

***GUIDELINES FOR COLLECTING
SEEDS FROM WILD PLANTS***

Introduction

This guidelines is intended to give a general information for germplasm collectors.

It is based principally on the author's personal experience and on literature written by various authorities on genetic resources and brief discussions with various consultants.

This is a living document, which will be updated and expanded periodically, and it is hoped that our partners throughout the world will contribute to this process.

Practise of exploration

The objective of wild seeds collecting expedition is to collect material with the maximum amount of useful genetic variability within a strictly limited number of samples.

Useful genetic variability is the alleles at a locus with a population frequency greater than 5%.

This basic strategy is recommended when information on the population genetic structure of the target species is lacking.

A successful mission is usually one which has been well planned.

Planning a seed collecting expedition from wild nature

Thorough research and planning before leaving on a trip is crucial. Field-based collecting can be an expensive undertaking and it is important to get the maximum benefit from fieldwork. A well organized programme with realistic targets is the best way of using time effectively.

Some specific points for seed collectors to consider:

- Stay focused on the aims and intentions of the collecting trip.
- Is the timing right for the species to be collected and do you have accurate locations of good populations?
- Do you have good knowledge of the target species and what it looks like?
- Do you have the right vehicle and is it fitted out to provide safe, comfortable travel?
- Do you have the correct licenses for collecting and for passing through designated lands?
- Consider your equipment needs: bags, secateurs, pole-pruners, collection books, plant reference books, tags and safety gear.
- Set goals about how much material you need so as not to waste time collecting too much or too little.

Make clear judgements about the material. If it is not mature, then leave it for a later time.

What makes a good seed collection for long term conservation?

1. Priority species (orthodox seeds, indigenous or endemic species, endangered, threatened or vulnerable, economically important species, species suitable for research, seed not widely available, rare seeds)
2. Accurately identified in field or from herbarium specimen
3. High quality seed with potential longevity of 200 years in seed bank storage
4. Sufficient sized collection to meet the intended uses.
5. Genetically representative of the species/population/individual sampled
6. Adequate associated data to meet the intended uses.

Logistical Planning

1. Partners in collecting
2. Collecting team
3. Itinerary
4. Duration
5. Transport
6. Equipment
7. Obtain permission

Technical planning

1. Basic strategy
2. Targeting a population for sampling
3. Sampling strategy
4. Seed collection techniques
5. Recording the data
6. Care of collections in the field

1. Basic strategy

- What to collect
- Why collect the material
- Where collect it
- When collect it
- How much collect

What to collect ?

- ❑ *Accurately identifying target species*
- ❑ *Learn about the species you collect - identification and herbarium specimens*
- ❑ *Collect native plants*

Accurately identifying target species

- You should have a very clear idea of the species you wish to collect and which of those are priorities
- Each seed collecting project has a distinct set of criteria for setting collecting priorities, but typically includes endemic, endangered and economically useful species
- Bring and use relevant identification guides, floras, or field guides, color photocopies of herbarium sheets of target taxa .
- Invite an appropriate taxonomist or specialist in the local flora to join the team if possible.
- Visit the potential collecting locations early in the season (ideally at flowering) to make herbarium specimens and to confirm identification with local specialists.
- Attention to inflorescence structure and their seed maturity patterns are also important in determining what to harvest.

Learn about the species you collect

You should gather as much information as you can on the target species you intend to collect, including:

- botanical description of target species
 - identifying keys
 - distribution of target species in the local area
 - flowering, fruiting and seeding times
 - whether the fruit/seed is located within hand's reach
 - approximate number of fruit per plant
 - approximate number of seeds per fruit
 - approximate time from maturity to seed shedding (weeks, months)
- because of the uncertainties of the weather, this will vary from year to year
- safety precautions (allergenic or poisonous plants)

Collect native plants

What is a native plant - They have adapted to the local soil, climatic, and biotic environments over the course of thousands of years.

A growing awareness and appreciation of the value of these plants from an aesthetic, agricultural, environmental and cultural point of view has created a demand for their seeds.

Why use native plants

- the easiest way to grow most plant species is from seed.
- the best plants to grow for re-vegetation are those that are local to your area.
- the best way to maximize and maintain genetic diversity is to propagate from seed.
- the most time, money, material and infrastructure efficient way of growing plants is from seed.
- the best way to ensure you get the plants you want is to collect your own seed.

Advantages of native plants

- add beauty to the landscape and preserve our natural heritage
- provide food and habitat for native wildlife
- serve as an important genetic resource for future food crops or other plant-derived products
- decrease the amount of water needed for landscape maintenance
- require very little long-term maintenance if they are properly planted and established
- produce long root systems to hold soil in place
- protect water quality by controlling soil erosion and moderating floods and droughts

Why collect seeds ?

- Collecting for taxonomic, phylogenetic and biosystematics research
- Collecting for genetic diversity study and conservation
- Collecting for immediate use in a breeding programme.
- Fill gaps in collection
 - Mandate species
 - Regional gap
- Recollection
- Danger of extinction

No matter what kind of wildflower seeds you intend to collect, you must keep a constant watch on the plants involved.

Where to collect seed ?

- Permission for collecting*
- Planning itinerary*
- Identify areas to collect*
- Evaluating the site*

Permission for collecting

Collections must be made with permission of any land owner or land management agency and adhere to the guidelines set by any applicable local state and federal laws.

This includes obtaining any required state and/or federal permits and adhering to the conditions set by the permit (s).

Copies of any required permits and/or agency authorization must accompany the seed collection

A permit is usually issued for a period of 12 months and may be subject to conditions that are intended to limit any impacts of native plant materials collection on the environment.

Planning itinerary

- Working out a provision route and time schedule is essential before embarking on a mission and a road map can be very valuable. In some regions, however, the maps are unreliable and allowance should be made for this.
- In general, the recently published road map with contours is very useful. Some very recent roads may not be indicated on the map and provision should be made to include these in the itinerary as they crop up.

The feasibility of following certain routes can be clarified with local contacts.

Identify areas to collect

- Where you collect seeds is just as important as why you collect.
- Determine the native seed collection project site or sites.
- Ecological considerations, permitting, land rights, and geography will determine where you collect.
- Find out the geographical and ecological areas where a species grows and from what areas it can be collected (literature, herbaria, field exploration).

