Collecting woody perennials

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Introduction

Woody perennials have increasingly been targeted for germplasm collecting in recent years. This is associated with an upsurge in interest in a wide range of lesser-known species which have potential for agroforestry and small-scale, non-industrial plantings. At the same time, the importance of using the best available germplasm for industrial forestry plantations has also become widely appreciated. For many tropical species at an early stage of domestication, the best available germplasm remains that obtained through collecting seeds from wild stands of known superior provenances of proved adaptability to specific sites. 'Provenance' refers to the geographic origin of a particular seed source (Turnbull and Griffin, 1985; but see Jones and Burley (1983) for a discussion of different definitions and their limitations). In addition to seed collecting for evaluation trials and for large-scale use, a major challenge is to collect and conserve *ex situ* the genetic resources of many socioeconomically important woody species which are endangered in their native habitats.

This chapter will provide some specific guidelines on the planning and execution of field collecting of woody perennials. Germplasm collecting of wild populations of woody plants will differ from collecting other wild species and field crops in a number of important ways.

- Most tree species are preferentially outcrossing organisms with high levels of intraspecific variation. These characteristics will affect sampling strategy and frequency ('Sampling strategy').
- Most tree species do not flower and fruit gregariously every year. They flower at intervals typically of two to three years but sometimes of up to five to eight years ('Importance of collecting in most seed years' and 'Planning and reconnaissance').

- In the case of tall trees and those growing in denser formations the fruits are usually in inaccessible parts of the canopy. Therefore, various specialized harvesting techniques may need to be employed ('Seed collecting methods and equipment').
- The ratio of fruit to seed mass is often high for woody species. Therefore, when undertaking extended expeditions to remote localities, it is often necessary to partially process and clean the fruit to reduce bulk and weight, and also help prevent fungal attack ('Handling, processing and transport of fruits and seeds').
- Though germplasm collecting of woody plants will usually be accomplished most efficiently by collecting seeds, in some situations collecting other plant parts may be desirable or necessary ('Vegetative germplasm collecting procedures').
- The majority of woody species have coevolved with root microsymbionts, which may be essential for their healthy growth and long-term survival. Cosampling of symbiont germplasm during seed collecting expeditions may thus be necessary ('Cosampling of root microsymbiont germplasm').

Sampling strategy

Chapters 5 and 6 deal with collecting strategies in detail. This section discusses both the general principles of sampling and the practicalities with specific reference to woody species.

Sampling objectives

Germplasm may be collected for research, conservation, immediate use and/or evaluation for future use. For evaluation (e.g. provenance trials) and *ex situ* conservation, the aim in collecting is to obtain a genetically representative sample. Therefore, collectors must be careful not to bias sampling when selecting seed trees. For some specific purposes it may be advantageous to impose a level of phenotypic selection. However, this will only be the case when highly heritable traits are involved, e.g. wood grain orientation, leaf oil composition, flower colour. Usually, phenotypic selection will be either genetically undesirable or a waste of time and resources, especially if the material will be grown in areas remote from, and environmentally unlike, the collecting site. Turnbull (1978) and Willan *et al.* (1990) provide useful discussions of sampling strategies for woody plants.

Sampling approaches

Information is increasingly becoming available on the pattern of genetic variation for tree species with extensive, more or less continuous distributions, e.g. many *Eucalyptus* spp. (Green, 1971; Boland, 1978; Turnbull, 1980; Moran and Bell, 1983; Moran and Hopper, 1987), *Acacia mangium* (Moran *et al.*, 1989a), *Casuarina cunninghamiana* (Moran *et al.*, 1989b) and many northern hemisphere conifers (e.g. *Picea sitchensis*: Falkenhagen, 1977; *Pseudotsuga menziesii*: Merkle *et al.*, 1988). The proportion of genetic diversity among populations of insect- and bird-pollinated Australian trees has been found to be double that for northern hemisphere wind-pollinated conifers and angiosperms (Moran, 1992). For many Australian trees with widespread distributions, the bulk of genetic diversity resides within populations. National Research Council (1991) reviews what is known of the structure of genetic variation in forest trees.

The present practice of gene pool sampling of widespread species for provenance or progeny trials usually involves collecting 10–20 individuals per provenance, with individuals spaced at intervals of not less than the distance of normal seed dispersal. A common general rule has been to keep a minimum of 100–200 m between sampled individuals. When collecting seeds of preferentially outcrossing species, it is advisable to avoid sampling reproductively isolated individuals, because these may hold a disproportionately high proportion of selfed seeds. If the collecting is for *ex situ* conservation, the number of sampled individuals should be increased wherever possible to 25–50 per population. For improvement programmes and seed orchards the number of individuals sampled may be $\geqslant$200 per population to avoid excessive narrowing of the genetic base at later cycles of selection. There have even been some suggestions of minimum numbers for *ex situ* and *in situ* conservation in excess of 2000 individuals (Krusche and Geburek, 1991).

The purpose of maintaining a reasonable distance between sampled trees is to avoid or reduce the likelihood of sampling related individuals. The degree of relatedness among neighbouring individuals will be influenced by several factors, including breeding system, stand size and history (recruitment events) and, in particular, the efficiency of pollen and seed dispersal. The sampling distance between individuals of species with poor seed dispersal (e.g. *Adansonia gregorii* and *Lodoicea maldivica*), those which form extensive clonal stands by root suckering (e.g. *Eucalyptus porrecta*, *E. ptychocarpa* and *E. jacobsiana*: Lacey, 1974; *Tamarix* spp.) and those which reproduce apomictically will need to be greater than that for species with efficient long distance seed dispersal by wind (e.g. *Alstonia* spp., *Casuarina* spp., *Cedrela toona*, *Securidaca longepedunculata*), water (e.g. *Acacia stenophylla*, *Eucalyptus camaldulensis* and most mangroves) or animal vectors (e.g. *Elaeocarpus* spp. and *Ficus* spp.).

