

Chapter

9

Collecting Seed from Non-domesticated Plants for Long-Term Conservation



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Summary

Seed collections made from non-domesticated plant populations for long-term conservation must be of the highest achievable quality, quantity and utility. To achieve this, collectors must make full use of published and local knowledge, must use a robust protocol which maximises the collection of high quality seed and associated herbarium vouchers, and must gather comprehensive field data which can be made available to future users. It is important that the seed collection represents, as far as possible, the genetic diversity of the target population and that the species and population from which sampling takes place are of priority and relevance to the particular project. The most appropriate sampling strategy for any given situation can be decided by reference to the ecology and distribution of the species; geography of the collecting region; likely breeding system and pollinators; natural seed dispersal mechanism; and seed quality indications. Seeds should be carefully sectioned, examined and assessed in the field for probable viability prior to collection. Allowing for all fieldwork tasks, an experienced fieldwork team may achieve a long-term average of between two and three large and high-quality seed collections *per* field day, making a massive contribution to the conservation of plant genetic diversity.

There is no substitute for common sense, based on biological knowledge, to guide the collection of seeds of wildland plants. (Young and Young, 1986)

Planning a Seed Collecting Programme

1. Introduction

Seed collecting is one of the most practical and effective ways to sample plant genetic diversity, and the resulting seed samples can become a valuable resource for species conservation, habitat restoration, plant breeding, and wider biological science.

Seed collecting is one form of germplasm collecting, which is usually undertaken to meet one of the following general purposes (Engels *et al.*, 1995):

- Immediate or expected need for propagation, trialling or research. Germplasm provides the material with which communities, farmers, foresters and scientists can maintain and develop crops, plantations, and breeding lines. If useful germplasm is missing or lost from existing collections, then there is often an immediate need to fill any gaps by additional collecting.

- Conservation purposes (e.g., rescue collecting), in the event of actual or threatened erosion of genetic diversity from plant populations of interest. Although there may not be any immediate demand from users for this germplasm, experience suggests that potentially valuable germplasm should be conserved before it is lost. Seed from taxa tolerant of desiccation and cold-storage (i.e., ‘orthodox’ seeds) can be conserved for many decades without measurable loss of viability. These collections additionally provide long-term insurance against loss of the *in situ* population due to disease, habitat destruction or other catastrophe.

To be a valuable long-term resource, a collection of germplasm needs to have the following attributes:

- Priority germplasm that has been accurately identified.
- Genetically representative of the species/population/individual sampled.
- High viability with acceptable longevity.
- Sufficient germplasm to provide to intended users.
- Acquired with all necessary consents and data to facilitate the intended uses.

This paper makes practical recommendations for collecting seed intended for long-term seed bank conservation. Advice is not included about desiccation- or low-temperature-sensitive (i.e., ‘recalcitrant’) seeds for which more specific recommendations (for tree species) are given in Thomsen (2000). Germplasm conservation from domesticated plant species is well described in Hawkes *et al.* (2000) although much of the general collecting advice given here is consistent with these sources.

2. Sampling Genetic Diversity

Biological diversity can be considered at the level of the ecosystem, the species or the gene, the basic unit of heritable genetic diversity. For the purposes of this chapter, attention will be concentrated on genetic diversity at the level of the species, which is of most relevance to seed collecting programmes. The variability that we observe among individuals (‘phenotypes’) results from the differences in their genetic composition (‘genotypes’) and in the interaction between the genotype and its environment. In a very few cases, collectors of non-domesticated species will have detailed genetic diversity data for the target species. However, in most cases, the collector will need to make a sampling decision based on the observed phenotypic variation, the environment, and knowledge about the nature and typical patterns of genetic diversity in plants.

2.1. Measuring genetic diversity

Our knowledge of plant genetic diversity comes principally from analysis of allelic variation. An allele is a form in which a gene may occur, and different alleles of a gene give rise to different expressions of a character. For example, different alleles for a 'flower colour' locus on a chromosome will determine whether the flower colour is red, yellow, etc. A diploid organism whose cells contain two identical alleles is termed homozygous for that character, one with two different alleles at the same locus is termed heterozygous. Several different alleles may be found at a given locus on the chromosome, and different alleles may be present in some individuals within a population and not others. Because many alleles code for particular isoenzymes (also referred to as allozymes or isozymes), cost-effective isoenzyme analysis can produce data on the number of distinct (polymorphic) alleles at a locus within a sampled species or population. This basic measure is termed 'allelic richness' (see Brown and Briggs, 1991). Obviously, when considering genetic variation, it is only possible to examine a few loci and to extrapolate from there as the true measure will be based on the average number of alleles for a large number of loci. In addition, isoenzyme data does not always identify the same patterns of variation as analysis of quantitative traits such as plant height, inflorescence length and inflorescence number (Gray, 1997) which are probably under selection. So, even if all isoenzyme genetic variation could be measured, it cannot be expected to completely reflect all the biological variation present. However, unlike direct analysis of DNA, isoenzyme analysis provides a measure of variation in the structural gene of a set of enzyme loci on the chromosomes, and it has the advantage of identifying actual protein changes and independent Mendelian polymorphisms scattered around the genome (Avisé, 1994). It therefore provides an excellent means to compare variation within species, and as an additional benefit, it is effective at handling large samples (Frankel *et al.*, 1995).

Increasingly, the simple isoenzyme studies described above are being overtaken by direct analysis of the DNA. Karp (2002) reviews the potential application of the current technologies, e.g., probe hybridisation, and PCR use with either generic or sequence-tagged primers. These techniques can yield useful information about the quantity, partitioning and evolutionary history of the genetic diversity present in the target genome, and this book includes a discussion of this work (Fay, 2003 – Chapter 5). As these analyses can yield huge quantities of data, some caution is needed in the interpretation of the information generated (Karp *et al.*, 1996), and advances in integration and analysis of molecular data sets will be required to enable these techniques to reach their full potential (Sobral, 2002).

In reality, germplasm collecting programmes can rarely afford to carry out comprehensive genetic sampling of the target taxa. If collectors need to collect on the basis of comprehensive genetic analysis, they may be faced with the dilemma that plant samples may initially have to be collected from across the species range in order to provide this detailed data. For known priority

species, collectors may therefore decide to initially make several germplasm collections. As projects develop, these initial collections can be used to provide information to refine subsequent sampling.

2.2. Distribution of genetic diversity

In the example of a large programme targeting genetic diversity at the species level, collectors will usually need to rely on broad generalisations established from analyses of patterns of variation. Useful information has been gathered by Fryxell (1957) and by East (1940) who demonstrated that out-crossing is widespread in plant species. Indeed Brown (1990), referring to Fryxell's data, estimated that only 20% of higher plant species reproduce predominantly by self-fertilization. As most of the detailed information on genetic variation is however from economically useful species, it is useful to compare the biology of crops with non-domesticated species. Marshall and Brown (1983), for example, showed that wild species (specifically forage plants) tend to differ from crop species in the following ways:

- Greater population differentiation on geographic and micro-geographic scale.
- Greater variation in flower heading, flowering and reliability of seed set.
- Greater variation in population density (i.e., they are usually in mixed communities).
- Greater range of breeding systems and a greater proportion of out-breeders.
- Greater number of perennials. Their populations show more complex age structure.

Hamrick *et al.*, (1991) assessed correlations between species traits (e.g., breeding system, life-form) and gene diversity (an estimate of the probability of two randomly-chosen alleles not being the same) measured among populations (Table 9.1). Clearly a knowledge of these traits, combined with good observation in the field, will help to predict the relative partitioning of genetic diversity in a target species. Brown and Marshall (1995) apply this understanding in a study of the effects of similar life history, ecological and genetic attributes, on sampling strategy. They highlight the effects of migration, population age structure, vegetative reproduction and fecundity. For example, at the level of the seed head, the authors note that (in contrast to wind pollination) animal pollination tends to result in fruit bearing seeds of the same male parent. Consequently, animal-pollinated out-breeders tend to show more population divergence than wind-pollinated out-breeders. Keys *et al.* (1995) also emphasised that the composition, length of flight season and relative abundance of pollinators may determine the extent to which self- or cross-pollination is achieved by a plant population.