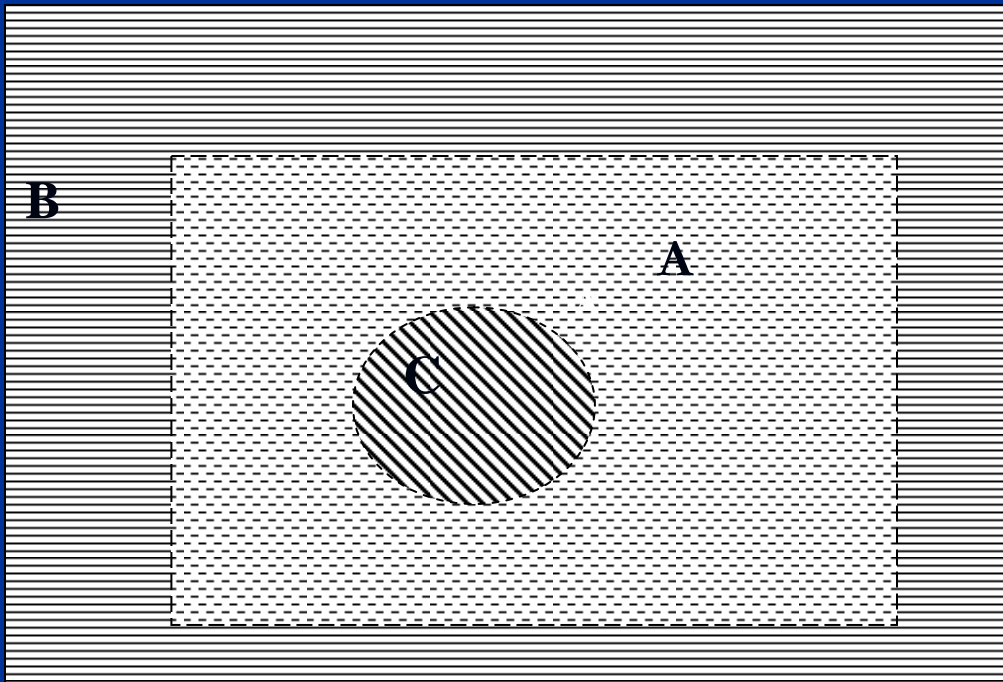
Never collect seed from a yard, lawn, garden, park, or any other obviously cultivated site!

Edwards, (1963) suggested that for an unknown species several provenances should be tried, including at least one from:

(A) **Optimum site.** A sample taken from the part of the natural distribution where a species exhibits its optimal development can be valuable but because of genotype environment, interaction, the phenotype in the natural stand may bear little relationship to the performance of the tree under different conditions.

(C) **Matching site.** The sampling of 'matching' sites involves a study of the environmental factors throughout the species natural range and attempting to locate an area, or areas, where there is a similarity with the site where the trial will be established.

(B) **Marginal site.** Populations near the boundaries of the natural distribution are often growing under environmental conditions which differ from those where the main populations occur.



Other, often severe, selection pressures may operate on the margins which can produce populations physiologically and morphologically very different from the other populations.

Marginal populations which tolerate a greater frequency of droughts are likely to be more adaptable to drier areas than the other populations. The idea of marginal sites assumes that the limits of distribution of a species are the result of limiting climatic or edaphic factors and that populations close to the boundary are those which have had maximum selection intensity applied to them through the limiting conditions.

The sampling of marginal sites is very important when determining the extent of genetic diversity in a species.

Advice

It is important to obtain a complete genetic representation, so sample from many plants, not just a single, or even several individuals.

Plant populations growing in unusually harsh conditions are very good candidates for collection.

Collection sites should be accessible so collectors can get to the site and move around to make collections.

Natural plant populations on unstocked or rested rangeland, forestland or riparian exclosures are excellent sites to make collections.

It may also be advisable to identify several sites with various elevations, aspects, or soils from which to collect.

Areas with heavy weed infestations should be avoided to prevent the unintentional gathering of weed seeds that could contaminate the collection. Do not collect from sites that have been previously planted, research areas, or from areas with threatened or endangered plants.

Suitable collecting sites should be identified through a combination of local knowledge, publications and advice from staff from relevant organizations (such as State herbaria, national parks, and State and local government departments).

Seed should be collected only in a wild land setting, such as a prairie, valley, hill, mountain foothill, mountain, etc.

Evaluating the site

In the interests of time and economy, the bio-systematic exploration of the species has frequently had to be combined with the collection of seed for provenance trials.

A single combined exploration and seed collection expedition cannot be expected to furnish all the answers on variation.

While a reconnaissance may provide valuable information on species distribution and variation, information relating to seed collection (timing, quantities) can be misleading since there may be heavy crop losses leading up to seed maturity caused by environmental conditions or predation by birds or animals for example.

Environmental data

- Geographical map
- Road map
- Topographical map
- Hydrological map
- Soil map
- Vegetation map
- Protected area map

When collect ?

- ❑ Timing of collecting expedition
- ❑ Check the maturity before gathering seeds

Observation is the best way to determine when seeds should be collected.

Timing of collecting expedition

- A key factor in planning is to time seed collecting to coincide with peak maturation of abundant fruit crops. Accordingly, the flowering and fruit pattern for the target species must be established.
- The timing between fruit maturation and seed shed varies considerably from species to species. Variation within a species can also be considerable over the natural geographical range associated with factors including latitude, altitude and distance from the coast.
- Seed development can vary within and between populations of the some species.
- Populations occurring along different altitudinal and longitudinal gradients may also vary in maturation times on a regional basis within species.
- Environmental factors, in particular temperature during the period leading up to maturity, also have a major influence.
- Most species do not flower and fruit gregariously every year and may typically flower at intervals of two to three years and more.
- Collecting of new species or species from new locations may require monitoring over more than one season in order to determine the optimum time.
- Clearly a range of seeding habits exists between species and generalizations are difficult to make with any certainty. Detailed observations on the phenology of flowering and fruiting are a desirable prerequisite in planning seed collections.
- Observing the plants through their life cycle is the next step. Timing of harvest is important because if seeds are collected too early, the seeds will be immature and have low seed viability. If seeds are collected too late, they can dehisce quickly and be lost.
- Seeds that are collected after dispersal are usually of low quality and are potentially costly to clean.
- Another potential timing problem is that seeds often do not ripen uniformly over the same flower stalk. Prolonged flowering and different stages of seed maturity limit uniform seed collection.

Check the maturity before gathering seeds

It is the best (and often essential) to collect fully ripe (mature) seed.

- Seeds that are hard, firm and dry (moisture content closer to 10%) are mature, immature seeds have a moisture content of approximately 60%;
- Harvest when the majority (> 60%) of seeds are mature
- Harvest when you have obtained permission to collect seed
- Color change, especially browning of stem just below seed attachment
- Changes in seed coat colour
- Brittleness of seed pod
- Opening of the seed pod
- Easy release from the plant.
- Some seed starting to fall
- Fruits splitting or breaking open
- Seeds rattling
- Seeds are often ripe about one month after blooming.
- Cutting test to determine the seed vigour (100 seeds) record the number of empty, aborted and disease infection seeds, picked randomly from the whole unit;
- Experimenting with germination tests is also helpful in determining at what stage seeds can be collected.

If the fruit are not ripe the seeds may not be fully developed and will not germinate !!!

Experience is the best teacher of recognizing seeds ripeness but the following should be a good guide to get started

Woody capsules

Upon ripening they generally:

- change colour from green to grey or brown;
- reach their full size (refer to guide books)
- turn dry or woody;
- form visible valves which may start to split apart to release seed (although some never open until picked or damaged).

Papery Capsules

Upon ripening they generally:

- Change colour from green to light or darker brown;
- Remove easily from plant
- Turn dry and papery;
- Split apart to release seed.

Follicles

Upon ripening they generally:

- Turn from a green to a hard brown or grey;
- May form discernible valves which may open or split.

Nuts

- Nuts often change colour, harden upon ripening, and are easily released from the plant.