In species occurring in small, disjunct populations, the major component of intraspecific variation will usually be among populations (e.g. Moran and Hopper, 1983, 1987). Accordingly, an optimal strategy to capture genetic variation will involve sampling relatively fewer individuals from as many populations as possible. This approach is also applicable to colonizing woody species (e.g. *Acacia cowleana*) which may occur as many small 'satellite' populations. Although within-population diversity may be highest in optimal areas central to the range of a species, marginal and isolated populations should also be sampled, as they will possess adaptations to extremes. Willan *et al.* (1990) suggest that one central and four peripheral populations is an absolute minimum number of provenances in first-stage trials of multipurpose trees (as, indeed, for *in situ* conservation), ranging up to $\geqslant$30 populations for species with extensive and ecologically diverse ranges.

Optimizing the mix of collecting effort between and within populations is often problematic in the absence of information on the pattern and distribution of genetic variation for the target species. Growth and morphological characterization can be used to assess the extent of variation and help provide the basis of sampling strategies. Preliminary coarse-grid isozyme or even DNA surveys may be used to help define more efficient sampling strategies for comprehensive seed collecting and evaluation of lesser-known tree species (Moran, 1992). These are discussed in Chapter 6. A knowledge of the breeding system of the target species and its pollen and seed dispersal mechanisms will clearly be important in the development of a collecting strategy for first-stage sampling.

Individual tree versus bulk provenance collecting

The choice of whether to collect and maintain seeds of individual trees separately or bulk them in the field will largely depend on the purposes for which the seeds will be used. Seed collecting on an individual tree basis is required for studies of genetic variation and tree improvement programmes. Bulk provenance collecting will often suffice for first-stage sampling for provenance trials of less well-known species. However, bulking of seeds of different individuals should only be done when the collector is thoroughly familiar with the species and is certain of the taxonomic identity of each individual seed tree included in the bulk. Bulk collecting is often favoured for extended expeditions sampling a large number of species. In such cases, it may not be logistically possible to maintain and clean a large number of individual tree seed lots.

Approximately equal amounts of seeds should be collected from each tree included in the bulk (assuming approximately equal germination percentages). The bulked seed lot should be thoroughly mixed. When sampling a given individual, fruits from all parts of the crown should be collected, because these may have been pollinated by different pollen sources. Bulked seed lots must not be biased by the inclusion of a disproportionate number of seeds from a few heavier seeding or more easily collected individuals. In at least some *Acacia* spp., the seeds in each pod are full sibs. Other things being equal, this means that in such species each tree's contribution to a bulk sample should come from as many different pods as possible.

Importance of collecting in mast seed years

In wild stands the amount of fruit borne by a particular individual is not only under genetic influence, but is also strongly affected by age (e.g.

in forest communities the heaviest crops are borne by mature or overmature dominants and emergents) and environmental factors (e.g. edge trees often bear heavily, while isolated individuals of outcrossing species may bear only very light crops). This may introduce a bias in sampling towards those individuals with precocious and/or heavier seeding habits. Such bias may to some extent be unavoidable, but it is often undesirable. An exception may be where flowers or fruits are the desired end-product, as in the case of nut or fruit species for human or stock food, honey species and ornamentals. Precocious and heavy-seeding genotypes are more likely to be less efficient wood producers. On the other hand, precocious genotypes can be used to shorten generation times in tree breeding programmes. Year-to-year variations in the factors that influence flowering and seed production, in particular climatic variables, also cause different trees to be represented as parents in different years (e.g. Houle and Filion, 1993). Undertaking seed collecting only in mast seed years, when many or most individuals are holding collectable numbers of seeds, will minimize these sampling biases.

Seed samples collected in years of sparse flowering and seeding will contain a higher proportion of empty or non-viable seeds and tend to be genetically less representative of the sampled population. For mainly outcrossing species, collecting in poor flowering and fruiting years and from isolated individuals may result in seed lots with abnormally high proportions of selfed seeds. These will usually give rise to genetically inferior plants, e.g. in *Eucalyptus regnans* (Eldridge and Griffin, 1983) and conifers (*Picea abies*: Eriksson *et al.*, 1973; *Pseudotsuga menziesii, Abies procera* and *Picea sitchensis*: Phillips, 1984). For such species there is a risk that provenance differences measured in field trials may be confused with or masked by collection year (i.e. percentage selfing) effects. Therefore, seed collecting in poor seed years is not only inefficient and wasteful of scarce resources, but may also be counterproductive.

Cultivated species

Zagaja (1983) has suggested that 'most of the fruit germplasm collections made in the past can be considered a biased sampling of the population', in that the aim of the collecting was to obtain material with particular characters, rather than to conserve genetic diversity. More recently, random population sampling of tree landraces has been recommended. Hawkes (1980, 1991) suggests that if the trees are being propagated by seeds, a bulk sample (of seeds or cuttings) of 10–15 individuals selected at random from throughout a village area or orchard is generally acceptable. When the trees are clonally propagated, they should be treated as a root or tuber crop, i.e. collected as vegetative material and each distinct morphotype (or traditionally recognized local variety) kept as a distinct sample. If seeds are collected from a clonally propagated cultivar at a given site, it can either be kept as single-tree samples (and given the same number as any vegetative sample) or bulked in a cultivar sample (and given a distinct number).

Collecting cultivated species will thus rely to a great extent on indigenous knowledge. For sampling to be appropriate to the situation, growers will not only need to be asked about the method of propagation of trees, the distinguishing features of different varieties and the total number of varieties in a village or district, but also about the genetic base of their material (i.e. whether seeds or other planting material came from a few or many mother trees) and their degree of adaptation to local conditions (i.e. how long they have been in the area) (Willan *et al.*, 1990). However, there is no less of a role for indigenous knowledge in collecting wild species. In particular, information on local uses can be critical in deciding on whether a given poorly known species might be worth collecting, a decision which would otherwise have to be made on the basis of probably inadequate field observations or simply taxonomic kinship to better-known species. Thus, the fact that the branches of a particular tree are said by local people to be lopped as fodder will be valuable information when collecting potential browse species. Without such information, one would have to actually observe animals browsing the foliage or guess that the foliage was palatable because that had proved to be the case for a closely related species.