Before developing a sampling strategy, it is also important to consider the types of alleles that can be identified in a sample. Marshall and Brown (1975)

Table 9.1 Associations between the characteristics of species and the genetic diversity among populations (from Hamrick *et al.*, 1991 with permission)

Characteristics	Proportion of genetic diversity among populations	
	Low	High
Taxonomic status	Gymnosperms	Angiosperms
Life-form	Long-lived woody perennials	Annuals
Geographical Range	No significant differences	
Regional distribution	Boreal-temperate species	Temperate and tropical species
Breeding system	Outcrossed, wind-pollinated	Selfing species
Seed dispersal	Gravity-dispersed and animal-attached seed	Gravity-dispersed seed
Mode of reproduction	No significant differences	
Successional status	Late successional species	Early and mid-successional species

classified four types of alleles (Table 9.2). This classification has underpinned much of the sampling strategy in the literature over the past 20–25 years.

In addition, it is important to distinguish between isoenzyme loci that are monomorphic (i.e., only a single allele found at a locus) or polymorphic (several alleles at a locus). Isoenzyme analysis of 406 plant taxa (Hamrick *et al.*, 1991), showed that, on average, 50% of allozyme loci in the genome are monomorphic. Furthermore, of the remaining polymorphic loci, on average, 78% of their diversity was maintained *within* populations. In combination, these figures demonstrate that the great majority of total genetic content of most plant species is present in a single ‘typical’ population (Center for Plant Conservation, 1991).

Table 9.2 The four types of alleles that can be identified in a sample (after Marshall and Brown, 1975)

Distribution of allele	Frequency of allele at a locus	
	Common (frequency >5%)	Rare (frequency <5%)
Widespread	Present even in small samples from a few populations. Probably collected whatever sampling approach is used.	Will be collected if sample sizes large enough. Choice of sampling strategy will have little effect.
Local	Local adaptations which are maintained by balanced selection and are, therefore, of interest to plant breeders and to restoration ecologists.	For example, newly arisen mutants or recombinations. May not reflect adaptation or fitness to local environment.

2.3. Developing a sampling strategy

From how many populations to sample?

The expected number of alleles in a sample increases in proportion to the logarithm of sample size. As shown in Table 9.2, initial samples will capture monomorphic and common polymorphic alleles, but alleles that are infrequent will not be fully represented in the sample. In terms of capture of alleles, there is therefore a declining 'marginal genetic benefit' as additional individuals or populations are added to the collection (Center for Plant Conservation, 1991). To practically and efficiently sample genetic diversity, it is most effective to firstly sample from the most accessible populations (Falk, 1991), but how many additional populations should be subsequently sampled? Brown and Briggs (1991) note the benefits of multi-population sampling where populations of a species have diverged into recognisable ecotypes, but the authors also recognise that sampling such additional populations of a single species may compete with conservation measures for other priority species. Brown and Marshall (1995) suggested that 50 populations per species would be ideal, but the author believes this could be potentially wasteful for the conservation of non-domesticated species. This can be illustrated with an extreme example (from Ceska *et al.*, 1996) in which isoenzyme data from ten populations of *Baptisia arachnifera* (Duncan) (*Fabaceae*) in Georgia USA suggest that 99% of the recorded isoenzyme diversity of the species could be obtained from just two selected populations.

A more achievable sampling approach is recommended by the Center for Plant Conservation (1991) which indicates that the sampling of five populations may be sufficient for representing even widespread species. This recommendation reflects advice from Brown and Briggs (1991) in the same book, where they recommend a maximum of five clusters of samples, each cluster representing one or two populations, and each cluster representing different edaphic and climatic zones. Interestingly, this sampling approach is consistent with advice from Wilkinson (2001) who suggests that similarity of habitat type may be more important than seed source proximity in determining restoration or re-introduction success.

Sampling within populations

Germplasm collecting strategies have for many years aimed to include at least one copy of 95% of the alleles that occur in the target population at frequencies greater than 5% (i.e., the 'common alleles' described above, see Marshall and Brown, 1975). This 5% limit is rather arbitrary in relation to ecological or evolutionary significance (Falk, 1991), but to either collect alleles that occur at lower frequency or to increase the certainty level drastically, increases the sample size that needs to be collected. From genetic theory, a sample representing 59 unrelated gametes is sufficient to achieve the objective. Thus the population sample should be from 30 randomly chosen individuals of an out-breeding species or 59 randomly chosen individuals of an inbreeding species (Brown and Marshall, 1995). In the light of this, and presumably

because the breeding system may be unknown, the author recommends the sampling of 50 individuals in a population as a benchmark figure.

Germplasm collectors may often achieve these figures whilst seeking sufficient seed for a useful-sized germplasm sample (see below). For example, data from the Millennium Seed Bank (MSB) collections (S. Linington pers. com.) show that 28% of the collections represent sampling from 50 or more individuals. The data also show that the average sampling level of each selected population is 70% with a median value close to 40%. In 21% of cases, sampling of the estimated population was 100% (where populations above 1,000 individuals were counted as 1,000). Sampling to represent allelic *frequencies* in the population is valuable where the collection is intended for direct use as an adapted population in a similar environment. To fully reflect the frequencies of alleles in a population, it would be necessary to increase the number of individuals sampled, to around 200 individuals (Marshall and Brown 1983). This may indeed be achievable: 10% of MSB collections represent samples from 200 or more individuals.

The genetic theory on which these recommendations are based assumes that seed sampling is carried out randomly and evenly. In practice, collectors can use one of four main sampling approaches (described in Box 9.1) to ensure that the collection is truly representative of the variation sought. For collection

Box 9.1 Sampling from individual plants within a population

There are several possible approaches to sampling individuals within the chosen population, as described by Brown and Briggs (1991).

1. Simple, random sample, by which each individual has an equal chance of being selected.
2. Stratified random sample, by which a random sample is taken from each of the distinct combinations of environmental conditions within which the population is found.
3. Systematic sampling, by which individuals are selected using a transect or grid approach, evenly spaced across the population.
4. Biased sampling, by which individuals are selected on the basis of appearance, e.g., branching pattern, performance, height, flower colour.

Biased sampling is used when selecting material for a specific breeding purpose, e.g., for agriculture, horticulture, or forestry. It is not usually appropriate for long-term conservation sampling of non-domesticated species, for which either simple, stratified or systematic sampling are all effective. Of these three, systematic sampling is the most practical for large populations in a uniform landscape, and is very effective for use by large collecting teams. Stratified sampling is optimum where the population includes contrasting soil types, aspects, moisture availability, etc. Simple random sampling can be useful for small populations in which most of the individuals will be sampled. Whatever approach is chosen, it is important that collectors record the sampling approach used.

Box 9.2 Recommended general sampling strategy for seed collections from non-domesticated plants

Goal: to obtain in a single population sample, 95% of all the alleles present in the population at a frequency greater than 5%.

- When population size permits, at least 50 individuals should be sampled in a non-biased manner. Preferably, samples should be greatly in excess of 50 as this will more closely reflect the allelic frequencies and will also maximise seed quantity.
- When assessing the number of individuals to be sampled, collectors are advised to be observant for species reproducing via rhizomes or stolons.
- If rare populations are encountered with 20 or less individuals and they are considered worthy of collection, samples from individual plants should ideally be kept separate. This will allow maximum genetic variation to be maintained in any multiplication step.
- If field observations suggest that there may be significant genetic differences between close stands of a species, either through selection or isolation, the stands should be harvested separately.
- Whatever sampling strategy is adopted, the method used should be recorded.

When should collectors increase sampling effort further?

- To reflect allelic and genotypic frequencies especially with a view to re-introduction. Note that a few populations may contain alleles that have important local adaptive value, and allele frequencies may also differ as a result of local adaptation (see Table 9.1).
- High variation or isolation observed between populations or individuals.
- Self-fertilising species.
- Herbaceous annual or short-lived perennial.

of seed from non-domesticated species for long-term conservation, either systematic or stratified-random sampling will usually prove practical for sampling from the required numbers of individuals.

A final consideration is the number of seeds that should be collected from each of the sampled individuals in the population. As a working minimum, a sample of 10 seeds per individual plant will help to ensure that each genotype is represented in the collection once losses due to storage, germination, maintenance and establishment have been allowed for. In practice, the benefits of having a large quantity of seeds in the final collection will often mean that many times this minimum quantity will need to be collected from each individual (assuming that this is possible within the safe collecting limits described later in this chapter). An additional benefit of sampling evenly from each target individual is that the collection will be truly representative of the genotypes present (which reflect adaptation to environmental conditions) and will not over-emphasise individual plants producing high quantities of seed at the time of sampling. The general sampling strategy recommended for seed collection from non-domesticated plants is shown in Box 9.2 above.

