carex vesicaria	6	7	1	British Isles ref [1]
Carex vulpina	5.5	6.5	1	British Isles ref [1]
Cicendia filiformis	8	9	1	British Isles ref [1]
Cladium mariscus	7.5	8.5	1	British Isles ref [1]
Eleocharis multicaulis	7.5	9	1.5	British Isles ref [1]
Eleogiton fluitans	7.5	8.5	1	British Isles ref [1]
Galanthus nivalis	2	6	4	British Isles ref [1]

	Average Flower Month (1 = January)	**Average Fruit Month** (1 = January)	**Difference in months**	**Flora and ref.**
Gladiolus illyricus	4	7	3	Mediterranean ref[2]
Hydrocotyle vulgaris	7	8.5	1.5	British Isles ref [1]
Kobresia simpliciuscula	6.5	7.5	1	British Isles ref [1]
Medicago arabica	6	7	1	British Isles ref [1]
Medicago polymorpha	6.5	7.5	1	British Isles ref [1]
Menyanthes trifoliata	6	8	2	British Isles ref [1]
Narcissus bulbocodium subsp. *bulbocodium*	3	4	1	Mediterranean ref[2]
Narcissus jonquilla	4	5.5	1.5	Mediterranean ref[2]
Narcissus papyraceus	2	4	2	Mediterranean ref[2]
Narcissus pseudonarcissus	3	6	3	British Isles ref [1]
Primula elatior	4.5	7	2.5	British Isles ref [1]
Primula vulgaris	2.5	5.5	3	British Isles ref [1]
Rhynchospora fusca	5.5	8.5	3	British Isles ref [1]
Ruscus aculeatus	2.5	9	6.5	British Isles ref [1]
Salsola kali	8	9	1	British Isles ref [1]
Sarcocornia perennis	8.5	10	1.5	British Isles ref [1]
Schoenoplectus lacustris	6.5	8.5	2	British Isles ref [1]
Schoenoplectus tabernaemontani	6.5	8.5	2	British Isles ref [1]
Scirpus sylvaticus	6.5	7.5	1	British Isles ref [1]
Thesium humifusum	7	8	1	British Isles ref [1]
Thymus pulegioides	7.5	9	1.5	British Isles ref [1]
Trichophorum cespitosum	5.5	7.5	2	British Isles ref [1]
Tulipa sylvestris	4	6.5	2.5	Mediterranean ref[2]
Vaccinium myrtillus	5	8	3	British Isles ref [1]
Vaccinium oxycoccos	7	9	2	British Isles ref [1]
Vaccinium uliginosum	5.5	8.5	3	British Isles ref [1]
Vaccinium vitis-idaea	7	9	2	British Isles ref [1]
Viscum album	3	11.5	8.5	British Isles ref [1]
Average for non-woody species			**1.8**	

Ref 1. Clapham, Tutin & Moore (1987).
Ref 2. Field observations, Jardim Botânico / Botanical Garden, Museus da Politécnica, R. Escola Politécnica 58, 1269-102 Lisboa, PORTUGAL (2009)

Appendix 4 Checklist of field equipment

General documents

Permits and authorisation in addition to personal and vehicular documentation

Clothes

Include suitable footwear for the terrain, water-proofs and a hat.
Gloves without finger ends are useful in cold locations

Navigation

Maps
Global positioning system (GPS) and batteries
Compass
Altimeter

Safety

Mobile phone and charger, two-way radio
Water – in hot countries inside a portable cool box
First aid kit
Sun protection
Insect repellent
Water bottles / hot water flask (for cold locations)
A spare set of vehicle keys

Habitat and species identification

List of targeted species
Floras and field guides
Magnifying lenses (10x, 20x)
EUNIS Habitat classification document (see Appendix 2)

Figure 17 General seed collecting equipment. (© RBG Kew)