Locating seed trees and seed stands

Planning and reconnaissance

A key factor in planning is to time seed collecting to coincide with peak maturation of abundant fruit crops. Accordingly, the flowering and fruiting pattern for the target species must be established. Information is needed on the time of the main flowering season and the time taken for fruits to mature. The period of maturation can vary considerably, even between closely related species. For example, in temperate-zone bipinnate acacias the maturation period varies from four to five months (*A. decurrens*) to 12–14 months (*A. mearnsii*). The time taken for *Eucalyptus* fruits to mature ranges from four to six weeks in *E. brachyandra* and *E. microtheca*, with fruits being shed shortly after maturation, up to 12–18 months for some species in the subgenus *Monocalyptus*. Some species may retain their fruits for several years after maturation.

It is also important to establish whether heavy flowering and seeding occurs annually, biennially or at irregular intervals related to climate and endogenous factors. Prolific flowering and heavy fruit set in many dry-zone species are dependent on particular rainfall conditions. For example, in *Acacia aneura* substantial quantities of fruit are only produced when flowering induced by summer rainfall is followed by good rains in winter (Davies, 1976). In subtropical dry-zone Juliflorae acacias (e.g. *A. colei* and *A. tumida*) substantial seed production has been observed to occur following heavy rainfall (totalling >350 mm) in the latter part of the summer rainy season. Examination of recent rainfall patterns using meteorological station data or remote sensing data can indicate those areas where good flowering and fruiting of dry-zone species are most likely to be occurring. Such information can greatly assist in planning seed collecting expeditions in arid zones and minimizing the time spent searching for seed crops.

Field reconnaissance of populations of the target species can help identify particular areas and individual trees from which seeds are most likely to be available. Reconnaissances are most warranted for species whose fruits take several years to mature and which bear heavily at irregular intervals (e.g. temperate *Pinus* spp. and *Picea* spp.). Reconnaissances are best undertaken either at the time of flowering or when the fruit crop is approaching full size and maturity. Newly formed, green fruit crops are easily overlooked. It should be borne in mind that reconnaissance missions, especially those to remote localities, are expensive and can only provide information on potential collecting sites. Developing fruit crops may abort or be destroyed by a variety of abiotic (strong winds, drought, fire) and biotic (disease and predation by mammals, birds and insects) agents.

Whether in planning or field reconnaissance, local people are often the major source of data on flowering and fruit development in target species, as well as on the distribution of species. These will include both forestry department staff and rural people making traditional use of the trees, either directly, for firewood, building, food or medicine, or indirectly, as fodder for livestock or nectar sources for bees.

In the field

Intraspecific variation in fruiting can occur at a regional level or on a finer scale. The collector will need to weigh up the relative merits of continuing to search in a particular area where seeding is generally sparse, as against moving to another region or aborting the mission. Quite often a population or a small group of neighbouring, and presumably closely related, individuals will be found holding heavy seed crops in a region where seed crops for that species are otherwise meagre or absent. Hence a seed collecting expedition should not be prematurely aborted if the first populations encountered are devoid of useful seed crops.

In more open plant communities, the ability to distinguish fruit-bearing trees of target species, especially from a distance, is a prerequisite for efficient seed collecting. This is a skill that should be developed by aspiring seed collectors. Fruiting trees can often be discerned from a distance through differences in the colour of their canopy. The canopy of trees holding a mature fruit crop will generally have a yellow, brown or reddish-brown tinge. Fruit crops are most easily spotted on sunny days, when the sun is at a low angle (i.e. early to mid-morning and late afternoon) and the light is behind the observer. Red wavelengths are more apparent in the late afternoon, making this the best time of day to locate fruiting trees of species with reddish brown or purplish fruits (e.g. *Acacia mangium* and *Argyrodendron actinophyllum*). A pair of binoculars or field glasses, with a moderate magnification of ×8 or ×10, is an essential piece of equipment for both locating and assessing crops on potential seed trees. Compact lightweight makes (i.e. 25 or 30 mm aperture) which can fit inside a pocket are especially recommended for work in hot climates. A critical safety aspect is that drivers should not also be involved in the task of locating seed trees while the vehicle is moving.

Once a potential seed tree is located it is important to quickly determine whether the seed crop is at the right stage of maturity and of sufficient quality and size to warrant collecting (Barner and Olesen, 1984c). For tall trees growing in forest formations, the first step usually involves scanning the canopy through binoculars to ascertain the extent and distribution of the fruit crop. This is followed by a search of the ground for fruiting material, which can be used to confirm species identity and to provide an indication of fruit maturity and the extent of insect attack or other damage.

During collecting it is important to continuously monitor the level of insect attack in a seed crop, as this can vary considerably between trees within a population (e.g. in tropical acacias such as *A. mangium* and *A. auriculiformis*). Seed collectors should be aware that some species of fig (e.g. *Ficus semicordata* from Nepal) may produce two types of tree, one bearing fruits with viable seeds and the other only wasp-infested gall figs (Amatya, 1990).

Seed collecting methods and equipment

The choice of seed collecting method(s) and equipment will depend on many factors, including:

- the number of seeds required;
- the relative size, number and distribution of fruits;
- fruit characteristics (including stage of ripeness);
- characteristics of the individual tree, stand and site.

Even within a population, a variety of collecting techniques may have to be applied to different individuals to reduce total collecting time. The use of high-technology machinery such as mobile hydraulic platforms and helicopters for collecting from wild populations of woody plants is only rarely warranted and these expensive options will not be discussed further.

The felling of individual trees for seed collecting purposes is not recommended. Such practices can erode the genetic resources of small populations and endanger their continued existence. If collecting is well planned, there is sometimes an opportunity to collect seeds from trees felled for other purposes. However, safety and such factors as rapid seed shed during warm weather often constrain the collecting that can be done in association with timber harvesting operations. In any case, for some species seeds or fruits collected from the canopy can be better conserved.