3. Targeting and Preparing a Fieldwork Programme

Before detailed logistical planning can take place, collectors will need to gather, compile and analyse information on the target taxa and the collecting region (termed ‘technical planning’ by Engels *et al.*, 1995). The resulting knowledge-base will be the foundation of a worthwhile trip to the field, and will help to ensure the usefulness of the collections made. Botanical information sources available to assist in this task include taxonomic databases, floras, monographs, conservation assessments, red lists, field guides, checklists and information from local people. The principal information sources for non-domesticated plants are listed in Prendergast (1995) and Frodin (2001). Defining priority taxa before a field trip will usually involve a great deal of research. Not only will species have to be prioritised according to various criteria (see Table 9.3 for examples) but, once selected, detailed information will be needed on localities and phenology. Herbarium data can only give the collector an estimate of when any particular species is likely to be suitable for collecting because this will vary from year to year depending on weather conditions. Where available, GPS coordinates on herbarium labels are invaluable, but descriptive locality information may become less useful through time. If possible, it is always useful to make contact with a botanist in the collecting area who can provide up to date information on potential collecting localities, and phenology of priority species.

Table 9.3 Examples of species characteristics important in prioritisation for collection and *ex situ* conservation

Species characteristics	Description
Orthodox seeds	Seeds which retain their viability after drying and cold storage, and which are therefore likely to be bankable.
Indigenous or endemic species	Species native to an area, and neither introduced nor a pan-tropical weed.
Endangered, threatened or vulnerable species	Species of restricted distribution or threatened on a local, national or global scale.
Useful species	Known to be valued/used by local people. Information may be available from databases such as SEPASAL* or in published sources.
Species targeted for research	Species required for research into germination, propagation or storage physiology.
Seed not widely available	Seed not already in seed banks or available from other sources.

* SEPASAL (Survey of Economic Plants for Arid and Semi-Arid Lands) is available online at: <http://www.rbgekew.org.uk/ceb/sepasal>

RBG Kew's Geographical Information System (GIS) Unit is currently exploring new methods of planning collecting trips, using information from herbarium sheets, digital maps and remote sensing data. It has already had some success in identifying habitats which are undergoing rapid degradation, and which are therefore a priority for seed collecting expeditions (see Moat and Smith, 2003 – Chapter 4). Kolberg (2003) – Chapter 11, has also used geographical information to prioritise collection of threatened species, in this case area and frequency of occurrence data. Further work in RBG Kew's GIS Unit will investigate the use of satellite data to provide real time information about phenology as a precursor to seed collecting missions. In addition, research into use of environmental data to predict species distributions is ongoing (Sawkins *et al.*, (1999); Moat and Smith (2003) – Chapter 4). Whatever tools are used, the most frequent result of a targeting and prioritisation exercise will include a list of target taxa that have been selected as priorities for collecting, together with information about their distribution, identification, and utility.

3.1. Logistical preparations

Following on from the targeting and prioritisation stages of planning, collectors need to prepare for one or more fieldwork trips. One of the most important factors for success is the composition of the collecting team. The following skills may be required in the team: plant identification; herbarium specimen preparation; seed physiology; seed collecting; photography; soil analysis; tree-climbing; off-road driving; camping; cooking; and translation of local languages. A provisional itinerary will also need to be agreed well before the trip, so that all equipment will be available for the agreed period and so that permission can be sought and obtained. Engels *et al.* (1995) clearly describes this process of 'logistical planning' which benefits greatly from previous collecting reports, and from discussion with residents or previous visitors to the target region. It is useful to describe these intentions in a short collection plan (e.g., the 'collecting proposal' of Engels *et al.*, 1995), which may be used to seek financial resources. It may also be useful for the important task of getting consent, and where appropriate, assistance, from the relevant authorities, landowners and other stakeholders. Box 9.3 lists the equipment that may be necessary for a seed collecting team during a short trip.

3.2. Obtaining consents and permits

As an absolute minimum, seed-collectors need to carry out their work in accordance with all local, national and international laws, and in accordance with the policies and procedures of their employing organisation. Only by careful preparation and good field-practice will collectors be able to comply with the appropriate local, national and international regulations concerning access to and transfer of plant material. Compliance with the Convention on Biological Diversity's (CBD) provisions on access to genetic resources and

Box 9.3 Equipment suggested for a two to three day seed-collecting trip

Equipment	Quantity suggested
Navigation	
Map (or photocopies)	1 per team
Compass	1 per team
Altimeter	1 per team
Global Positioning System unit	1 per team
First aid and safety	
Medical box (See Engels, <i>et al.</i> (1995) for suggestions)	1 per team
Water bottle	Each person
Plant identification	
Identification guides if available	1 set per team
Hand lens	Each person
Seed collecting and labelling	
Binoculars	2 per team
Secateurs/hand pruners	Each person
Tree pruners	1 per team
Penknife/pocket knife	Each person
Leather gloves	Each person
Collecting buckets	Each person
+ belts or rope to tie buckets around waist	Each person
Cloth (cotton) bags: sack	5 per team
Cloth bags: large, medium, small	20 of each per team
Paper bags: large and medium	10 of each per team
Cardboard envelopes	10 per team
Polythene bags large, small	10 of each per team
Numbering tags	200 per team
Herbarium specimen preparation	
Herbarium press and straps	1 per team
Filmsies or newspaper	100 sheets per team
Blotters or absorbent paper	100 per team
Portable stove and stand	1 per team (for longer trips)
Data recording	
Assessment checklist	50 per team
Field data sheets	50 per team
Camera and plenty of film	1 per team
Clipboard, field notebook, pencils	1 each person
Transport of material	
Cardboard boxes	5 per team (packed flat until required)
Adhesive parcel tape	1
String	1
Sealable container for seed drying	1 per team
Silica gel for seed drying	1 kg

benefit-sharing is described in Cheyne (2003)– Chapter 1, and the particular challenges relating to the acquisition of traditional knowledge from local communities is described in Laird and Noejovich (2002). Typically, the most successful projects reflect the interests and concerns of local, scientific and government stakeholders and incorporate consultation, participation and benefit-sharing beyond that required by the authorities. In addition to the provisions of the CBD, seed collectors need to pay strict attention to restrictions under the Convention on International Trade in Endangered Species (CITES) and Plant Health conventions which control the transfer of listed plant material from one country to another.

3.3. CITES

Seeds of all CITES Appendix 1 species, and seeds of Appendix 2 cacti species from Mexico, are currently (January 2003) subject to CITES controls. In addition, dried herbarium specimens from all Appendix 1 and many Appendix 2 species are controlled, and further measures are in place, for example, to monitor import of listed species into the European Union. It is essential that an up to date CITES list is consulted before, during and after fieldwork to ensure that no attempt is made to export controlled material without the relevant permits. Current lists of controlled taxa can be found in CITES publications or public web sites e.g., the UK CITES authority (<http://www.ukcites.gov.uk/>) or the CITES secretariat (<http://www.cites.org/>). Transfers of listed plant material between two CITES registered institutions are currently exempt from licensing provided that CITES registration labels are clearly displayed on the shipments.

3.4. Plant health

In order to reduce the threat to national agricultural, forestry and horticultural industries, imports of seed from economically important taxa may be restricted by governments under the International Plant Protection Convention. Collectors may need to obtain import permits and also phytosanitary permits from the exporting country which may require an application, visual inspection of the seeds, and payment. Sufficient time should be allowed at the end of the fieldwork to follow this procedure. Frison and Jackson (1995) give further information about plant pests and the required plant health documentation.

3.5. Invasive species

There is a significant risk that plants introduced into a new environment could become invasive and damaging. Although collectors cannot predict the ecological behaviour of non-domesticated germplasm in other environments, collectors have a clear responsibility to ensure that they do not promote the spread of invasive species. Good field practice will ensure that weeds and pests are not unknowingly moved between sites, and reference to some of the invasive plant or weed publications such as national weed lists or the Global Compendium of Weeds (<http://www.hear.org/gcw/>) will help to prepare

collectors for known invasive species that could be encountered in the field. There is also a risk that seed samples subsequently distributed from the seed bank could become invasive in other regions. For this reason it is important that collectors fully and accurately document the collection, the environment, and associated species as described in this paper.