Seed pods

Upon ripening they generally:

- Change colour from green to light or darker brown (collect pea-flowers at this stage, just before they split open and eject their seed – consider bagging fruiting branches to capture seed);
- Pods should be collected just as the pods are beginning to open. Collecting the entire pod is advisable because this allows the seed to continue ripening in the pod as it dries.
- Reach their full size (refer to guide books);
- Turn dry and brittle;
- Start to split apart and curl to release seed (collect acacia seeds at this stage)

Drupes

Upon ripening they generally:

- Release with gentle pressure.

Grains

Upon ripening they generally:

- Change to a brown colour
- Grain is removed easily from seedhead
- Whole seedhead becomes dry & brittle
- Many species have differential ripening of a period of time

Achenes

Upon ripening they generally:

- Change to a slight brown colour
- Release easily with slight pressure

Cones

Upon ripening they generally:

- Turn from soft and green to hard and brown

Berries

Upon ripening they generally:

- Change colour from green to attractive blue/purple/red (collect at this stage);
- Change from hard to soft and pulpy;
- Are removed easily from plant with a gentle shake.

Proper seed harvesting is aided by an understanding of seed ripening, dispersal mechanisms, and the influences of weather on the timing of seed maturation. Flowering and fruiting dates vary from year to year.

- **if collected too early**, the seeds may not be fully developed and you may affect the ability of the seed to germinate
- **if you wait too long**, the seeds might be taken by the wind or the local wildlife

How much seed to collect?

- ❑ Ideal quantity required for long term conservation collections of seed
- ❑ Making a collection of sufficient size/seed number
- ❑ Collecting high quality seed

Estimate the quantity of seed required from the collection, to help determine the appropriate sampling strategy

Ideal quantity required for long term conservation collections of seed

You should decide how much seed you will need of each species and the likely number of plants that will need to be sampled to obtain this amount.

In good seeding years it may be desirable to collect more than your current requirements and place the extra in storage for the poorer seed years.

The number of seed sources sampled will depend on the extent of the natural distribution, the genetic diversity of the species, and the resources available to sample and carry out the research.

It is important to note in the field whether the seeds that are being collected have viable embryos. Many instances occur where a high percentage of seed consist of only an empty seed coat or have been heavily parasitized. In this situation a larger seed collection would be necessary.

Remember that seed put in storage must be fully mature and handled with more care during the extraction processes.

The size of the seed collection will ultimately depend on the purpose of the collection, collection timing, the size of the plant population, the quantity and quality of seeds that each plant produces and the taxa itself.

Obviously situations exist where this size of a collection would not be practical or could negatively impact the population.

- For high quality conservation collections, in general, we recommend sending a minimum of 2,500 seeds per population from 50 individuals randomly sampled throughout the populations distribution.
- Base collection (ideally 500 seeds) kept in case of loss of wild population
- Developing an effective germination protocol (100 seeds)
- Viability monitoring over the anticipated 200 years lifespan of the collection (650 seeds)
- Duplication at another bank for safety (at least 1150 seeds)
- Distribution to users (5000 seeds)

In summary, the amount of seed collected must ideally be sufficient to maintain, secure, and make available samples from the collection over the desired storage period.

Providing that it can be carried out without threatening the survival of the natural population, the *ideal quantity* to collect for long-term conservation is between 10,000 and 20,000 seeds collect from a large number of individuals (100-500).

Making a collection of sufficient size/seed number

To calculate how much is enough, you should have some idea of the following:

- No. of plants required
- Losses in field and nursery
- Germination percentage
- No. of seeds per kilo
- Yield of seeds from fruits
- Yield of fruits from plants

Some of this data may not be available, so a guess must be made.

However, with experience, it should be possible to get good estimates, which should be recorded to help further calculations.

Calculate the cost of collection which is determined by; the distance to the seed source, the number of people involved, their productivity, their pay per day, and the amount of seeds actually collected. Think of the whole rotation period of a source.

Collecting high quality seed

For long term conservation purposes, it is important to collect seed

- At the optimum stage of development (have viable embryos). This will be assessed by observing phenology and seed development
- With a high proportion of fully developed (filled) and undamaged seeds. This will be assessed by careful physical examination of a sample of fruits and seed.

Influences on seed quality

- Field practices (seed collector's methods)
- Extraction methods
- Storage (post harvest handling)
- Development of anaerobic conditions around the seeds caused by their own respiration. This is due to storing in plastic bags or tight packing.
- Prolonged time interval from collection of samples to propagative facilities under conditions conducive to fungal and bacterial growth.
- Factors effecting initial seed quality (excessive temperature)

2. Targeting a population for sampling

- ❑ *Assessing the population*
- ❑ *The concept of provenance*
- ❑ *Selection of provenances*

Assessing the population

- What is the extent of the population?
- How many individual plants are there?
- Is the population damaged in any way?
- Is the population of wild origin?
- Is the population at reproductive stage?
- Do sub-populations exist?
- Sample the population randomly

Choosing target populations and identifying the most suitable population(s) will be up to the knowledgeable botanists and plant ecologists working in consort with the scientists.

The most important factors in deciding whether to collect from a population are as follows:

- The population is likely to be genetically distinct (defined, for example, by soil, climate, altitude, pollinator's range, physical barriers to genetic mixing).
- The population has not already been adequately sampled and conserved by the seed bank(s).
- The population is wild, self-sown and not planted or cultivated.
- Survey the population to estimate its size and health.
- A seed collection will be most representative of the population when an individual plant produces many seeds and when there are more than 50 reproducing individuals which can be sampled randomly and evenly in this population.
- The seeds are ripe, i.e. preferably still on the plant, and about to be shed.
- Collectors should take time to monitor seed maturation, insect damage and other damage levels throughout the population before making the seed collection
- Small populations (less than 50 individuals) or those that will yield less than 1,000 viable seeds in a collection following the sampling strategy above should only be collected if they are priority species for collection and if more productive populations are not available. **In no case should you take more than 10% of a population's yearly seed output.**
- Where populations are suitable and the quality and quantity of seed is adequate, it may be possible to make collections of a number of different species from the same site.

- It is often helpful to make a preliminary visit to the site to assess the populations, to confirm the identification, to estimate the likely harvesting date and potential seed production.
- These may recommend the collection of only 20% of the available seeds from a population at any one time.
- Whatever the method, collections should always be conducted in a manner that does not damage existing vegetation or other resources.
- For high quality "conservation collections" sampling of a population should be made in a manner that optimizes capturing the genetic diversity of the population without harming the plant populations' long term viability.
- Collectors should try to leave the population in as good a condition as it was when they arrived.
- Ideally, at least 50 % of the seed crop at a given site is left intact to allow for natural recruitment and regeneration of the native population.

Formula for assessing population size:

100,000 / (# seeds/fruit x # fruits/plant) = minimum number of plants required in the population

The concept of provenance

Provenance relating to seed material, otherwise known as 'place of origin', is the geographical area and environment in which parent plants grow and within which their genetic constitution has been developed through natural selection.

The idea of provenance implies that genetic patterns of variation are associated closely with the ecological conditions in which the species evolved and that some morphological or other traits can be recognised to characterise them.

The 'ideal' provenance based on Barner (1975) is:

- composed of a community of potentially interbreeding plants of similar genetic constitution (and of significantly different genetic constitution from other provenances)
- sufficiently large for the seed collection to provide sufficient seed to meet objectives
- defined by means of boundaries wherever possible

The ease of delineating the boundaries of provenances depends on the natural distribution pattern of the species. If a species is restricted to a single site or the distribution is limited and discontinuous, the term 'provenance' may be synonymous with 'site' and can be readily defined.