Removal of fruits from the canopy from the air is possible. Kitzmiller (1990), for example, describes collecting from large inaccessible stands by means of a cone rake attached to a helicopter. However, such a technique is clearly unlikely to be used very often by germplasm collectors. Most techniques for harvesting fruits and seeds in fact fall under the following three headings:

- collecting fallen fruits and seeds;
- removal of fruits from the canopy using ground access;
- climbing into the canopy.

The series of Technical Notes of the DANIDA Forest Seed Centre are particularly valuable in this context. The most relevant are listed separately in the References section. A checklist of specialized equipment is given in Box 23.1.

Box 23.1
Specialized equipment for collecting seeds of woody species

- Tree measuring instruments – diameter tape and height measuring instrument, e.g. clinometer.
- Calico collecting sheets (e.g. 2 × 2 m).
- Large, heavy-duty, canvas and nylon tarpaulins (for seed collecting, transport and camping).
- Secateurs.
- Rifles (plus cleaning equipment) and ammunition.
- Pruning saws (bow saw, commando saw with rope, long-handled pruner plus spare blades).
- Throwing ropes.
- Tree climbing equipment.
- Tree ladders.
- Chain-saw (chain-saw spanner, file, spare parts, fuel and oil).
- Safety gear (steel-capped boots, gloves, hard hat, safety belt).

Collecting fallen fruits and seeds

Collecting fruits and seeds from the ground is suitable for species with non-winged, heavy fruit growing where ground vegetation is sparse. Recently shed fruits and seeds may be raked or swept up and sieved clean. There is also the option of sucking material up with a portable car vacuum cleaner, light domestic vacuum cleaner (Bastide and Gama, 1980) or portable vacuum harvester.

For most research purposes, collecting fallen fruits and seeds is not generally recommended because of:

- uncertainties regarding their source;
- risks of contamination from morphologically similar seeds of nearby related species;
- their lower physiological quality, compared with those obtained by other methods, due to collecting a higher proportion of immature, empty and unsound seeds, and early onset of deterioration or germination;
- greater risks of contamination of the fruit or seed surface with soil-borne pathogenic fungi.

The main attractions of collecting from the ground are that large numbers of seeds can be gathered quickly and cheaply, without the need for highly skilled technicians or specialized equipment. Accordingly, this method is better suited to bulk provenance seed collecting for operational plantings. For example, the fruits of some northern temperate genera (e.g. *Castanea, Fagus* and *Quercus*) are commonly collected from the ground for large-scale plantings (FAO, 1985). The same is true of teak and *Gmelina arborea*. For bulk provenance collecting of hard-seeded species (e.g. *Robinia pseudoacacia, Acacia mearnsii* and *Prosopis tamarugo*) the seeds can be sieved from soil collected from under source trees and stands.

For research and *ex situ* conservation purposes, ground collecting of fallen fruits and seeds may be suitable for some dry-zone and rain-forest species.

Dry-zone species

There is often little ambiguity regarding the source tree of fallen fruits or seeds in plant communities in which individual shrubs and trees are widely spaced, as is the case in arid and semiarid zones. The large seeds and fruits of several dry-zone tree species are amenable to being collected from the ground. Examples are *Prosopis* spp. in Latin America (Ffolliott and Thames, 1983) and *Acacia sclerosperma, Owenia* spp. and *Terminalia arostrata* in Australia. Shed fruits may lodge in and around the base in bushy species. Large quantities of fruits of *Atriplex* spp. and *Acacia victoriae* are sometimes available in such situations.

Rain-forest species

There are several reasons why ground collecting of fallen fruits and seeds may be the preferred method for obtaining material of some rain-forest species.

- Many tree species in lowland tropical rain forests occur as scattered individuals within the forest, thereby allowing unambiguous identification of the source tree of fallen fruits.
- In thick rain-forest communities it may be difficult to sight fruit crops from the ground, reducing harvesting options.
- In some rain-forest species (e.g. *Flindersia*) the fruiting units are

sparsely and evenly distributed over the entire canopy, further restricting harvesting options.

- Fully mature, fallen fruits may be the easiest to handle and store. For example, de Muckadell and Malim (1983) found that fully mature fruits of dipterocarps were less susceptible to desiccation and rotting during handling and storage. The same authors reported that seedlings raised from such seeds demonstrated more vigorous initial growth.

A number of rain-forest leguminous species with large, hard-coated seeds (e.g. *Intsia* spp. and *Parkia javanica*) are well suited to ground collecting and this considerably extends the collecting period for such species. For most other tropical species the timing of collecting is critical. The first seeds to fall to the forest floor are often of poor quality. An indication of the soundness of fallen fruits can be obtained by cutting through the fruit with a pair of sharp secateurs and inspecting the seed embryo. Change in fruit colour can also be an important indicator of seed quality. For example, green and yellow fruits are preferred to brown fruits in *Gmelina arborea* (Woessner and McNabb, 1979), while greenish-purple fruits are preferred in *Terminalia platycarpa* (L. Thomson, unpublished data). Depulped fruits of *Azadirachta indica* (neem) collected at the yellow-green stage have been found to be suitable for long-term storage whereas seeds from more mature, yellow fruit rapidly lose viability (P. Tompsett, pers. comm.). Fruits containing sound seeds should be collected as soon as possible after shedding to minimize fungal, insect and animal attack and to reduce the incidence of mortality and germination. Early germination can be a major problem for species with recalcitrant seeds such as *Shorea platyclados*, *Syzygium suborbiculare* and *Macadamia* spp.

Seed collecting from the canopy using ground access

The harvesting technique adopted will depend on the species, plant form and stage of fruit maturity. Ground-based harvesting techniques include:

- hand picking or stripping;
- beating and shaking;
- sawing of small branches with long-handled pruning saws;
- sawing of branches using flexible saws;
- shooting down branches with rifles.

Hand picking or stripping

Hand picking is used for harvesting the fruits of shrubs and low-branching tree species in which the fruits are firmly attached to the stem. Hand picking is well suited to collecting fleshy-fruited dry-zone species such as *Boscia senegalensis*, *Grewia* spp., *Maytenus senegalensis*, *Securinega virosa* and *Vitex diversifolia* in West Africa and *Capparis* spp., *Carissa lanceolata*, *Eremocitris glauca*, *Eremophila* spp.

and *Santalum* spp. in Australia. Plants in some shrub genera (e.g. *Olearia*, *Cassinia* and *Maireana*) have light, fluffy seeds and fruits which may be suitable for harvesting from the stem with the aid of a portable car vacuum cleaner. This technique not only gives a very clean product, but also ensures that only fully mature seeds are collected.