Field Techniques

1. Identification of the Target Taxon

One of the most important tasks in the field is to confirm the identity of the target taxon before committing the team to carry out a germplasm collection. It is critical to the value of the seed collections that the species can be accurately identified, and assistance from botanists familiar with the local flora can be a great asset in the field. Floras are often incomplete and, in many cases, are more appropriate to identification in a herbarium. Most useful, where they are available, are plant identification guides or local guidebooks with plenty of colour illustrations. Photocopies or digital images of herbarium specimens may also be useful for identifying target species. If opportunistic collections are to be made, identification tools will be needed in combination with an up to date list of seed bank collections for the region and previous expedition reports.

2. Collecting High Quality Seeds

Seed collections for long-term conservation should be made at the optimum stage of development, and should be substantially free from insect damage or empty seeds. These two components of seed quality can easily be assessed by collectors in the field, and will be discussed in more detail below.

2.1. Timing of seed harvesting

Hay and Smith (2003) – Chapter 6, describe the importance of selecting the optimum seed harvesting time in order to maximise longevity in storage. At the optimum stage of development, orthodox seeds will have acquired desiccation tolerance and yet will not have lost viability due to ageing. The optimum harvesting stage can be assessed by observing the phenology and seed development of individuals within the plant population, and data can be systematically recorded using a pre-collection checklist e.g., Box 9.4.

In contrast to crop species, the timing of harvest from non-domesticated plant species presents several challenges for the seed collector. Among their many characteristics, non-domesticated species may present:

Box 9.4 Example of a checklist for assessing a potential seed collection

Taxon identified, and apparently similar taxa distinguished? Yes/No

Approx. area of population: X(metres, km)

Approx total number of individual plants present and accessible:
 1–10, 11–50, 51–100, 101–1,000, > 1,000

Is there any evidence that seeds have been disturbed/damaged by fire, herbicide etc? Yes/No

Do distinct sub-populations exist? Yes/No

(If yes, consider how to sample separately or from the most suitable)

Assessing readiness for collection (use the following indicators)

Seed colour change

Seed hardness

Fruit development, colour, texture, odour (e.g., ripe fleshy fruits for animal dispersal)

Detachment of seed within the fruit (e.g., rattling of *Prosopis* sp. and similar legumes)

Detachment of the dispersal unit from the mother plant

Readiness of population for collecting: give percentages or circle the most frequently occurring state:

Vegetative/In flower/Immature seeds/Around natural dispersal/Post dispersal

Estimate the number of individual plants at natural dispersal stage:

Physical quality and availability of seed

From a sample of seed, give % or circle the most frequent condition:

Filled seeds *Empty seeds* *Infested seeds* *Other damage*

Average number of seeds per fruit/dispersal unit:

Average number of fruit/dispersal units per individual:

Estimate the number of potentially viable seeds collectable if 20% of the available seed is sampled:

Sampling approach to be used:

Simple and random

Stratified random

Systematic

Biased sampling

- Population heterogeneity. Due to genetic and environmental variation, a non-domesticated plant population may display varied phenology and physiology. In this context, environmental cues such as rainfall events may not result in an identical and synchronous response from the individual plants, which may commence flower and fruit development on different dates.
- Indeterminate inflorescences, i.e., the flower stalk continues to grow after onset of flowering so that several stages of development are evident on a single individual. Diagrams in Judd *et al.* (1999) illustrate inflorescence-types in terms of flowering sequence.
- Shattering seed heads, i.e., seed may be freely liberated from the seed head, and in addition, may be shed over a long time-period.

It is necessary to carefully assess the phenology and physical seed quality of any given population prior to collecting in order to ensure that the collecting is likely to be successful. Smith (1995) noted that the use of fruit colour-changes as a morphological marker for collection-readiness is not consistently useful in wild species. However, collectors that are fortunate to have experience of seed development and phenology of the target taxon will recognise the optimum stage for seed sampling with confidence. Recommendations in Schmidt (2000) describe an approach for assessing reproduction in trees, and the use of maturity indices in species for which detailed studies have been carried out. This normally equates to a collecting window of opportunity post mass maturity (i.e., maximum dry weight) and post abscission, when the seeds have begun to equilibrate with the surrounding (drier) air and are losing water. Typically this occurs at around the time of natural dispersal (Hay and Smith, (2003) – Chapter 6). As Schmidt (2000) notes, ‘dispersal in itself is an indication of seed maturity’ and collectors can safely assume that seeds in the process of natural dispersal are suitable for collection for long-term conservation, except in the case of recalcitrant seeds.

2.2. Assessing the physical quality of seeds

Not all flowers are pollinated effectively, and not all that are pollinated will develop into seeds (Snow, 1982; Schmidt, 2000). Furthermore, developed seeds are not always filled (embryonic) and not always free from damage by insects and pathogens. All empty seeds and most damaged seeds will be found to be inviable in germination tests. The frequency of empty and damaged seeds varies according to the population, species, and season, but data from the RBG Kew seed collections, sub-samples of which have been routinely X-rayed after cleaning, shows that some families tend to exhibit high levels of inviable seeds. Although it is often possible to remove a proportion of these inviable seeds at the cleaning stage, it is good practice to avoid a high proportion of inviable seeds in the field collection. Collectors need to recognise and assess the proportion of empty or damaged seeds before making a collection.

The proportion of empty seeds at seed harvest has been studied by Rayachhetry *et al.* (1998) for a single species *Melaleuca quinquenervia* (Cav.) S.T. Blake (*Myrtaceae*). They found a threefold difference between the frequency of filled seeds in fruit clusters resulting from seven distinct flowering events. A broader dataset from Linington *et al.* (1995) shows a high frequency of empty (i.e., non-embryonic) seed collections in the *Poaceae* (13%), *Cyperaceae* (7%) and *Combretaceae* (6%) within cleaned, X-rayed sub-samples of Kew Seed Bank collections. Many accessions from other families contained a proportion of empty seeds, e.g., 73% of all studied *Tiliaceae* accessions contained some empty seeds, with an overall average of 19% empty seeds per cleaned accession from this family. So why are empty seeds so frequently encountered? Empty seeds are probably the result of resource deficiency (Fenner, 1985), although lethal gene combinations may also have an effect. Fenner suggests that the apparently wasteful practice of producing empty seeds may be offset by the value of the non-fruiting flowers providing additional pollen and nectar resources to attract and feed pollinators.

Damaged seeds can occur due to attack by insects or microbial pathogens. Damage from these sources can occur at different stages in the seed life-cycle, e.g., prior to dispersal, whilst on the ground surface, and also after incorporation into the soil seed bank. The principal insect orders that affect seeds are reasonably well known, and include the *Coleoptera* (beetles, especially weevils and bruchid beetles), *Lepidoptera* (moths and butterflies) and *Hemiptera* (bugs) (Bonner *et al.*, 1994). Linington *et al.* (1995) recorded insect-damaged seeds in 26% of *Leguminosae* collections examined after cleaning, and damage levels exceeded 50% in some *Acacia* and *Mimosa* accessions. This data is consistent with the observations of Miller (1994) who recorded up to 53% bruchid infestation of seeds of *Acacia tortilis* prior to dispersal.

Microbes (bacteria, viruses and fungi) can also cause significant losses to seed crops (Bonner *et al.*, 1994) but, as with insect predation, the proportion of seed affected can vary greatly according to the species, the year, and the environment. In the most severe infections, collectors may be able to recognise discoloration and/or malformation of the seed coat or embryo. In addition to the reduced viability expected for these seeds, they may act as agents for transmission of the pathogens into the remainder of the seed collection. It is known, for example, that endophytic fungi can remain viable under seed bank conditions. Thus, it is a great advantage to prevent heavily infected material becoming part of the initial field collection.

