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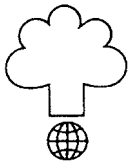
The role of ex situ conservation of trees in living stands

by

Ida Theilade



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Cover photo:
10-year-old *ex situ* conservation stands of *Eucalyptus tereticornis* (right) from Australia and *Pinus caribaea* (left) from Nicaragua. Kivamarukanga, Tanzania. H. Keiding phot. 1989.

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Danida Forest Seed Centre (DFSC) is a Danish non-profit institute which has been working with development and transfer of know-how in management of tree genetic resources since 1969. The development objective of DFSC is to contribute to improve the benefits of growing trees for the well-being of people in developing countries. DFSC's programme is financed by the Danish International Development Assistance (Danida).

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Acronyms

CAMCORE	Central America and Mexico Coniferous Resources Cooperative
FAO	Food and Agricultural Organization of the United Nations
FNCRDC	Forestry and Nature Conservation Research and Developmental Center
IUCN	The World Conservation Union
IPGRI	International Plant Genetic Resources Institute
IUFRO	International Union of Forest Research Organisations
NRC	National Research Council
UNEP	United Nations Environment Programme
WCMC	World Conservation and Monitoring Centre

1. Introduction

Protected areas make important contributions to forest conservation, protect many forest values, and represent very considerable achievements towards conserving forest genetic resources. It is clear, however, that existing protected areas are not, in themselves, sufficient to achieve or sustain all forest conservation goals. Many are in a wrong place, of inadequate size, too disconnected from their surrounding environment, and inadequately protected from pressures which compromise their conservation value (Kanowski 2001). It is estimated that more than 8000 tree species are endangered according to the World Conservation and Monitoring Centre (WCMC 2001). Only about 12% of these are recorded in protected areas and only 8% are known to be in cultivation (IUCN 1999). At the same time habitat destruction is happening at ever increasing rates all over the planet. If we are to believe the habitat-species curves, and there are no reasons not to, we are to loose thousands of species in the coming decade. Nowhere on earth is habitat destruction as imminent as in tropical forests. Thousands of tree species will depend on conservation outside protected areas, that is in managed forests, agricultural landscapes, or *ex situ* in botanical gardens, arboreta, seed banks or gene field banks. This paper presents experiences with *ex situ* conservation of tropical trees in living stands (gene field banks) and discusses some of the inherent opportunities and drawbacks.

Photo 1. Thousands of tropical tree species will depend on conservation outside protected areas. Photo: Ida Theilade

2. The aim of *ex situ* conservation stands

The purpose of *ex situ* conservation stands is to keep genetic resources in a secure area for future utilisation. However, *ex situ* conservation in this pure form is rarely found. Some botanical gardens and arboreta have started collections of threatened species mainly for conservation purposes but these collections often consists of very few individuals. In forestry, *ex situ* conservation stands often consist of a larger number of individuals but the long-term objective is mainly combined with an immediate and far more utilitarian purpose.

Humans have always moved valuable plant material whenever they migrated to new areas. However, it was first with the colonial period between 1850-1950 that an era of plant exploration and introduction of unprecedented extent unfolded. During colonial times numerous *ex situ* populations of tropical trees were established to test promising exotic or indigenous species. The first rubber trees in Singapore and the teak and *Cinchona* plantations in India were all *ex situ* plantings but established at a time where no one had to worry about, least say knew the word, conservation. This contrasts with today, where natural forests are diminishing and, often it is uncertain whether a given natural stand will be available in a decade or two. Consequently, most *ex situ* plantings of trees, in particular tropical trees, can be said to function both as 'gene conservation stands' and 'gene field banks'.

The most frequent functions of *ex situ* stands in forestry are to serve as seed sources, for utilisation in tree breeding programmes, for testing of promising exotic or indigenous tree species or for research and educational purposes. Most plantings termed '*ex situ* conservation stands' are established with one or more of these objectives in mind.

It is estimated that only about 100 tree species are conserved adequately *ex situ*. These are almost exclusively species whose genetic resources have been assembled for domestication programmes, with which almost all substantive *ex situ* forest conservation activities are associated (NRC 1991). The purpose(s) of a particular *ex situ* conservation stand is of course all-important for decisions related to silvicultural management of the stand, which again will decide how many and which alleles are conserved and which may, intentionally or unintentionally, be lost.