If the fruit crop is sparse, fruits may be picked or stripped directly into a bag or other receptacle. For heavier crops, or where larger samples are required, it is usually more efficient to lay out a calico sheet or nylon tarpaulin on the ground adjacent to the plant and either hand-strip, or pick and drop fruits directly on to the sheet.

Secateurs can be very useful for cutting small fruit-holding branchlets, especially for those species with woody fruits which release their seeds on drying, but extreme care is required in their use. The retention of some leaf and twig material results in an open packing arrangement, giving better air flow around the fruits and thereby facilitating drying and reducing the hazards of mould and decay. However, for finer-foliaged *Casuarina* species (e.g. *C. cunninghamiana*, *C. equisetifolia* and *C. junghuhniana*), the cladodes (fine green stems) should be removed from cone collections as upon drying they easily break into short segments that are very difficult to separate from the samaras (winged seeds).

Thick rubber gloves are recommended when collecting fruits from species with caustic or irritant surfaces (e.g. *Arenga pinnata*: Masano, 1990; *Grevillea pyramidalis*) or which have toxic pulps (e.g. *Melia azedarach*). Leather gloves may provide some protection when collecting seeds of species with very large spines or with recurving brambles (e.g. *Acacia* subgenus *Senegalia* and *Acacia cuspidifolia*). For many spiny species fruits may be stripped along the branch using a continuous hand movement in the same direction as the spines are orientated.

Beating and shaking

Many shrubs and trees, including those from leguminous genera such as *Acacia*, *Leucaena* and *Sesbania*, have non-woody fruits which, when fully mature, are only very weakly attached to the bush. Fruits of these species can be very easily and efficiently collected by beating. The dry, mature fruits are beaten, usually with sticks, onto a large sheet or tarpaulin laid out beneath the bush. Sometimes, finer stems can be shaken vigorously to dislodge the mature fruits.

Beating has been found to be a very useful technique for seed collecting of many dry-zone *Acacia* spp. (Thomson and Cole, 1987), especially spiny or thorny species. However, near perfect synchronization of collecting with fruit ripening is required for optimal application of this technique. Nearly mature green fruits are not easily dislodged and may be damaged by beating, whereas fully mature and dried fruits are quickly lost from the bush during warm windy weather. Hence the period when fruits can be collected by beating is often rather limited, typically about two weeks for *Acacia* spp.

Shaking the bole may also be an effective technique for dislodging weakly held, fully mature fruits or seeds onto large sheets spread underneath the canopy. This technique has proved effective for *Cordia alliodora* (Stead, 1979) and *Acacia citrinoviridis* and *A. neurocarpa.*

Long-handled pruning saws and pole pruners

A variety of long-handled tools may be used to increase the canopy volume from which fruits can be harvested. These are illustrated in FAO (1985) and Robbins *et al.* (1981). A well-designed, long-handled pruning saw is an extremely efficient tool for removing fruit-bearing branches. The pole should be strong, rather rigid but light. An appropriate grade of 6 cm aluminium pipe is the preferred pole material. The optimum pole length is about 6 m. The pruning saw blade should be made from high-quality steel alloy, 35–40 cm long. It should be downward curving, with aggressive teeth set. It should be rigidly attached to the pole with a minimum of two fixing points, but the mounting should enable ready interchanging of blades in the field. Some commercially available pruning saws come with a cutting hook on the upper side, an arrangement that can be useful and eliminates the temptation to use the saw blade as a hook. Very long (8–10 m) poles with a hook can be used either to remove individual large fruits or to shake branches and loosen dry fruits, as is done very effectively by nomadic herders in dry tropical Africa for many acacias (e.g. *A. albida* and *A. nilotica*).

In some instances, pole pruners (consisting of 'parrot-beak' snippers mounted on a long pole) can be efficient harvesting tools and have the advantage of minimizing damage to the canopy. Pole pruners are most useful for small trees (< 10 m tall) in which the fruiting units are large, with individual fruits or bunches of fruits distributed rather sparsely and evenly over the canopy.

Flexible saws

Various types of flexible saw can be used to sever fruit-bearing branches. They are illustrated in FAO (1985), Robbins *et al.* (1981) and Robbins (1984b). A weighted nylon cord is thrown over the desired branch and the saw, with heavier polypropylene cords tied at each end, is then drawn up into position. Once the saw is in position a person at each end of the rope can work it across the branch and quickly sever branches up to about 15 cm in diameter. The operation of one particularly efficient type of flexible saw is illustrated in Boden (1972). A potentially hazardous situation can arise if a flexible saw breaks in mid-use, as the broken blade may come hurtling back in the direction of the operators. Accordingly, flexible saws should be well maintained and regularly inspected for any signs of weakness.

Flexible saws are well suited for collecting seeds of medium-sized trees with a clear bole for 8–18 m and horizontal or semihorizontal branch habit, growing in open habitats. Examples include *Acacia sieberiana, Brachystegia spiciformis, Eucalyptus camaldulensis, Khaya* spp., *Sterculia quinqueloba* and *Sterculia africana.*

Rifle with telescopic sight

Severing fruit-bearing branches with a high-powered rifle is the preferred technique for collecting research seed samples from taller tree species. This method has being widely used by the Commonwealth Scientific and Industrial Research Organization (CSIRO)'s Australian Tree Seed Centre for sampling taller eucalypts and acacias and undoubtedly could be applied to many other tall tree species. However, extensive training and extreme care are essential. The technique has been described by Green and Williams (1969) and Kleinig and Boland (1977). The following account is derived from Boland *et al.* (1980).

The selection of branches and of the position along the branch at which the shots are to be placed is critical. It is important to select branches that will fall unobstructed to the ground and to position the shots at a point along the branch so as to take advantage of branch weight and leverage. In particular, horizontal and semihorizontal branches are more easily severed than erect branches.