The problem of delineating provenances is much more difficult with species that occur over an extensive area—during initial sampling, provenance boundaries may have to be set in an arbitrary way in the absence of hard information on geographic variation.

Selection of provenances

The term provenance is used to serve as a marker to identify the local population and the population boundary is therefore the provenance boundary. *Turnbull and Griffin* (1986) make the point that it is rarely possible to delineate natural provenance boundaries on the basis of gene exchange.

Variation within these widely distributed species may sometimes be as great as the variation from between closely related species. Other species have a more limited distribution which, however, may sometimes consist of isolated provenances adapted to specific environmental conditions.

The area constituting a local population, provenance, or region of provenance, is determined arbitrarily on the basis of local ecological conditions and meeting the criteria of minimum number of sampled plants.

In natural habitats, especially where they cover extensive areas in underdeveloped regions, it is often difficult to find an appropriate name to indicate provenance. It is common practice to name the provenance after the river, nearest road, town, geographic feature, which may be some distance from the actual collection site. A single name is frequently insufficient to convey the exact location of a population of plants.

There is no standard way of assigning provenance names and they frequently indicate a general area only. Lack of precision in applying locality names must be compensated for by the provision of latitude and longitude co-ordinates, an accurate altitude or a map showing the collection site in relation to local features. It is essential that the location of the collection be sufficiently precise to enable others to return to the location.

The choice of provenances to represent species should involve a careful, detailed study of the climatic, edaphic, and other factors within the natural distribution.

Once in the field minor adjustments were made to the locations according to seed crop abundance, lack of human disturbance to the stand, and convenience of access. For species with a very restricted and disjunct distribution, it may be necessary to sample all sites even for use in a species trial.

For species in which comprehensive provenance trials have already been conducted, the published results are an important source of information when determining which provenances to focus on.

Because of the frequent limitations placed on resources, there is a trade-off between numbers of provenances collected and numbers of plants sampled per provenance.

It is frequently a question of whether to collect from a few provenances with a large number of plants per provenance as against a large number of provenances with limited plants per provenance.

Sampling provenances within species can be split according to two distinct requirements:

(1) Sampling methods for species introduction trials.

For the first requirement, where there is little known about the species variation, several provenance collections should be made to include:

- sampling from that part of the natural range where the species appears to be growing best.
- part of the range that most closely matches the climate for which the seed is required.
- marginal sites within the natural range.

(2) Wide-ranging sampling of many provenances to represent part or whole of the distribution for use in provenance trials.

Requirement (wide-ranging sampling for provenance trials) the number of sources sampled will depend on the extent of the natural distribution, the diversity of the species, ease of access, seed availability, time available, money, staff resources, and other resources available to mount a collecting expedition. A knowledge of the breeding system of the target species and its pollen and seed dispersal mechanisms will assist in determining the collection strategy.

3. Sampling strategy

- ❑ What we need to do ?
- ❑ How do this?

For many potential *users* and *uses* of the collection, it is important to maximize the number of alleles present within the sample, by capturing the greatest proportion of those alleles represented in the field population.

The optimum sample size per collection site would be the number of plants required to obtain the alleles in a population that occur at 5 % frequency or more.

According to Brown and Marshall (1995), at least one copy of 95% of the alleles occurring in the population at frequencies of greater than 0.05 can be achieved by sampling from:

- 30 randomly chosen individuals in a fully outbreeding sexual species,
- 59 randomly chosen individuals in a self fertilizing species.

What we need to do ?

- Give priority to species which can be most easily utilized, or those endangered
- Choose target area based on distribution patterns, focus on *centres of diversity* ('*place of origin*')
- Sample as diverse geographical and ecological sites as possible
- Put the emphasis on collecting more sites rather than larger numbers of plants per site.
- To sample from in excess of 50 individuals, from within a single population, where available
- To look for populations with larger numbers of plants.
- Plants that are more closely related are more likely to contain similar genes, so you collect less genetic variation when you collect from closely related plants.
- When plants do not all flower and fruit in the same period, you may want to visit the seed source several times. Plants that flower at different times often have different genes, and you want to collect as many different genes as possible.

- Keep the distance between plants to more than 5 meters and ensure that there is an isolation distance of 100 m of plants of same species.
- Collect equal amounts of fruits or seeds from each plant. In this way, you will ensure that the genetic information of each seed plant is equally represented within the seed lot.
- When most of the seeds of your collection come from the same plant, then more of the genes in your seeds will be similar.
- Fully ripened mature seed is going to have the highest viability, the most vigor, and greatest longevity in storage.
- While it is sometimes possible to collect immature fruits, whose seed finishes ripening while attached to the harvested stems in the collection bag, this collection method should be avoided for important conservation collections.
- Depending upon the purpose and ultimate use of the seed collection, collections can be made consisting of seed from all individuals sampled within the population – packaged collectively - or the collection can be made along
 - Maternal lines where seeds from each individual sampled are kept separate.

How do this?

- Individual plants
- Covering whole habitat
- Random by transects:
 1. Random sampling
 2. Systematic sampling
 - line transect
 - belt transect
 3. Stratified sampling
- Based for conspicuous phenotypes

Individual plants

The seed collected from individual plants is usually combined or 'bulked' together to form a seed lot and mixed to an homogeneous blend.

Similar quantities should be collected from each plant so that no individual is favourite or underrepresented in the genetic make-up of the seed lot.

The way we sample individual plants at the collection location strongly influences the genetic quality of seed collected.

Ideally, look for local plants that are in healthy and viable natural populations and are large enough to provide sufficient seed by sustainable and responsible collection methods (sampled from at least 50 individual plants).

Ensure the target species is uniformly distributed, with a mature seed crop of preferably at least moderate quantity.

Make sure of plant identification. If there are any doubts about identity it is essential to keep seed separate until it can be accurately identified from a voucher specimen.

Collect seed from at least 10 to 20 widely spaced, healthy parent plants (not diseased) across the population.

Wherever possible, aim to collect from genetically unrelated plants, thus increasing the capture of genetic variability of the population; that is, from plants that are unlikely to be breeding with one another. This may be difficult for many reasons – and be aware that even in large, natural populations there may be high levels of inbreeding and genetic structuring.

Collect seed only from plants separated from one another by a distance of at least twice the plant height.

Avoid collecting from reproductively isolated individual plants, even if they carry heavy seed crops. If you don't have any option but to collect from isolated plants, then make sure you bulk this seed with that from other local plants (of that species) to achieve increased genetic diversity in the mix.

Collect seed from sites with similar conditions (rainfall, soil, altitude and aspect) as the re-vegetation site.

Do not take more than 10% of the seed of any individual plant.

Covering whole habitat

Sample area	Number of plants
<1 m ²	1
1-10 m ²	2-10
10-100 m ²	11-100
100-0.1 ha	100-1000
0.1 ha-1 ha	100-1000
1 ha-10 ha	1000-10000
10-100 ha	1000-10000
>100 ha	>10000

Random by transects:

1. Random sampling

Random sampling is usually carried out when the area under study is fairly uniform, very large, and or there is limited time available.