Use of the 'cut-test' to assess seeds

To minimise the proportion of inviable seeds that are collected, one of the most important tasks for collectors is to carefully examine a representative sample of seeds. This information gives the collector a chance to select techniques to maximise the collection of high quality seeds (e.g., by avoiding certain parts of the plant population), or if necessary, to seek a more productive collecting opportunity at another time or place. Fenner

(1985) rightly notes that ‘many fruits which are damaged internally by larvae have the same external appearance as sound fruits’. As this can also be the situation with empty seeds (particularly from the *Cyperaceae*), close external and internal examination of a sample of seed is always required prior to collection. Lippit *et al.* (1994) and Linington *et al.* (1995) have demonstrated the value of X-ray images in reliably assessing damage to seeds, but seed collectors do not have access to X-ray equipment in the field. It is sometimes possible to separate empty or damaged seeds of some species by water flotation or winnowing in gentle air currents whilst in the field, but the results of these steps will always need to be validated in the field by internal examination of the seeds. In most field situations, seeds can be easily sectioned using secateurs, scalpels, scissors, nail clippers or other sharp blades. Tiny seeds can be held on adhesive tape during sectioning. Although some enthusiasts may wish to take field microscopes in the collecting vehicle to examine longitudinal and transverse seed sections, either a $\times 10$ or $\times 20$ hand lens is usually adequate for this purpose. With care, seeds that are known to be non-toxic can be crushed between fingernails or teeth if sectioning is difficult. *In conclusion, the ‘cut-test’ is the only simple and reliable technique for providing accurate, quantitative seed-quality data in the field.*

To apply this in practice, collectors should examine representative seed samples from several well-spaced individuals in the population. This data is vital for two purposes: firstly it provides evidence of whether the seed quality is acceptable, and secondly, collectors may be better prepared to select a collecting technique that minimises the presence of any empty or damaged seeds in the collection. Again, a pre-collection checklist (Box 9.4) can be useful to record this data in a systematic way.

3. Making a Seed Collection of Sufficient Quantity

What is the minimum quantity of seed required for a long-term conservation collection of seeds? Each step in the maintenance and conservation of the collections, distribution of samples, and ultimately, if necessary, regeneration of the collection, will require a sample of seed. Each of these steps will be considered in turn. Finally, safe limits to collecting will be addressed.

3.1. Sufficient seeds for conservation

The core or ‘base’ part of the collection that is conserved as insurance against loss of the natural population needs to retain sufficient genetic diversity, taking into account any potential losses, when establishing a breeding population of the species. Based on the genetic theory cited in this chapter, several seeds sampled from each of at least 50 individual plants would be needed to minimise the founder effect, random genetic drift, and to ensure the greatest possible chance of survival of the species from this sample. As a working minimum, 500 seeds would ideally be required for this purpose.

In the event that a seed collection has been diminished by supply requests, or the viability of the seed collection has fallen below a critical level (the ‘regeneration standard’, often 75–85%), curators need to consider the possibility of recollection or regeneration of the collection. Regeneration has the attendant problems of impracticality and cost. In addition, there is a risk of hybridisation with similar material, outright loss, random genetic drift (where rarer alleles are lost due to the sample size being too small) and selection (due to differences between the original habitat and the regeneration site). It is clear that regeneration is best avoided unless it can be done within the original environment of collection (see Frankel *et al.*, 1995).

3.2. Sufficient seeds for maintenance

Properly maintaining (curating) a collection can only be assured if there is sufficient seed to establish a successful germination protocol and thereafter to periodically monitor the viability of the collection. Assuming that dormancy can be successfully overcome within two 50-seed germination tests, and monitoring takes place at 10 and then 20-year intervals, 750 seeds would ideally be required to maintain a collection for 200 years (Prendergast *et al.*, 1992). It should however be noted that as viability falls, statistically demonstrating that a collection is above or below the ‘regeneration standard’ may increase the number of seeds required in a viability test. Also note that the use of eRH measurements to determine moisture status (Probert *et al.*, 2003 – Chapter 20) is non-destructive and means that seeds no longer need to be set aside for such assessments.

3.3. Sufficient seeds for duplication

One of the great benefits of making a conservation seed collection is the possibility that a proportion of the collection can be easily stored at a second location, thus reducing the risk that the entire collection could be destroyed by a single catastrophic event. Assuming that duplication is practised at one or two other locations, the initial collection may need to be twice the quantity required by a single seed bank. Many germplasm programmes maintain very small ‘emergency’ sub-samples of their collections at an additional location to reduce still further the risk of catastrophic loss.

3.4. Sufficient seeds for distribution

Each seed conservation programme will have anticipated the needs of users expected to request samples through the collection’s lifetime. For example, a sample of 5,000 seeds would permit one sample of 50 seeds to be supplied on average every second year (approximately the request rate at the Millennium Seed Bank) within the 200 year expected life-span of the collection.

Providing that collecting can be carried out without threatening the survival of the natural population, the *ideal* quantity to collect for long-term seed bank conservation is therefore between 10,000 and 20,000 seeds. These quantities can often be collected within only a few collector-hours of work. As an example, Figure 9.1 shows that around 40% of the collections made by one of

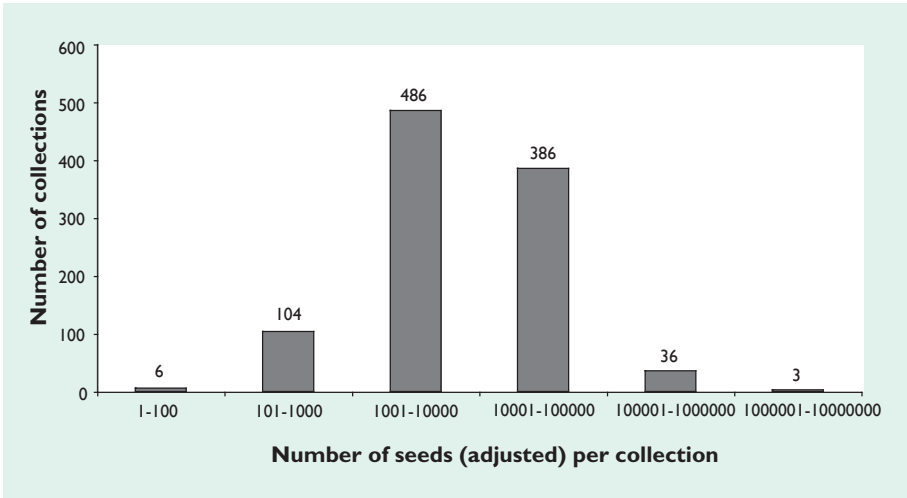


Figure 9.1 Adjusted seed quantities recorded from 1,022 seed collections made by a RBG Kew seed collector during 26 expeditions. Adjusted seed quantity is a statistical under-estimate of the filled, potentially viable seeds in a collection.

the RBG Kew seed collectors have an adjusted (i.e., potentially viable) total of over 10,000 seeds. Before making small collections of maybe 500–2,000 seeds, collectors must consider carefully whether other more productive populations could be collected instead.

3.5. Setting a safe limit to seed collecting.

There are situations when a harvest of 10–20,000 seeds may not be safe for the natural population to withstand. If the target species or population is thought to be at risk, detailed observation of the target population(s) will be required before a safe level of seed harvesting can be estimated. Experimental work may also be necessary to understand the relative importance of factors such as seed or pollen limitation, resource deficiency, animal predation, and seed bank dynamics affecting the survival of the population. For example, Pavlik *et al.* (1988) illustrates the use of demographic data of seed, seedlings and adult plants in order to help rank the endangerment status of three species of perennial endemic plants. For rare or threatened species, collectors will need to apply especially robust protocols such as described in Center for Plant Conservation (1991), as revised by Guerrant and Fiedler (in press).

However, seed collections must always be limited to the level that will reasonably avoid any impact on the long-term survival of the wild population. Detailed data on harvesting from non-domesticated plant species is scarce, but Maze and Bond (1996) provide interesting estimates of a safe harvesting level for two *Protea* species that depend on seedling establishment after fire to maintain their

population (in this case, at optimal density for commercial flower production). Modelled flower harvesting rates of 50% in *Protea repens* and 85% in *P. neriifolia* were successfully validated against experimental harvests. Although the evolutionary impact of this harvesting is unknown, these figures imply that, at least over the timescale of a few generations of seedling establishment and population renewal, a substantial proportion of the reproductive output could be collected without causing harm. Further evidence comes from studies of two extreme situations: masting and seed-limitation, which will be considered in turn.

Masting

Erratic seed production or ‘masting’ is seen in many tree species and can make seed practically unobtainable except during mast years. Seed predators respond to mast events, and their response suggests that species most prone to seed predation show strongest masting behaviour. Therefore one can assume that predation is influencing these plants to ensure that, at least in some years, predators are overwhelmed by the available seed and there is ample surplus available for regeneration and establishment (Fenner and Kitajima, 1999). By extension, there may be a surplus available for conservation collecting during mast years.