The marksman should be positioned at a point so that the line of sight is at right angles to the selected branch and the rifle should be steadied against a fixed object such as a tree or the side of a vehicle. The best results are achieved by shooting to sever the bark on the underside of the branch with the first shot. In this way, branch hang-ups are avoided. Subsequent shots are placed at intervals across the branch. The recommended equipment is a .308 calibre rifle fitted with a ×4 to ×8 magnification telescopic sight and soft-nosed bullets.

Limbs with strong, fibrous bark may be difficult to dislodge completely because the severed branch may remain attached or hanging by a ribbon of bark. This is especially a problem for certain eucalypts (e.g. *E. globoidea*) and tropical acacias (e.g. *A. aulacocarpa* and *A. mangium*), which have very strong, fibrous barks. It is essential to shoot down any hung-up branches before moving on as these constitute a major hazard. Shooting from a different angle or position will often bring down branches which are otherwise difficult to dislodge.

Collecting by climbing into the canopy

Climbing may be a suitable alternative method of harvesting fruit from tall trees when rifles are either not available, not permitted (legal restrictions, closely settled areas) or not especially suitable (e.g. conifers and many rain-forest species). Well-trained climbers, operating with appropriate ancillary safety equipment, can make climbing a reasonably efficient and safe operation (FAO, 1985). Nevertheless, Landsberg and Gillieson (1982) have noted that conventional tree climbing with the aid of climbing spikes and safety belt is usually slow and can be hazardous.

Single-rope techniques for obtaining access to the canopy of tall trees have been found to be especially useful in the tropics (Perry, 1978; Perry and Williams, 1981; Landsberg and Gillieson, 1982). There is a wealth of published information on various aspects of tree climbing techniques, equipment and safety, which should be studied carefully by prospective climbers (Seal *et al.*, 1965; Pitcher, 1966; Dobbs *et al.*, 1976; Yeatman and Nieman, 1978; Whitacre, 1981; Barner and Olesen, 1983a, 1984a,b; Robbins, 1983a,b; Ochsner, 1984; FAO, 1985; Perry and Williams, 1985; Stubsgaard, 1987).

Handling, processing and transport of fruits and seeds

Chapter 20 gives information on the theory and practice of seed handling and storage in the field. Some specific examples are presented here to illustrate the main general points as they regard woody perennials.

Handling of fruit and seed samples

Fruits must be handled carefully after harvest to ensure maintenance of seed-lot viability. They must be kept in an environment that minimizes deterioration prior to seed extraction or return to base, with regular inspections, every two to three days or so, to allow early detection of deterioration due to fungi and insects.

The fruits of species with orthodox seeds are best stored under ambient, well-ventilated conditions in coarse cloth sacks, calico bags or securely tied calico sheets. In contrast, the fruits of species with recalcitrant seeds may need to be stored under cool, humid conditions, though it should be remembered that temperatures of $<20°C$ can cause chilling damage to recalcitrant seeds of tropical species.

The difficulties in handling the seeds of recalcitrant species may be illustrated by *Syzygium suborbiculare*. Seeds in the fleshy fruits of this species may germinate soon after collecting (61% after ten days) while being stored in a calico bag. In the field it may be possible to slow germination by storing the fruits in a moist, sterile medium such as peat, with added fungicide, under some kind of refrigeration (Turnbull *et al.*, 1986). The collecting of recalcitrant seeds must be accompanied by detailed forward planning and preparations for their immediate use as they have a limited storage life, even under optimum conditions. For example, de Muckadell and Malim (1983) have recommended that the time between seed collecting and sowing of Malaysian dipterocarps with recalcitrant seeds should not exceed six to seven days.

Field processing of fruit and seed samples

For extended collecting expeditions, it is usually necessary to extract the seeds or at least partially process the collected material in the field in order to reduce bulk and weight.

Methods of seed extraction will depend largely on the characteristics of the fruit. The first step for species with orthodox seeds is to dry the fruits in a sunny, well-ventilated environment. Sun drying has been found suitable for fleshy-fruited *Anthocephalus chinensis.* The seeds may be extracted later by rubbing them against a roughened surface and sieving (Chacko, 1981). In dry, sunny weather fruits can be dried on sheets or tarpaulins (check for holes!) spread on the ground in open locations. All sides of the sheet should be properly weighed down to avoid it being lifted by gusts of wind. Seeds should not be dried in close proximity to ant nests and sometimes application of insecticide around the drying area (not to the seed) may be necessary to prevent ants from removing seeds.

For bulk cone collecting of tropical pines a heavy-duty canvas tarpaulin of 5 × 10 m has been found satisfactory (Robbins, 1984a). The cones need to be raked frequently and released seeds should be collected each day. A calico sheet (2 × 2 m) has proved ideal for individual tree collecting of *Eucalyptus* species. Prior to seed extraction the fruit-bearing branchlets from each tree sampled are held in a separate sheet, which can be opened up for drying in sunny weather and quickly tied into a secure bundle in the late afternoon or for transport. It takes one or two sunny days to extract the seeds from the capsules of most *Eucalyptus* spp.

In cold and/or rainy weather it may be necessary to dry fruits under cover. A polythene tent can be quickly set up in the field for this purpose. Care should be taken to avoid excessively high temperatures building up in such structures. In some locations it may be possible to have access to open-sided sheds (e.g. hay sheds) or glasshouses for drying fruit samples.

Many tree species have dehiscent fruits which readily release their seeds upon drying. For most of these species (e.g. *Eucalyptus* spp.), the fruits will dry more quickly if cut with some twig (with or without leaves) attached. Some fruits shatter energetically upon drying, scattering their seeds widely. Care needs to be taken when drying the fruits of these species (e.g. *Acacia difficilis, Goodia lotifolia* and *Petalostigma* spp.) to ensure that their seeds do not inadvertently contaminate other seed lots. At the other end of the spectrum are species whose fruits require mechanical force to release the seeds. Even when the pods have been fully dried, the seeds of many *Acacia* species and other woody legumes may remain firmly attached to the pod or enclosed in sections of pod. Two species in the latter category, *Pterocarpus indicus* and *Dendrolobium umbellatum*, can be stored and germinated in this state. Various mechanical methods have been used to fully extract such seeds. However, though some examples of these will be given, it must be stressed that the recommendation is that only manual methods be used for seed lots destined for long-term gene-bank storage (Chapter 20).