When using random sampling techniques, large numbers of samples/records are taken from different positions within the habitat.

A quadratic frame is most often used for this type of sampling. The frame is placed on the ground and the plants inside it counted, measured, or collected, depending on what the survey is for.

This is done many times at different points within the habitat to give a large number of different samples.

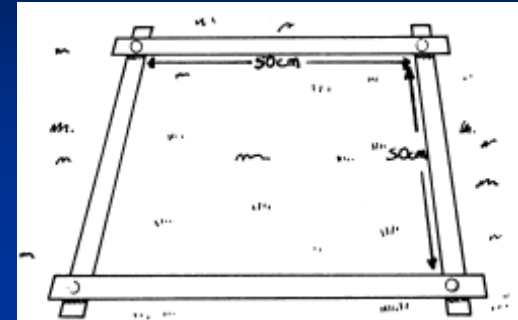
In the simplest form of random sampling, the quadratic frame is thrown to fall at 'random' within the site. However, this is usually unsatisfactory because a personal element inevitably enters into the throwing and it is not truly random.

True randomness is an important element in ecology, because statistics are widely used to process the results of sampling.

However, this is unsatisfactory because a personal element enters into the throwing and it is never completely random.

True randomness is an important factor, because many of the common statistical techniques used to process results are only valid on data that is truly randomly collected. This technique would also only be possible where quadrats of small size are being used. It would not be possible to throw anything larger than a 1m quadratic and even this might pose difficulties.

It would be impossible to throw anything larger than a 1m² quadrat and even this might pose problems. Within habitats such as woodlands or scrub areas, it is also often not possible to physically lay quadratic frames down, because tree trunks and shrubs get in the way. In this case, an area the same size as the quadrat has to be measured out instead and the corners marked to indicate the quadratic area to be sampled.

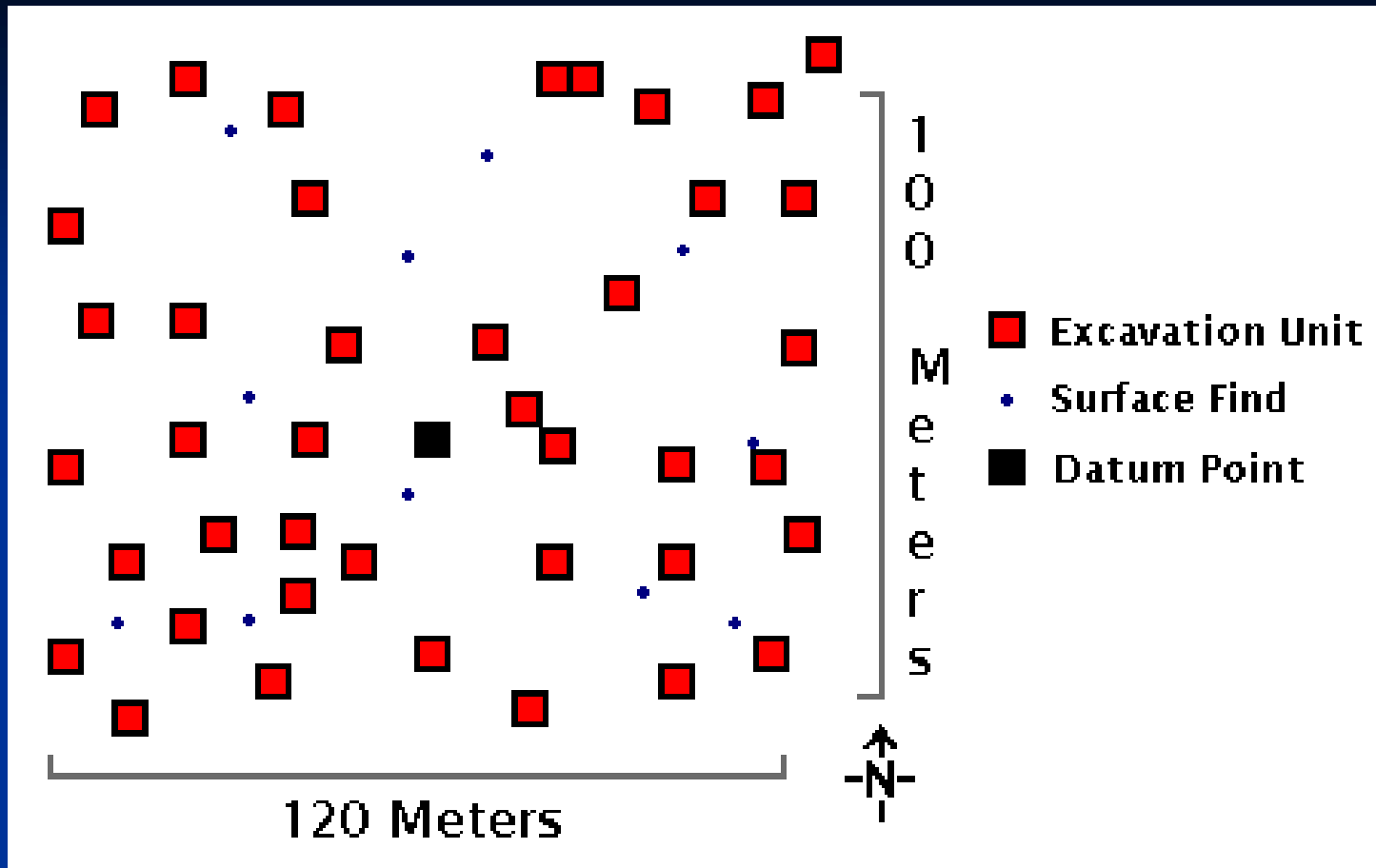


A better method of random sampling is to map the area and then to lay a numbered grid over the map. A (computer generated) random number table is then used to select which squares to sample in. ([Random number table](#)). For example, if we have mapped our habitat, and have then laid a numbered grid over it as shown (Figure - below), we could then choose which squares we should sample in by using the random number [table](#)