Seed limitation

Seed limitation in natural populations is most prevalent in early successional species and in early successional habitats such as sand dunes (Turnball *et al.*, 2000). Particular care must be taken when collecting seed of rare annual plants from these habitats, as J. Harper (unpublished data) has indicated, for example, that *Vulpia membranacea* (L.) Dum. and *Mibora minima* (L.) Desv. appear to be almost wholly reliant on annual seed production for population renewal. In these cases it would be wise to collect only during years experiencing above-average seed production.

In the course of setting up a general sampling protocol in the early 1970’s, the Kew Seed Bank received various suggestions of safe harvesting thresholds ranging from 10% to 95% of the available seed, depending on the life form, habitat, and the seed longevity of target species. Current advice is that collection of a maximum of 20% of the seed available on the day of harvest should be unlikely to cause long-term harm. Clearly, if any specific data is available on longevity, fecundity, masting, and establishment, this data should be analysed to set more specific safe limits for collecting. In addition, collectors and conservation managers should be alert to the possibility that collecting teams working for other programmes could be active in the same area and target the same population in the same year. Good communication between agencies and alertness to harvesting signs in the field are important additional safeguards.

4. Seed Collecting Techniques

The collection of seed from non-domesticated plant species requires care, resourcefulness and determination. Non-domesticated species collected on a commercial scale may occasionally be harvested mechanically with machinery adapted from crop harvesters, but here attention is given to the manual techniques that are most commonly used by collectors. The many available collecting techniques can be grouped into the five categories described below. Techniques specifically for forestry tree seed collection can be grouped in the same categories, but frequently require additional use of rope and climbing techniques (see Schmidt, 2000).

4.1. Plucking of whole fruits

This is the most basic and the most flexible of techniques, and is often suitable for the situations in which:

- Target fruits can easily be selected by eye (e.g., due to colour or texture change of fruit coat).
- Non-target (e.g., immature or damaged) fruit cannot be excluded from the collection by any other technique available.
- Fruits are easily accessible to the collectors who can use buckets or similar containers tied around the waist to release both hands for collecting, holding branches, etc.

Plucking has many benefits when making small seed collections, especially from fleshy and many-seeded indehiscent fruits. However, inexperienced teams often resort to plucking fruits without considering more effective techniques which may be more suitable for the situation.

4.2. Stripping entire seed-heads

This is a popular technique for collecting seed from grasses. Typically, a panicle is grasped at the base with a gloved hand, which is pulled upwards dislodging many or all of the seeds present. Collectors routinely collecting grass seed from large stands may choose to use or adapt containers with short tines. This equipment can be very efficient at removing seed from the grass panicle (see for example, Young and Young, 1986). However, this technique does not give the collector much opportunity to select optimum panicles for harvesting, and in addition may introduce a significant proportion of pre-dispersal stage seeds into the collection. Such seeds might need further post-harvest ripening which can be time consuming and is best avoided. The stripping technique is most suitable for the following situations:

- Dense, mono-specific stands of grass with no weed or other species present.
- Panicles completely and consistently at natural dispersal stage.

4.3. Pruning clusters of fruit

This can be a very effective technique (typically for trees) when:

- Seed is clustered at the distal (terminal) part of branches.
- The species is abundant and a small associated loss of branch and foliage can be tolerated.
- Seed is beyond reach of the collectors and has to be obtained using tree-pruners.

The seed clusters are then individually assessed for damage before seeds are added to the collection.

4.4. Collecting from the ground

Often, collectors encounter ample seeds below a tree or bush, but only rarely is it suitable to collect for long-term conservation. The most evident, and frequent harm that seed could suffer will be damage due to pests or pathogens whilst on the ground. The collector must also consider carefully the possibility that the seeds may have been on the ground for several months, and could even date from the previous years' seed production. Such seed will have lost significant viability due to ageing, and its life-span in storage will be reduced. For this reason, great care must be taken to inspect the available seed, to assess any variation in the fruit, seed coat and seed internal tissues, and to estimate the date of natural dispersal. The presence of aged seed in the collection should be carefully noted so that seed bank staff can allow for this in germination tests. In general, collect from the ground only when:

- The mother tree(s) can be determined without doubt.
- It is certain that you will collect seeds that have dispersed recently.
- Seeds have not suffered significant damage from pests or pathogens.
- Other techniques or collecting options are unsuitable.

4.5. Shaking branches

Occasionally, collectors will find that careful shaking of branches will dislodge the best available seed, which can be captured in buckets or on a tarpaulin held or laid beneath the plant. One great advantage of this technique is that badly developed or heavily damaged seeds may have different dispersal properties, and may be retained on the mother plant during light shaking. Collectors should start with gentle taps, and carefully check each sample of seed dislodged as shaking becomes more vigorous until the optimum technique has been identified. Heavy beating of branches is undesirable because damage may be caused to the tree, and other plant material and associated insects may land in the containers, necessitating additional cleaning of the collection. Light, plumed seed from *Bombacaceae* and *Asclepiadaceae* will often be carried away by air currents if this technique is used in even gentle wind. However, it can be useful in the following situations:

- Dehiscent fruits which are liberating medium-large seeds.
- Seeds which have irritant plumes (e.g., *Cercocarpus* in the *Rosaceae*) or heavily armed (e.g., spiny) trees such as *Prosopis* (*Leguminosae*).
- Level, open ground suitable for tarpaulin use.

A variation on this technique (when there is frequent access to the collecting site, and when seeds would otherwise be lost) is to fix a mesh bag loosely over the pre-dispersal seed heads so that seeds are captured as soon as they are shed, and can then be periodically removed. This has been recommended, for example, by Lippit *et al.*, (1994) for *Fouquieria splendens*.

4.6. Additional notes for collecting practice

- A record should be monitored of the number of individual plants sampled. When working as a team, a good estimate of the total number of individuals sampled can thus be obtained.
- Collections should be made into buckets, cloth or paper bags, and each person's sample should be carefully checked before combining into a single population collection.
- The collections of dry, ripe seed should be secured into cloth or paper bags for transit. Any awned seed or hooked fruit that would damage or get stuck in cotton bags should be stored in cardboard boxes or strong paper bags.
- All containers of seed should be labelled inside and out with the appropriate collection number, and securely sealed. Cotton bags with draw-strings have the advantage that they can be tightly tied around the neck, and can easily be opened and re-sealed to check the condition of the seed collection.
- Fleshy fruits should be collected directly into strong plastic bags or tubs with as much air as possible. The bags should then be packed in some kind of rigid plastic container. This should ensure that the fruits are not squashed and also will prevent them getting too hot and fermenting during their journey. The seed may need to be removed from fleshy fruits either during or immediately after the field trip.

5. Voucher Specimens

Germplasm collectors usually take voucher material which can be used to name and classify the associated germplasm collection. Although photographs and vegetative material can occasionally be valuable for this purpose, herbarium specimens are the most frequent voucher material chosen to compliment seed-collecting activities. Whatever material is chosen, it is vital that the voucher adequately represents the plant population from which seed was collected, and that it is accompanied by accurate and relevant data (see next section). The voucher will always

provide a material link to the seed collection, so that if the voucher is re-named in the future, the seed collection can also be kept up to date. The simplest and most secure way to ensure that the voucher specimen is always closely linked to the germplasm collection, is to use an identical collection number for both. Another option is to include a suffix to the collection number that signifies either seed, photograph, herbarium, spirit or vegetative material from a single botanical collection. Clearly, any voucher material needs to be collected with caution and if there is any evidence that the collecting of herbarium specimens or vegetative material could itself harm the population, collectors will need to use photography and a careful written description of the plant instead.

5.1. Selecting herbarium vouchers

Collectors are encouraged to take at least three duplicate herbarium specimens to accompany each seed collection made, so that vouchers are available for curation, naming and study in several herbaria. Collectors need to select the very best material that (1) truly represents the seed collection, and (2) provides adequate material for subsequent identification and study by herbarium taxonomists.