Seed / herbarium specimen collecting equipment

- Backpack
- Passport data forms
- Camera and batteries (film if needed)
- Binoculars
- Different sizes of paper, cloth and plastic bags (but see note under section 4.1)
- Label tags
- Stapler
- Washbasins/sieves/tray (metal to reduce static problems)
- Large sheet of white paper
- Forceps and mounted needles
- Scissors and secateurs (long-handled pruner e.g., for collecting chasmophytes)
- Leather gloves
- Clipboard, field notebook, voice recorder or handheld computer (PDA)
- Pencils and permanent markers
- Pocket knife
- Trowel and containers for soil samples
- Measuring tape
- Silica gel (for seed drying and also useful when collecting samples for DNA extraction)
- Large plastic bags to store herbarium material for a few hours
- Large folders for pressing herbarium specimens
- Portable Press
- Newspapers for drying herbarium specimens

Others

- Car (4x4, with sufficient storing capacity and spare parts)
- Sunglasses
- Flashlight

Appendix 5 Summary of sampling recommendation

Collecting	Outcrossing	Selfing / apomict
Number of populations	Few	Many
Number of individuals	Many	Few
Number of fruits / seeds per individual	Many	Few

Appendix 6 Guide to seed numbers that might be required for a collection

Extrapolated from Way (2003)

Seed collection	5000
Base sample of viable seeds sufficient to maintain diversity (assume out-breeder and maintaining allelic frequencies of sampled population)	1000
Seed loss through storage (assume 100% initial viability and 75% regeneration standard)	1250
Seeds for duplication (assume minimum of 3 attempts at regeneration using 200 seeds)	600
Monitoring (assume non-destructive moisture monitoring and 2x50 seed initial germination test and then tests based on 50 seeds every 10 years up to 100 years	550
Seeds for distribution to users	1600

Appendix 7 The volume of cleaned seeds that contain at least 5000 seeds

Length of the seeds	Total Volume	Examples
< 1 mm	5 cm^3 aprox.	*Sedum, Saxifraga*
1-3 mm	10 cm^3 aprox.	*Biscutella, Thymus, Trifolium*
3-5 mm	25 cm^3 aprox.	*Salvia, Pistacia*
5-10 mm	75 cm^3 aprox.	*Retama, Ferula*
> 10 mm	Estimate individual number	*Quercus, Pinus*

Appendix 8 Incidence of empty and insect-damaged seeds found in 4070 European seed collections from different plant families (data from Millennium Seed Bank, RBG Kew)

Family	No. of seed collections	% of collections with empty seeds	% of collections with insect damage
Aceraceae	7	100.0	0.0
Anacardiaceae	3	100.0	0.0
Aquifoliaceae	3	100.0	0.0
Araceae	1	100.0	0.0
Celastraceae	2	100.0	0.0
Cornaceae	6	100.0	16.7
Cupressaceae	12	100.0	8.3
Cynomoriaceae	1	100.0	0.0
Fagaceae	1	100.0	0.0
Globulariaceae	5	100.0	40.0
Hippuridaceae	2	100.0	0.0
Rutaceae	2	100.0	0.0
Zannichelliaceae	2	100.0	0.0
Betulaceae	14	92.9	7.1
Rhamnaceae	8	87.5	0.0
Grossulariaceae	4	75.0	0.0
Juncaginaceae	4	75.0	0.0
Myricaceae	4	75.0	0.0
Oleaceae	8	75.0	50.0
Santalaceae	4	75.0	0.0
Verbenaceae	4	75.0	0.0
Rosaceae	213	74.2	14.6
Illecebraceae	14	71.4	0.0
Typhaceae	13	69.2	0.0
Balsaminaceae	3	66.7	0.0
Corylaceae	3	66.7	33.3
Menyanthaceae	3	66.7	0.0
Molluginaceae	3	66.7	0.0
Potamogetonaceae	24	66.7	4.2
Dipsacaceae	22	59.1	36.4
Cistaceae	32	56.3	21.9
Onagraceae	32	56.3	3.1
Lamiaceae	163	54.6	11.7
Poaceae	383	53.8	8.6
Alliaceae	28	53.6	0.0
Malvaceae	28	53.6	21.4
Cyperaceae	193	51.3	5.2
Boraginaceae	45	51.1	2.2
Apiaceae	234	50.9	24.8