20	17	42	28
74	49	04	19
94	70	49	31
22	15	78	15

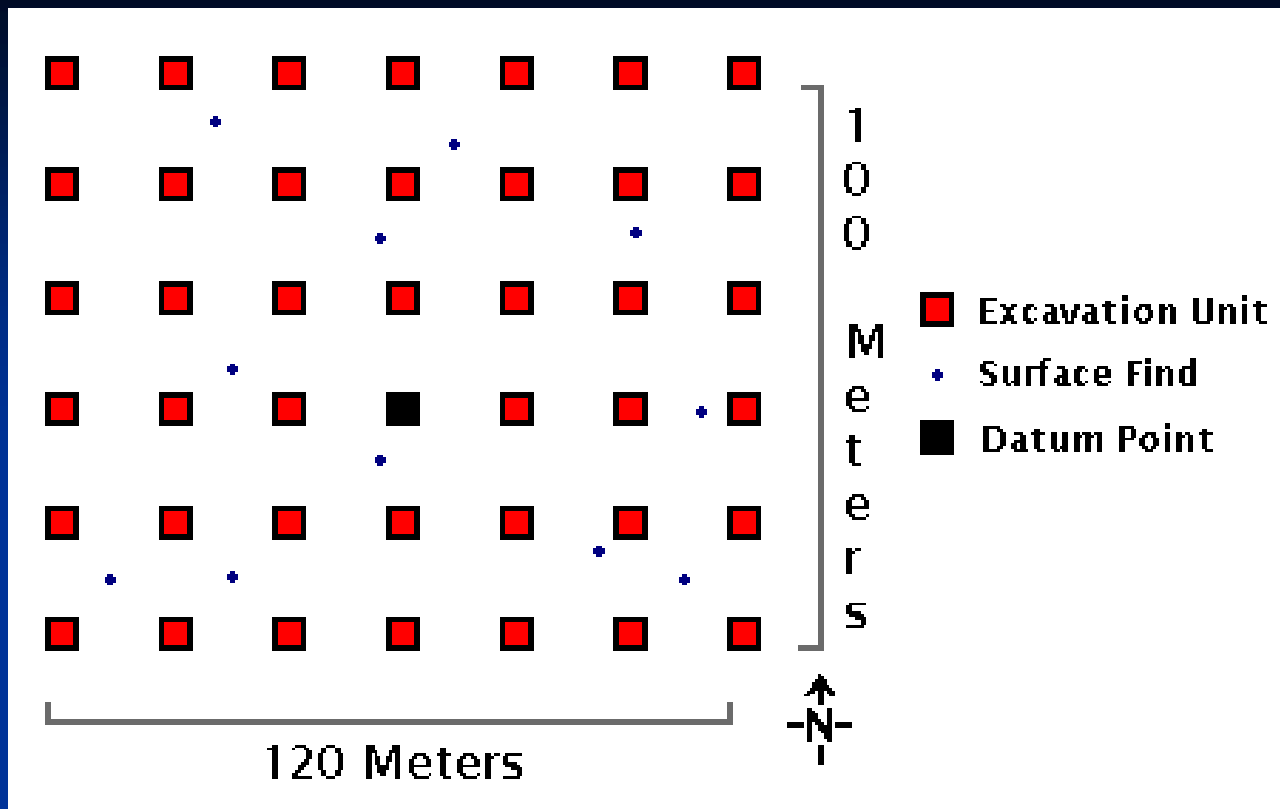
If we look at the top of the first column in the random number [table](#), our first number is 20. Moving downwards, the next two numbers in the random number table would be 74 and 94, but our highest numbered square on our grid is only 29. We would therefore ignore 74 and 94 and move on to the next number which is 22. We would then sample in Square 22. We would continue in this fashion until we had obtained enough samples to be representative of the habitat. There are other methods for selecting numbers from a random number table, but this is the simplest.





A random sampling strategy is the least biased sampling method. Using this method, the locations of excavations are determined by generating a list of random coordinates and excavating units at those coordinates.

This method introduces the least sample bias. However, as shown above, random sampling can provide uneven coverage or concentrate units in areas away from surface finds



In a systematic sampling strategy, the goal is to provide equal, and *unbiased* coverage of a suspected site.

This method is useful for determining the boundaries of a site.

In constructing a systematic sampling strategy, excavation units area usually distributed across the study area in a way that will provide equal coverage to the entire area. In this case, the 5x5 meter squares are evenly spaced at 20 meter intervals across the site.

2. Systematic sampling

Systematic sampling is when samples are taken at fixed intervals, usually along a line. This normally involves doing transects, where a sampling line is set up across areas where there are clear environmental gradients.

For example you might use a transect to show the changes of plant species as you moved from grassland into woodland, or to investigate the effect on species composition of a pollutant radiating out from a particular source .

Line Transect Method

A transect line can be made using a nylon rope marked and numbered at 0.5m, or 1m intervals, all the way along its length. This is laid across the area you wish to study.

The position of the transect line is very important and it depends on the direction of the environmental gradient you wish to study. It should be thought about carefully before it is placed.

You may otherwise end up without clear results because the line has been wrongly placed.

For example, if the source of the pollutant was wrongly identified in the example given above, it is likely that the transect line would be laid in the wrong area and the results would be very confusing.

Time is usually money, so it is worth while thinking about it before starting.

A line transect is carried out by unrolling the transect line along the gradient identified. The species touching the line may be recorded along the whole length of the line (continuous sampling).

Alternatively, the presence, or absence of species at each marked point is recorded (systematic sampling). If the slope along the transect line is measured as well, the results can then be inserted onto this profile.

Why use line transects?

Line transects are used when you wish to illustrate a particular gradient or linear pattern along which communities of plants change. They provide a good way of being able to clearly visualise the changes taking place along the line. Depending on how detailed the line transect is, they can usually be accomplished fairly quickly.

Line transects do not produce as much information on the relative densities of individual species as a **belt transect** would do. A line transect tells you what is there, but gives limited information on how much of it is present. If detailed density information is required then a belt transect must be carried out instead.

Belt Transect Method

This is similar to the line transect method but gives information on abundance as well as presence, or absence of species. It may be considered as a widening of the line transect to form a continuous belt, or series of quadrats.

In this method, the transect line is laid out across the area to be surveyed and a quadrat is placed on the first marked point on the line. The plants inside the quadrat are then identified, their abundance estimated, while it is usual to estimate the percentage cover of plant species.

Cover is the area of the quadrat occupied by the above-ground parts of a species when viewed from above. The canopies of the plants inside the quadrat will often overlap each other, so the total percentage cover of plants in a single quadrat will frequently add up to more than 100%.

Quadrats are sampled all the way down the transect line, at each marked point on the line, or at some other predetermined interval (or even randomly) if time is short. It is important that the same person should do the estimations of cover in each quadrat, because the estimation is likely to vary from person to person.

The height of plants in the quadrat can be recorded and the biomass of plants can also be measured by harvesting all the plants inside the quadrat and then weighing either fresh, or dry weight in the laboratory.

This is obviously a very destructive method of sampling which could not be used too often in the same place. Sampling should always be as least destructive as possible and you should try not to trample an area too much when carrying out your survey.

3. Stratified sampling

Stratified sampling is used to take into account different areas (or strata) which are identified within the main body of a habitat. These strata are sampled separately from the main part of the habitat. The name 'stratified sampling' comes from the term 'strata' (plural) or stratum (singular).

For ease of understanding, the term 'unit' will be used in the following explanation, rather than stratum.

Individual habitats are rarely uniform throughout their extent. There are often smaller identifiable areas within a habitat which are substantially different from the main part of the habitat.

For example, scrub patches within a heathland area, or areas of bracken in a grassland

One of the problems with random sampling is that random samples may not cover all areas of a habitat equally. To continue with the example of bracken patches in a grassland, if the area was random sampled, it is possible that none of the samples might fall within the bracken patches. The results would then not show any bracken in the habitat.

Clearly this would not be an accurate reflection of the habitat. In this sort of situation, stratified random sampling would be used to avoid missing out on important areas of the habitat. This simply means identifying the bracken as a different unit within the habitat and then sampling it separately from the main part of the habitat.

While the bracken area clearly needs to be taken into account, it is nevertheless important to avoid overemphasising its significance within the habitat as a whole. Its importance is kept in context by locating a proportional number of samples directly within the bracken unit. The proportion of samples taken within the unit is determined by the area of the unit in relation to the overall area of the habitat.