The large, hard-coated seeds of some phyllodinous *Acacia* species (e.g. *A. anaticeps, A. pachycarpa, A. platycarpa* and *A. wanyu*) can be separated from the pod by placing the pods between a heavy-duty tarpaulin and running the wheels of a vehicle over them several times. For a large number of hard-seeded leguminous species (e.g. many species of *Acacia, Adenanthera, Albizia, Cathormium* and *Dendrolobium*: Searle, 1989), mechanical threshing has proved a suitable technique for separating the seeds from the pod. The Australian Tree Seed Centre has modified the 15 cm flailing thresher described in Doran *et al.* (1983) to make it belt-driven by a small 5 horsepower (HP) generator or electric motor and lighter and more readily assembled and disassembled in the field. The portable thresher has been successfully used for field processing of bulky *Acacia* seed samples, in both arid zones and the humid tropics. Operators wear protective breathing apparatus to minimize inhalation of the irritating dust produced during threshing. For *Acacia mangium*, threshing early in the morning when the pods are slightly moist can reduce the dust hazard. In contrast, the pods of *Acacia crassicarpa* need to be brittle dry to be sheared apart in the thresher: they are best processed in mid-afternoon. In the field, clean seed lots can be obtained from the threshed mixture by flotation, with viable seed sinking (Doran *et al.*, 1983). Wet processing also alleviates the dust hazard. There has been no experimental study of the effects of flotation seed cleaning methods on seed viability, but it is not recommended for long-term conservation (Chapter 20).

The extraction of seeds of tropical pines may be considerably hastened by rotating the partly opened cones in a tumbler. In Thailand, a manually operated tumbler consisting of a rotating drum (50 cm diameter, 240 cm long) made of iron rods at 10 cm intervals was very effective for *Pinus kesiya* and *P. merkusii* (Granhof, 1984).

A number of woody perennials (e.g. *Banksia* spp.) have massive serotinous cones and their seeds are only naturally released after exposure to fire. In the field the seeds of many *Banksia* spp. can be extracted by briefly (5–10 minutes) barbecuing the fruits on a grill over a fire, or by setting the cones alight for those species with persistent perianths or styles.

Removal of the pulpy part of fleshy fruit while still wet is usually done with a knife, and is very laborious. Lauridsen (1986) achieved rapid ($\leqslant$24 hours after collecting) and complete depulping of mature *Gmelina arborea* fruits by threshing, mashing or tumbling them to loosen the skin, followed by thorough washing. Searle (1989) reported successful depulping of a wide range of fleshy-fruited tropical trees using a concrete mixer and varying combinations of sand, rocks and water.

Clean seed lots can be obtained by sieving or winnowing to remove unwanted material. A set of interlocking sieves of different mesh size, with a base tray, is essential equipment for seed collecting of most woody plant species, especially those with round seeds. In order to obtain reasonably clean seed lots in the field, the sieve set should include a sieve of a mesh size which will just allow all seeds of the target species to pass through and a sieve which will just retain more than 95–99% of those seeds. A funnel and scoop can facilitate the sieving operation. It should be borne in mind that for some species seed size can vary both between years and between areas. For example, *Acacia mangium* seeds from Maluku Province, Indonesia, are smaller than seeds from other areas. In some species there may also be marked variation in the size of seeds collected from a given tree or population. The round seeds of *A. citrinoviridis* may vary between 3 and 6 mm in diameter from one individual to another. If examination of cut seeds shows that the smallest do not have a fully formed embryo and are not likely to be viable, these seeds can be sieved off.

Storage and transport of samples

After extraction and cleaning, dry orthodox seeds should be stored in calico bags out of direct sunlight and away from other heat sources. If seeds have been dried to acceptable levels of moisture content, the bags should be placed in air-dry containers. The seeds of some species (e.g. *Eucalyptus deglupta*) lose viability rapidly at ambient temperatures and samples are best maintained under refrigeration (about 4°C) after extraction.

During extended field missions it may be necessary to periodically send samples back to base. This would apply to extensive provenance collecting of species which fruit over a brief period and which have fruit crops that are bulky or that can only be processed slowly. Periodic dispatch of material from the field may also be required for recalcitrant seeds or in very humid areas. Some seeds and fruits require special packaging for long distance transport. The large pear-shaped fruits of the bamboo *Melocanna baccifera* perish rapidly and require careful packing in dry sand or charcoal for transport (Zabala, 1990). The fruits of *Gmelina arborea* need to be handled carefully to avoid damage to the skin and subsequent rapid fermentation. Fruits should be transported in open weave cloth sacks out of direct sunlight (Lauridsen, 1986).

When sending material back to base from the field it is vital that all necessary documentation and instructions are securely attached to the outside of the container and clearly visible. Documentation to accompany consignments of plant germplasm being transported across national borders will include an export permit, import permit and phytosanitary certificate. It is also advisable to include explicit instructions for handling at the receiving end (Willan, 1988). Collectors should be aware that even brief fumigation with methylbromide can be fatal for seeds with high moisture content (e.g. recalcitrant seeds) and many conifers.

Vegetative germplasm collecting procedures

Collecting seeds may not be possible or feasible for some woody species, so that collecting vegetative material may constitute the only available means of germplasm acquisition. Also, for certain purposes it may be desired to clone interesting phenotypes of mature trees directly (e.g. 'plus trees', which are individuals displaying outstanding vigour, disease resistance, outstanding bole form or good fodder value). However, the movement of vegetative material poses a greater quarantine hazard than the movement of seeds. Woody species may be collected vegetatively as:

- root cuttings;
- shoot cuttings;
- *in vitro* meristems;
- seedlings.