Family	No. of seed collections	% of collections with empty seeds	% of collections with insect damage
Adoxaceae	2	50.0	0.0
Amaranthaceae	6	50.0	0.0
Berberidaceae	2	50.0	50.0
Buxaceae	2	50.0	0.0
Cannabaceae	2	50.0	50.0
Colchicaceae	2	50.0	50.0
Empetraceae	2	50.0	0.0
Plumbaginaceae	22	50.0	9.1
Polemoniaceae	4	50.0	0.0
Staphyleaceae	2	50.0	50.0
Polygonaceae	56	48.2	3.6
Asteraceae	440	47.7	14.8
Liliaceae	15	46.7	6.7
Valerianaceae	16	43.8	0.0
Violaceae	19	42.1	0.0
Ericaceae	34	41.2	2.9
Chenopodiaceae	149	40.9	1.3
Ranunculaceae	129	37.2	9.3
Clusiaceae	38	34.2	0.0
Asclepiadaceae	3	33.3	0.0
Cucurbitaceae	3	33.3	33.3
Geraniaceae	24	33.3	8.3
Paeoniaceae	3	33.3	100.0
Parnassiaceae	3	33.3	0.0
Thymelaeaceae	3	33.3	33.3
Ulmaceae	3	33.3	0.0
Rubiaceae	40	32.5	7.5
Plantaginaceae	13	30.8	15.4
Caprifoliaceae	17	29.4	0.0
Alismataceae	24	29.2	4.2
Asparagaceae	7	28.6	0.0
Convolvulaceae	14	28.6	21.4
Melanthiaceae	7	28.6	0.0
Resedaceae	7	28.6	14.3
Lythraceae	11	27.3	9.1
Brassicaeae	194	26.8	7.7
Callitrichaceae	4	25.0	0.0
Convallariaceae	4	25.0	0.0
Dioscoreaceae	4	25.0	0.0
Tamaricaceae	4	25.0	25.0
Euphorbiaceae	17	23.5	5.9
Caryophyllaceae	235	21.3	8.1
Papaveraceae	40	20.0	12.5

Family	No. of seed collections	% of collections with empty seeds	% of collections with insect damage
Primulaceae	45	20.0	0.0
Fabaceae	266	17.7	18.0
Scrophulariaceae	188	17.6	3.7
Amaryllidaceae	23	17.4	13.0
Campanulaceae	69	17.4	5.8
Linaceae	23	17.4	13.0
Crassulaceae	18	16.7	0.0
Solanaceae	18	16.7	0.0
Iridaceae	13	15.4	15.4
Urticaceae	7	14.3	0.0
Juncaceae	67	13.4	1.5
Gentianaceae	55	12.7	7.3
Hyacinthaceae	24	8.3	16.7
Droseraceae	15	6.7	0.0
Saxifragaceae	31	6.5	0.0
Araliaceae	4	0.0	25.0
Aristolochiaceae	1	0.0	100.0
Asphodelaceae	4	0.0	25.0
Butomaceae	2	0.0	0.0
Capparaceae	1	0.0	0.0
Ebenaceae	1	0.0	0.0
Elaeagnaceae	2	0.0	50.0
Elatinaceae	2	0.0	0.0
Eriocaulaceae	2	0.0	0.0
Frankeniaceae	1	0.0	0.0
Haloragaceae	2	0.0	0.0
Hemerocallidaceae	1	0.0	0.0
Lentibulariaceae	5	0.0	0.0
Nymphaeaceae	1	0.0	100.0
Orchidaceae	1	0.0	0.0
Oxalidaceae	1	0.0	100.0
Pinaceae	2	0.0	0.0
Polygalaceae	2	0.0	0.0
Portulacaceae	3	0.0	0.0
Ruscaceae	1	0.0	0.0
Salicaceae	4	0.0	0.0
Taxaceae	1	0.0	0.0
Tiliaceae	2	0.0	0.0
Trilliaceae	2	0.0	0.0
Zygophyllaceae	1	0.0	0.0