For example, say the grassland area is 200 m² overall, with the bracken patch occupying 50 m² of this total area. The bracken therefore accounts for 25% of the total grassland area. Say it has been decided that a total of 12 samples need to be taken in order to accurately reflect the composition of the whole habitat. Then 3 of those samples (one quarter, or 25%) would be located within the bracken unit and 9 (three quarters, or 75%) in the general grassland area.

There is a standard formula for calculating the number of samples to be placed in each unit. This is:

$$\text{The number of quadrats sampled in the unit} = \frac{\text{the area of the unit} \times \text{the total number of quadrats to be sampled}}{\text{total area of the habitat}}$$

For example, in the illustration given above this would be:

$$3 = \frac{50 \times 12}{200}$$

4. Seed collecting techniques

Collecting methods vary for each species.

- **Plucking of whole fruits**
- **Stripping entire seed-heads is most suitable for:**
 - Dense, mono-specific stands of target species with no weed or other species present.
 - Inflorescences which are completely and consistently at the natural dispersal stage.
- **Shaking it off the stem**
- **Clipping the stem with scissors or small scythes just below the spikelet**
- **Collect ripe seeds by gently rubbing inflorescence over a bag or tray.**
- **Collect ripe seeds by laying tarpaulins or plastic sheeting beneath the plants.**

The most basic and flexible of techniques; hand picking has many benefits

■ **Cutting:**

Used for herbaceous plants, especially grasses, this involves gathering all the stems of one plant in one hand, and then cutting the seed heads with a sickle in the other hand. Wear leather gloves for protection from sharp blades!

■ **Stripping:**

Grass seeds can be harvested by stripping seed off the stem or by clipping the seed culm (stem) just below the spikelet. The seeds of many broadleaf herbaceous plants can be collected by holding a bag or tray under the plant and shaking seeds from the plant. Gloves should be worn for this.

Beating:

Used for shrubs, this method involves gently tapping branches with a stick to dislodge seeds onto a tarp spread under the plant.

■ **Shaking:**

Herbaceous plants and wildflowers with seeds in pods or capsules are easily collected by collecting the entire pod or capsule or by shaking the inflorescence over a tray to catch the seeds.

For species that dehisce explosively, the entire inflorescence must be cut before maturity and allowed to dry in mesh bags. Pods from species having spike-type inflorescences (lupine and penstemon) may be stripped in the same manner as grasses.

The pappused (parachute-type) seeds of many species in the Composite (sunflower) family can be swept or brushed into bags if timing of collection is ideal. For very small annual plants, the simplest method may be pulling the entire plant and bagging in cloth or paper bags.

Seeds of many woody, non-fleshy-fruited plants are collected by holding a tray or bag under the branches and flailing the branches with a stick or tennis racket, knocking the seed into the receptacle.

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

1. Per single flower inflorescence (e.g. *Tulipa* with one capsule, but also a single flower of *Clematis*). In some cases for e.g. *Tulipa* the seed number of a single flower is the number of the total plant (species with a single shoot).

2. Per multiple flower inflorescence, per single shoot (e.g. *Vicia cracca*, the umbel of *Daucus carota*, the panicle of a grass species or of the shrub *Sambucus racemosa*).

3. Per multiple flower-stem or per fertile frond (in the case of horsetails per fertile shoot/stem). Species from disturbed habitats have very often multiple-flower stems, for e.g. Compositae, Chenopodiaceae, Umbelliferae or Cruciferae.

This category can be also interesting to multiple stem bushes as Ericaceae. This category is defined as the seed number per single stem (branched or unbranched) above ground. A single stem with a root point is defined as one ramet.

4. Per ramet or total individual plant (e.g. a tree or an annual herb).

5. Per square metre. The reproductive capacity of one species per m² can be calculated as the potential seed number per species to 100 % cover in the vegetation unit.

The reproductive capacity of a population is characterized as the number of seeds produced by one species per 1 m² at its one hundred-percent cover per one season.

Basic rules in wild seeds collection

- Try to collect from fruit in the middle or upper portions of the plant rather than the lower portions.
- Because seed heads mature sequentially, you might collect at the site again in a few days.
- Transfer seed to the appropriately labelled paper or cloth bag immediately after collection, allowing it to dry without further handling.
- Never collect seeds from rare or endangered species—collect only from plants that you find growing abundantly in a given area, to ensure that you do not eradicate an isolated population.
- Gather fruits from the ground only if they have recently dropped.
- Seed harvested from different areas is best kept separate. It is always possible to combine several seed sources at a later date for a larger purchase if seed source is not critical.
- When harvesting seed of native grasses it is important to avoid contamination with the seed of noxious weeds and non-native grasses.

- Thoroughly check the area for the presence of non-native species before harvesting,
- Foot access only is recommended in these areas.
- Collect from as many plants of a single species as possible throughout the collection area, but never less than 10 plants, 50 plants a minimum for herbaceous species.
- Collect each species for the project several times throughout the seed ripening period; early, mid and late ripening seed.
- By starting seed collection efforts at lower elevations and following maturation up slope the optimum seed collection period can be extended. If seeds of the target species have shattered on south- or west- facing slopes, seed of the same species may still be available for collection on north- or east- facing slopes.
- Avoid collecting the same species from the same site in consecutive years.
- Collecting seeds that have been pollinated is best. This means nature has done the work for you - the wind, bees, and the insects have done the pollination. The opposite of this is hybridization where breeders decide parents and try to develop a plant with 'new and improved' characteristics. Open-pollination will create plants just like the original. However, every so often an unusual plant might occur.

5. Recording the data

A plant collection without accompanying data is of no use to the scientific community.

Keep a careful record of collection data and field observations in a field notebook using a consistent, clear, and legible style.

The collection of data associated with the species or populations from which seed is taken is a vital contribution to knowledge about these plants.

Existing information on floral biology and ecology should be recorded and additional supplementary data should be collected.

To reduce the amount of writing needed in the field book and on labels, collect as many different plants as possible from the same site.

- Label all samples legibly and unambiguously. Make sure all samples are tagged.
- If any special or significant sampling methods were used, note what was done.
- Note any pest problems associated with the parent plant at the time of collection.
- If possible, make arrangements with the propagation facility before sample collection.
- Submit samples to the propagation facilities **as soon as possible!** Delays may have deleterious effects on sample viability.
- Always use two labels, one fixed to the outside of the container or bag, and one inside together with the seed.
- The labels and ink should be waterproof. For identical seedlots, write total number of containers together with container number, e.g. 1 of 3.