To adequately represent the seed collection, one or more of the collectors that participated in the seed collecting should select the voucher material. Some general guidelines to follow when selecting a voucher are:

- Whatever the level of morphological variation in the population, it is important to try to select material from a 'typical' individual from which seed was actually collected.
- In the case of large plants, the required number of duplicates should be removed from a single representative individual.
- In the case of small plants, and most annual plants, a group of similar, representative, individual plants should be selected and press at least one plant should be pressed for each duplicate required.
- If seed has been collected from an extremely variable population, or one showing highly stratified variation, it is good practice to collect additional, separately numbered, voucher specimens to illustrate the range of variation in the population.

To select herbarium material adequate for identification, specimens should ideally include examples of all organs present including flower, fruiting structure, vegetative parts, roots of annuals and bark of trees. Box 9.5 lists the key features of a good herbarium specimen. Collectors wishing to learn the correct technique should either accompany an experienced botanist taking specimens in the field, or should try to attend a training course such as those run by RBG Kew and other botanical organisations. Ideally, the specimens should be dried daily using portable driers to maintain as much as possible of

Box 9.5 Key features of a good herbarium specimen (after Herbarium Techniques Course, RBG Kew, and Bridson and Forman (1998))**Good herbarium specimens should be:**

1. Carefully selected plant material.
2. Well preserved.
3. Accompanied by an unambiguous collection number.
4. Accompanied by good collection data.

Careful selection of plant material

Material should be fertile. Material should be representative of the population (collect an average specimen and note range, collect a range of phenotypes) and of the individual (collect some of top, middle and base if it is not possible to collect the whole plant). Try to include the following:

- Underground parts if possible.
- Bark/wood.
- Heterophylly (e.g., juvenile foliage with stipules).
- Developmental stages (leaf buds, young leaves, flower buds).
- Male and female flowers.
- Different flower forms.
- Points of attachment, i.e., preserve arrangement of organs.
- Loose collections are useful. Place them in an envelope or capsule as extra material.

Use discretion regarding the amount of plant material to take.

Prepare at least three duplicates for each collection.

Look at and plan sampling of plant before cutting.

Well preserved

Araceae and fleshy parts are best preserved in spirit.

Palms and *Pandanaceae*: the base of leaves is important.

Protect delicate tissues using wax paper.

Special conditions for collecting and preserving plant parts in different families are detailed in Bridson and Forman (1998).

Collection Numbers

Avoid the danger of number duplication: Keep collection numbers simple e.g., Smith 260

Consider producing a field collecting book with sequential numbers printed in it.

Write the collection number on jewellers tags (small labels) which can be tied to each of the herbarium specimens and will not become detached from the specimens in transit or during mounting.

Good collection data

Minimum:

- Locality, including country.
- Latitude, longitude and datum/map used (but indicate if this information cannot be made public).
- Altitude.

Box 9.5 Continued

- Habitat.
- Description of plant – concentrate on things that are lost in sampling, e.g., smell, colours, life form, three dimensional structures.
- Collector's name.
- Collection number.
- Date of collection.

Additional data:

- Name (e.g., vernacular).
- Ecology (associated species).
- Detailed morphological data (see below).
- Frequency of occurrence.
- Economic data.
- Conservation status.

Morphological data:

- Habit/height/spread.
- Underground parts if not collected.
- Stems and trunks – buttresses, bark, latex, etc.
- Stipules.
- Fresh size, shape, colour of inflorescence, flowers, fruits, seeds.

the shape, colour and characters of the living plants. Where drying equipment is not available, the specimens should be kept warm and dry and the absorbent paper should be changed frequently.

If the individuals from which seed was collected do not contain adequate vegetative and reproductive material to use as a voucher, it is essential that collectors **do not** select more complete voucher material from a distinctly different part of the same population, or from another population, as this will not be representative of the seed collection. If necessary, other herbarium specimens can be collected (with a different collection number) but they should not normally be used to name the seed collection. A practical option may be to grow a voucher from the seed collection which, at least for herbaceous plants, is a practical and inexpensive technique. Disadvantages may include significant delays whilst the collection is germinated, propagated and grown to flowering stage. In addition, a specimen cultivated from seed in a glasshouse may demonstrate atypical growth and the herbarium specimen may not attract adequate attention from botanists revising wild specimens of the taxon. A preferred option for a tree or shrub without voucher material may be to get it identified in the field by a specialist, or tagged with marker tape so that adequate herbarium specimens can be collected at a later date.

At the end of the trip, the labelled, dried, herbarium specimens will be available for verification by a specialist able to check the field identification. It is good practice for one of the duplicates to be retained by the specialist for their herbarium. Other duplicates can then be distributed to appropriate local, national or international herbaria as permits allow. If printed labels are subsequently to be produced for all the duplicates, it is useful for labels to include the verified plant name and also the initials of other herbaria receiving duplicates as cited in Holmgren (1990).

6. Data Associated with the Seed Collection

The collection of data associated with the species or populations from which seed is taken is a vital contribution to knowledge about these plants. Associated data falls into several categories: habitat information (such as rainfall, altitude, slope, landform, aspect, geology, vegetation physiognomy, associated species, and soil characteristics) has important applications for restoration ecology and re-introductions. Population characteristics (phenology, number of plants, % of population producing seed, pollination and dispersal mechanisms, predation, etc.) are useful data for conservation authorities, and information about the plants themselves (form, height, flower/fruit morphology, etc.) is required by taxonomists. A comprehensive review of the data requirements for field germplasm collections of either crop or non-domesticated plant species was carried out by Moss and Guarino (1995). They set out the importance of clear data-recording to an agreed format, ideally using published descriptors which will maximise the usefulness of the collection in the long term. An example of a simple field data form suitable for long-term conservation seed collecting is shown in Figure 9.2. Electronic data recording in the field has now become routine for some projects, and the initial caution about relying on expensive and cumbersome electronic equipment has been eclipsed by new palm top devices. This has enormous potential for herbarium collectors, but may result in modest benefits for germplasm collectors who, as Moss and Guarino (1995) note, are usually time-limited by the collecting, not by the data-recording step in the field.

7. Post Harvest Handling of Seed Collections

Seeds collected with surface moisture, or with green foliage, should, as soon as possible, be spread out on newspaper to dry naturally, either outside in the shade or in a well ventilated room. Recently abscised and under-ripe fruits which are still humid must be handled particularly carefully, as high temperatures or fast-drying could prove lethal to such seeds. Care should be taken with all seed collections to prevent overheating, for example by being left in a vehicle in full sun. Exposure to such sustained high temperatures can badly damage seed collections. Attempts should be made to maintain ventilation around the collections at all times and the collecting vehicle

➔

Please use **BLOCK CAPITALS** Genebank accession Number:

Please complete all the priority fields labelled in **bold**

Please circle relevant descriptors shown in *italics*

Date Collected

DD	/	MM	/	YY
----	---	----	---	----

Seed Collection Number

Collector(s)

Location details

Country:	State:	County/District:

Lat. *dg/min/sec*

Long *dg/min/sec*

Elevation (m)

GPS used (YES/NO)

GPS Datum used or

If no, please see over

HABITAT DATA

Habitat and Associated Species

Modifying Factors *Mown* *Burnt* *Grazed* *Flooded* *Trampled* other:

Land Form <input style="width: 100%;" type="text"/>	Slope° <input style="width: 100%;" type="text"/>
Land Use <input style="width: 100%;" type="text"/>	Aspect <input style="width: 100%; text-align: center; border: 1px solid black;" type="text" value="N NE E SE S SW W NW"/>
Geology <input style="width: 100%;" type="text"/>	Soil Colour <input style="width: 100%;" type="text"/>
Soil Texture <input style="width: 100%; text-align: center; border: 1px solid black;" type="text" value="Clay silt sand other:"/>	

COLLECTION DATA - **If plant has been identified by a specialist, please see over**

Family <input style="width: 100%;" type="text"/>	No. of Plants Sampled <input style="width: 100%;" type="text"/>
Genus <input style="width: 100%;" type="text"/>	No. of Plants Found (approx) <input style="width: 100%;" type="text"/>
Species <input style="width: 100%;" type="text"/>	Area sampled (sq. metres) <input style="width: 100%;" type="text"/>
Sub species/var. <input style="width: 100%;" type="text"/>	Number of pressed specimens <input style="width: 100%;" type="text"/>

Seeds collected from: *Plants* *Ground* *Both*

Plant Habit: *Tree* *Shrub* *Liana* *Erect herb* *Creeping herb* *Climbing herb* Plant Height (m)

Does the pressed specimen have the same reference as the seed collection?