Root cuttings

A small number of woody species and populations have lost the ability to reproduce by sexual means. Examples include the Kalamunda (Western Australia) population of *Acacia anomala* and western Victorian populations of *Casuarina obesa*. These now only have the capacity to reproduce clonally by root suckering. More importantly, many other tree species with considerable socioeconomic value and high levels of genetic diversity (e.g. *Acacia albida, A. ampliceps, A. melanoxylon, Casuarina glauca, Pterocarpus indicus*) possess the ability to reproduce clonally by means of root suckering in addition to producing seeds. Collecting germplasm of these species can be undertaken by sampling suitable woody root material if seeds are not available.

To collect root cuttings, roots lying near the soil surface are followed outwards from the tree and segments are taken from them of diameter 8–15 cm and length 25–30 cm. After shaking off excess soil and washing, these are stored in a moist, sterile medium (e.g. peat moss) in plastic bags away from direct sunlight and other heat sources. For longer field trips it may be desirable to keep the root cuttings under cool storage (5–10°C). Root cuttings are normally cut into smaller sections (5 cm) for planting.

Root cuttings have several advantages over shoot cuttings. They are more robust and easier to handle, yield a higher percentage establishment and provide physiologically juvenile plants with better developed root systems.

Shoot cuttings and* in vitro *techniques

Shoots suitable for taking cuttings should be healthy and of sufficient size to allow later division and trimming. The last fully mature growth flushes from the exterior canopy of the tree or shrub are ideal. It may be possible to stimulate the formation of adequate shoots by scoring the trunk in some species; these shoots could then be collected during a second visit (D. Boland, pers. comm.). Leafy shoots should be wetted as soon as possible after excision and leaf area reduced (by 75% or more) to minimize transpiration stress. Soaking for 10 minutes in soapy water, a 0.5–1.0% bleach (sodium hypochlorite) solution and/or a fungicidal and insecticidal dip will help reduce contamination. Cut ends can then be coated with a sealant such as melted low-temperature paraffin wax. Cuttings should be stored in closed plastic bags in a cool location (<22–25°C) out of direct sunlight. It is preferable not to tie cuttings up in bundles, as this may lead to bruising. Aronson *et al.* (1990) give details of a method for the long-distance transport of cuttings under tropical conditions which involves placing the proximal cut ends in an aerated biogel solution in a polythene sack, the whole covered by a moistened paper sack. Green budwood cuttings of rubber trees are packed in boxes or cartons in single layers separated by 1–2 cm of damp, aged sawdust. Shoot material will be difficult to maintain in good condition for more than several days and should be rapidly transited to base for best results.

For the majority of woody species there are difficulties in rooting leafy cuttings taken from mature individuals. One solution is to collect scion material from mature trees in the field for grafting on to seedling stock plants of the same species. This approach has been used for various pines and *Terminalia superba*. It has proved useful for *Triplochiton scleroxylon* plus trees in southern Nigeria (A. Ladipo, pers. comm.) and high cineole phenotypes of *Eucalyptus camaldulensis* from northern Queensland, Australia (J.C. Doran, pers. comm.). It is fair to say that, for most forestry species, the best technique for collecting and regenerating shoot cutting has yet to be identified.

For extended collecting expeditions to remote localities it is likely that simple *in vitro* collecting techniques for buds and shoot apices, especially when coupled with micrografting, will prove more useful in the future than collecting large shoot cuttings, which are more difficult to transport and prone to rapid deterioration during transit. Various *in vitro* collecting approaches have been shown to be successful or are being explored for a wide range of woody genera including *Acacia*, *Citrus*, *Cocos*, *Eucalyptus*, *Gossypium*, *Pistacia*, *Prunus* and *Olea*. Information on *in vitro* techniques is given in Chapter 24 (see also FAO, 1993; 1994).

Seedlings

Collecting seedlings (wildlings) may constitute a useful method for those woody species which are either viviparous, have recalcitrant seeds, are shy seeders or otherwise present difficulties in seed collecting. It is evident that germinated seeds and seedlings should be planted as soon as possible after collecting.

In a small number of woody plants, notably various mangrove species in the genera *Aegiceras*, *Avicennia*, *Bruguiera* and *Rhizophora*, the seeds germinate while still attached to the mother plant. Therefore, germplasm sampling of such viviparous plants usually entails collecting germinated seeds. Following collecting, germinated and germinating seeds should be kept in a moist but well-aerated environment, i.e. they should not be allowed to desiccate nor should the water be allowed to become stagnant. A reduced light environment is desirable, but live plants should not be stored in total darkness for more than two or three days.

For some tree species collecting live seedlings may be a more feasible method than collecting seeds. One such example is *Oreocallis brachycarpa*, a fast-growing, valuable timber species from Papua New Guinea. This tree occurs in remote localities, sets seed in the wet season when access is very restricted and produces winged seeds that are shed as soon as they mature. Collecting in remote areas is expensive and resources may not be readily available for follow-up collecting if seed crops are immature, already shed or absent. In such cases it can be prudent to collect live seedlings. Howcroft (1978) collected large numbers of seedlings (100–500 per population) over a broad area in stands of *Araucaria cunninghamii* and *A. hunsteinii*, for which seeds were not available. These seedlings were used to establish *ex situ* conservation stands in more accessible locations.

Cosampling of root microsymbiont germplasm

The majority of woody plants form root symbioses with ectomycorrhizal and/or vesicular-arbuscular mycorrhizal (VAM) fungi. In addition, some woody angiosperms (some 200 species in 24 genera and eight families) form root nodules in association with the actinomycete *Frankia*. Root nodules are also formed by the bacteria *Rhizobium* and *Bradyrhizobium* on species in the family Leguminoseae and the genus *Parasponia*. These symbioses, which are often close genetic associations between host and microorganism, can be essential for good plant growth. Cosampling of plant and root microsymbiont germplasm during field missions is thus sometimes necessary, especially when the target area is remote, because funding for separate root microsymbiont germplasm collecting is often not available. It should, however, be noted that approaches to sampling of root microsymbionts generally require a high level of experience and specialist skills. Chapter 26 provides an introduction to the subject.

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