Seed sampling form

- Family, genus, species (scientific name, common name), infra-specific
- Provenance name
- Number of plants sampled
- Number of plants found
- Number of plants collected from: 1-10 / 10-100 / 100+
- Seed crop quantity: Heavy / Medium / Light
- Seed harvesting (early, mid, late season)
- Seeds collected from (plants, ground, both)
- State of seeds (moist, dry, both, other)

Location description form

- Seed source location
- Country
- Site description: terrain, slope, aspect, soil type (colour, texture and pH)
- Accessibility (distance, accessibility road, collection permit)
- Habitat and associated species

Collection data

- Date collected
- Collection number
- Collector's name
- Geographical coordinates: Latitude/Longitude/Altitude
- Map reference

Additional data

- Herbarium data
- Size of population: 1-10 / 10-100 /100+
- Abundance of population and species:
- Protected area map
- Vegetation map
- Photographs

Equipment which may needed

Seed collection

- Seed containers (field). Sacks and bags (can be re-used).
- Seed containers (despatch). Cotton bags, canvas sacks (despatched with seed).
- Tree markers, e.g. plastic tape.
- Climbing equipment. Foot spurs or tree bicycles or ladders. Safety belt, safety ropes, safety helmets, tool lines.
- Seed cutters, e.g cone hooks, cone rakes, pruning shears, secateurs (hand).
- Plastic sheeting (heavy gauge) for protection when storing fruits, extracting seed, etc.
- Binoculars for studying tree crowns, fruit development, etc.
- Walkie-talkie radios (special permission may be needed).
- Insecticidal and fungicidal powders for seed protection (use with care).
- Axe, saw, machete, knife.
- Rope, string, labels, felt marking pens.

Site description

- Notebook, description forms.
- Maps (outline copies also for plotting).
- Compass.
- Altimeter.
- Meteorological equipment (hygrometer, max/min thermometer).
- Soil survey equipment (auger, colour charts, pH test kit).
- Tree measuring equipment (altimeter, diameter tapes, bark gauge, etc.).
- Camera and equipment (wide angle lens).
- Tape recorder (battery powered).
- Spade.

Specimen collection

- Botanical presses (may be made locally).
- Drying papers (local newspapers will do).
- Plastic bags.
- Specimen bottles.
- Preservative fluid.
- Increment borer (for wood samples).
- Carpenter's brace and auger (for resin samples).
- Insulated container (e.g. ice box).
- Hand lens.
- Insecticide spray (for herbarium material).
- ALSO Medical supplies, camping equipment, vehicles and equipment as appropriate.

Transport

- Two spare tires, pump and pressure gauge
- Puncture repair kit with lots of patches
- Heavy duty hack & tire leavers
- Petrol cans, large funnel & plastic tubing

Medical supplies

- Insecticide sprays
- Insect repellent creams
- Antibiotic tables
- Antacid tablets for minor stomach upsets
- Pain killer such as aspirin, Panadol, baralgin
- Bandages
- Ban aid
- Water purifying tablets
- Snake bite serum

6. Care of collections in the field

- 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.
- Care should be taken that seed collections do not overheat, for example by being left in a vehicle in full sun. Exposure to such sustained high temperatures can badly damage the seed collections.
- Attempts should be made to maintain ventilation around the collections at all times and the collecting vehicle should be parked in the shade, or at the very least, the windscreen shaded.
- Damp collections should, as soon as possible, be spread out on newspaper to dry naturally, either outside in the shade or in a well ventilated room, before dispatching material to institute.
- Do not pack samples with excessive moisture or allow samples to sweat in the bags for an extended period of time. This promotes fungal and bacterial growth and accelerates the decline to sample quality.
- In a few cases 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 ready for shipping. In most cases, it is best to leave the task of cleaning the collections to institute, processing staff who have the full range of facilities necessary to carry out this task.

- Try to cushion material so it won't be crushed.
- Use “breathable” containers such as paper envelopes or bags. Do not use plastic bags.
- Vegetative materials deteriorate quickly post harvest and quick transfer from field to the propagative facilities. Facility is imperative to ensure maximum viability.
- Additional care must be taken during transport since they are easily damaged.
- Clean samples to remove poor quality seeds.

Fleshy fruits may require careful handling and partial cleaning. Rapid dispatch is recommended.

There are two basic options:

- **Pack the whole fruits** in strong plastic bags with as much air as possible. The bags should then be packed in some kind of rigid plastic container. This should ensure the fruits are not squashed and also do not get too hot and ferment too much during their journey.
- **Remove as much flesh** from the fruits as possible before transit. This can be done under cool running water using a sieve. The seeds should then be left to air dry *for a little while* before sending. Dry carefully on material that will not stick to the seeds (do not use newspaper). They should then be packed as dry seeds, i.e. in cloth bags.

Collecting for use in a breeding programme

Breeders and plant introduction workers often have very specific requirements for particular traits.

One possible way of obtaining desired traits is to systematically screen available gene bank holdings of the target species and related taxa in the primary and secondary gene pools.

Characterization and evaluation data may be available for particular collections (or parts of collections), but the distribution of desired traits in the gene pool is often not known in details.

However, their presence can often be predicted with some confidence from consideration of the eco-geographical passport data associated with the accession.

- If material adapted to particular climatic conditions is being sought, only those eco geographic regions satisfying these requirements will be targeted (homoclimate strategy).
- Searching for germplasm with tolerance to abiotic stress should start in the areas where the species has been exposed to the stress factor for a considerable period.
- In the case of pest resistance, collecting will be focused on areas where there is a long documented history of coexistence of the host and the pest.

In summary, when collecting for specific traits, the target area should be chosen after studying the available eco-geographic and pest information (including information on the distribution of alternate hosts), analysing existing characterization and evaluation data and/or screening gene-bank accessions for the desired (or correlated) trait.

Relatively small areas should be selected for careful, intensive sampling. Comparatively large random samples should be collected from populations likely to harbour the desired traits

In addition to a population sample, a selective sample (e.g. of healthy individuals in an infested field) can be collected if the expression of the target traits is obvious and stable.

Collecting seed from rare and threatened plant species

When collecting rare and threatened species, the collector needs to think about a number of factors before going into the field. To start with, it is necessary to think about what seed samples will be used for.

Once in the field the collector needs to consider a number of other factors:

- 1) For developing germination and propagation protocols, or to examine seed behaviour, use existing *ex situ* material if available. If wild populations must be sampled, begin with small collections from the largest and most secure populations. For developing reintroduction protocols, make the smallest collections possible to address the management questions being posed in experimental reintroduction.
- 2) To increase the probability of successful, self-sustaining populations of threatened plant species, collect from as large and diverse an array of founders as is prudent. If possible, collect and maintain separately seeds from each maternal line.
- 3) Where possible, spread the collection out over two or more years, especially for small populations.
- 4) For species with 50 or fewer populations, collect from as many populations as possible. For species with more than 50 populations, collect from as many as possible up to 50. For populations with 50 or fewer individuals, collect from all known individuals; for populations with more than 50 individuals, collect from 50.

5) For populations of species with extremely low overall numbers, particularly those (a) that have 10 or fewer reproductive individuals and a poor history of recruitment, or (b) are known to be in rapid decline, collection of seed should be made at the discretion of the licensed collector. The decision about how much to collect should be based on as much information as possible, including species autecology, nature of threat, ex situ conservation facilities and knowledge base available etc.

6) Record as much additional information about the population as possible, including:

- _ Total number of plants
- _ Number of juvenile plants
- _ Number of adult plants
- _ Number of dead plants
- _ % of plants flowering/fruiting in the last season
- _ Seed production (per fruit and per individual)
- _ Threats to habitat
- _ Threats to species

7) Avoid destructive sampling. Consider carefully whether herbarium vouchers are essential to the naming and classification of the plant sampled. Do not take herbarium samples if it will reduce the populations capacity for survival . take photos and detailed notes instead.

Conclusions

The most economical collection method is that of seeds of good quality which are collected from carefully selected seed sources in well planned collection expeditions, with well trained personnel and under professional supervision.

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