If not, enter details of collector, reference, where lodged and date collected

Notes to assist identification of pressed specimen e.g., flower colour, odour, presence of closely related species

Common name(s) of plant:

Photograph taken: *Digital 35mm* Reference Where image will be filed

Figure 9.2 Example of a field data form suitable for long-term conservation seed collecting.

For a completed seed collection:

If GPS **not** used, please state method of obtaining lat. and long:

Map Publisher

Series

Scale

Map Co-ordinate

Map Date

If collection has been identified by a specialist please complete sections below:

Material Identified:

Identified by Organisation

Date identified

should be parked in the shade, or at the very least, the windscreen should be shaded. In general, the seed collections should be kept in a cool, dry place prior to dispatch to the seed bank but they should not be frozen. See also Probert (2003) – Chapter 19.

Cleaning of seeds may be carried out in the field but in most cases it is best to leave the task of cleaning the collections to seed bank processing staff. These staff will have a wider range of equipment to clean seeds without causing harm to the collection, and they will have binocular microscopes to view cut-tests and to better inform the decision about cleaning technique. In a few cases however, where, for example, seeds have been collected fully mature within dry, bulky fruits or capsules, it may be relatively straight-forward and rapid to carefully open the fruits and to separate the seed by hand. A gentle breeze may be effective for the gentle winnowing of partially cleaned seed collections but collectors should certainly consider the cost-benefits before attempting to clean seed whilst in the field.

Fleshy fruits may require partial or full cleaning if they are over-ripe, or have been damaged or crushed during the collecting. Using a sieve and cool running water, as much flesh as possible should be removed from the fruits. The seeds should then be left to air-dry on a fine wire mesh or thick filter paper until surface-dry before being packed and transported in cloth bags.

Carrying Out a Successful Collecting Expedition

1. The Itinerary

A good collecting itinerary is not a rigid framework for carrying out fieldwork. Of course, it will include some priority routes and locations which form the backbone of the fieldwork programme. These locations may include target plant populations which are being actively monitored, or where accommodation or assistance has been arranged, for example. However, providing that there has been sufficient time to gather survey information and consents for access, a good itinerary will also include many optional potential collecting locations and exploration sites, clustered within reach of the main route. As the fieldwork progresses, as the phenology of the target populations becomes clearer and as targets are reviewed, these additional locations can be included in the daily itinerary. Local advice is invaluable in informing decisions about changes to the itinerary, for example whether roads are passable, and whether fuel, water or accommodation is available in remote areas. A well-prepared itinerary, combined with flexibility in the field, will maximise the chances of a productive trip.

2. Recognising an Adequate Seed Collection

The most difficult moment for a collector of seed is often when deciding whether a population meets the minimum standards for seed-sampling. Use of a pre-collection checklist such as shown in Box 9.4 will help collectors carry out the assessment. However, the quality and quantity thresholds for many criteria including, for example, seed availability below which a collection is not worthwhile, will depend to an extent on the project objectives, priorities and constraints. If the assessment provides evidence that a population does not meet minimum criteria for seed-sampling, there are several alternative options open to collectors, set out below:

- Seek another population of the same species on the same trip.
- Return to this population on a later date.
- Take herbarium specimens and detailed notes to confirm the identification.
- Assess the next target species available.

An optimum collecting programme will include primary and secondary target taxa. If the primary target taxa cannot be collected, it is very efficient to be able to make collections from secondary targets, which themselves may not be regularly available to a collecting team and may become valuable outputs from the expedition. Using this systematic but flexible approach, the team can remain productive in all situations and will become well-prepared for future collection trips.

3. Team Work

Good co-ordination and teamwork is always required during collecting. In most collecting missions, the whole team will need to help collect seeds, which is usually the most labour-intensive activity. Each member of the collecting team will need to be assigned appropriate tasks and equipment. Through encouraging discussion within the team and participation in the various tasks, teams can become more effective at their work. In particular, it is worth ensuring that each person is able to participate in data recording and also in the voucher specimen selection and preparation during the trip as this will help to develop important observation skills.

It is useful to take a second set of vehicle keys and to carefully choose the best location to park the expedition vehicle. The leader should set meeting points and timings, and 'fall-back' plans if anything should go wrong during the collecting. In addition, the leader should also check on inexperienced collectors frequently to ensure that everything is going to plan and to identify any capacity-building needs for the team. If a team is sharing the sampling of a single collection, it is useful before the collecting starts for the team to develop clear visual cues (a 'search-image') for the target seed head which will ensure that everyone selects the correct seed. If this image cannot easily be carried in the collectors' heads, an example of the correct seed head can be carried in the hand. This will help to ensure that the samples can be successfully combined at the end of the collecting.

Health and safety of the collecting team should be paramount at all times. If there are any specific hazards to the team, the danger should be assessed and a decision taken on minimising the risk. For example, in many situations the leader may decide that each team member should carry and know how to use a first aid kit, radio, and compass or GPS. In difficult or hazardous terrain, collectors will need to work in pairs or maintain close visual contact. If the target plant has any toxic/irritant or thorny parts, the collectors will need to take care not to injure themselves during the activity. Appropriate clothing should be worn, and gloves, tarpaulins, etc. used to minimise contact with any hazardous material. In drylands, the most frequent problems are heat-exhaustion (resulting from dehydration) and injuries from collecting equipment.

Finally, it is important that collectors are alert for any rare or fragile plant or animal communities, and historical or cultural artifacts in the area which could be damaged by the chosen collecting technique. Care should be taken not to increase the risk of wild fire (e.g., by parking a hot vehicle over combustible grasses). Weed seeds should be cleaned from seed bags or clothing before leaving a site, so as not to introduce them to new areas. If there is a significant risk of damaging the environment, the collecting plan should be modified or the collection attempted elsewhere.

4. Collecting Rate

Collectors should allow adequate time for all the activities necessary to make a high-quality seed collection. For example, two collectors might require the following indicative time to make a non-domesticated seed collection for long-term conservation:

Identification of taxon and any apparently similar species	15 mins
Assessment of population, physical seed quality, and potential collecting techniques	15 mins
Seed collecting	15 to 120 mins
Herbarium specimen preparation, photography, and specimen labelling	15 mins
Geo-location and completion of field data forms	15 mins

However, time should also be allowed for associated activities such as travel to site, exploration, survey, dialogue with landowners and local people, vehicle maintenance, meal and rest breaks, plus the post-harvest care of seed collections and herbarium material. In conclusion, it is evident that a long-term average of between two and three high-quality, large seed collections per field day may be achievable by a pair of collectors. The collection rate will also be strongly influenced by a number of other factors (Table 9.4):

Table 9.4 The principal factors that influence collecting rate in the field

Factors	Collecting rate	
	Higher rate	Lower rate
Composition of team	Several experienced collectors*	One or two inexperienced collectors
Terrain	Easy access to site and within collecting site	Difficult terrain
Objectives of collecting program	Broad and general plant diversity collecting programme	Highly targeted programme
Literature available	Specialist and/or recent floras available to consult	Poorly documented flora, little or no recent publications
Target plants	Weedy and annual plants	Large shrubs and trees

*Experience from RBG Kew seed collecting missions suggests that a team of between two and four people sharing a single vehicle is ideal for most situations. In remote or dangerous terrain, a second vehicle is essential together with radio communication and additional field supplies.

5. Reporting and Tracking

It is the collector's responsibility to ensure that the seed, voucher specimens and data resulting from the trip arrive safely and completely at the seed bank, and also to ensure that colleagues are made aware of any special conditions required for the processing, study or use of the material. Particular care should be taken to respond to all undertakings to share data, material and information from the trip. The collection numbers should be included in all reports so that these can be referred to for seed requests, enquiries, etc. The trip report may be prepared in two stages: a brief initial report detailing the activities carried out and summarising the itinerary travelled, followed later by a more detailed final report including the quality and availability of collections as a result of testing. The report(s) should be sent to all the participants, landowners, authorities and stakeholders involved.

In conclusion, common sense, based on biological knowledge will continue to underpin decisions about seed collecting from non-domesticated species (Young and Young, 1986). The very best seed collections are made as the result of good preparation, careful field assessment and robust collecting techniques. Provided that the collecting, documentation and labelling is adequate, and the seed collections are safely delivered to the seed bank within a few days of the fieldwork, the seed collector can be confident that a valuable biological resource has been created for the benefit of future generations.

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