3. Early, and some recent, experiences with *ex situ* stands

3.1 Hardwoods in Indonesia

In the early part of the twentieth century the Dutch established plantings of a number of hardwoods in Indonesia. The Dutch hardly conceived these plantings as conservation stands. At the time of establishment these plantings were meant as silvicultural trials to evaluate species for plantations. Nevertheless, these stands of which many are 60 years old by now, may provide valuable experience on establishment and management of *ex situ* conservation stands. Indeed, today the Indonesian stands are often referred to as *ex situ* conservation stands in the literature (Subiakto *et al*. 2001, Sidiyasa *et al*. 2001).

Since 1937 the Forest Research Institute, then the Forestry and Nature Conservation Research and Developmental Center (FNCRDC) established eight demonstration forests in western Java, harbouring dipterocarp collections of 5 genera and 41 species from Sumatra, Bangka, Java, Kalimantan and Maluku. Various studies have been conducted from these demonstration forests including growth, yield, pest and disease and flowering patterns. Currently, dipterocarp stands at the demonstration forests have become important seed sources for planting programmes. Thus, even though actually designed for research purposes, some of the plots are now considered valuable field gene banks.

Table 1. Selected dipterocarp collections at FNCRDC demonstration forest (for full list, see Subiakto *et al*. 2001).

Species	Planting site	Planting year	Origin	Number of trees left	Natural regeneration
Dipterocarpus gracilis	Darmaga	1957	Sumatra	16	Few saplings
Dipterocarpus haselthii	Carita	1957	Java	8	Few saplings
Dipterocarpus tempehes	Haurbantes	1940	Kalimantan	21	None
Dryobalanops lanceolata	Haurbantes	1954	Kalimantan	2	No fruit, flowering only
	Pasir Hantap	1973	Kalimantan	6	Do not flower yet
	Darmaga	1987	Kalimantan	0	None
Hopea bancana	Haurbantes	1954	Sumatra	57	Few saplings
Hopea mengarawan	Haurbantes	1954	Sumatra	4	Plenty
		1958	Sumatra	34	Few
		1974	Sumatra	0	None
Shorea acuminatisima	Haurbantes	1940	Kalimantan	2	No fruit, flowering only
Shorea javanica	Pasir Awi	1958	Java	0	None
Shorea laepifolia	Haurbantes	n.a.	n.a.	0	None
Shorea leprosula	Haurbantes	1940	Kalimantan	3	Plenty
Shorea macrophylla	Haurbantes	1940	Kalimantan	0	Plenty regeneration. Poles up to 20 cm dbh.

Photo 2. *Ex situ* conservation stand of tropical pines in Zambia. Photo: Allan Breum Larsen.

As there is an increasing trend of encroachment and illegal logging even in protected areas, it has been argued that the safest way to conserve dipterocarp species is by *ex situ* conservation (Subiakto *et al.* 2001). However, as the demonstration forests also show, maintenance of dipterocarps *ex situ* is by no means simple (Table 1). Besides loss of a number of stands, the majority of remaining stands has been diminished to a very few trees. To serve a purpose for conservation these stands will have to be infused with new material in order to maintain a minimum of genetic variation.

Furthermore, most of the stands have reached maturity, which raises the question about regeneration. For some stands this will be easy as plenty of natural regeneration is found within the stand. But for most stands there are only few seedlings or none at all. How are these stands going to be regenerated and what will be the costs?

Regeneration may for some species be overcome by simply collecting seed to establish a new plot. But some species will prove problematic to regenerate. This is amply illustrated by a species like *Dryobalanops lanceolata.* Three *ex situ* stands of this species were established in three different sites (Table 1). One stand was lost, one stand, established in 1973, is down to 6 trees but has not flowered yet. In the third and oldest stand from 1954 flowering is observed but no fruiting. This either shows how many years some of these long-lived trees take before flowering is initiated or it shows that flowering and fruiting is somehow disrupted in the new environment. How is the conservation officer going to proceed from here?

3.2 Tropical pines from Central America

Natural populations of *Pinus caribaea*, *P. oocarpa* and *P. tecunumanii* in Central America are under intense pressure from agriculture, grazing, overexploitation and fire. Therefore a network of *ex situ* conservation stands of the three pine species was established in the late 1970s (FAO 1985). DFSC and FAO recently assessed stands in 8 different countries to document their conservation status.

135 *ex situ* conservation stands with a total area of 950 hectares were included in the study. The survey showed that stands had been successfully established and had relatively good survival. About 20% of the stands were lost. Fire was the over-riding cause of lost and disturbed stands while encroachment and illegal cutting damaged a number of stands to varying extent. The majority of stands were smaller than the 10 ha recommended at the time of establishment. Nonetheless, most stands were considered to have a sufficient number of individuals to secure an acceptable level of genetic variation.

About half of the *ex situ* stands fulfilled requirements for isolation of stands from possible contaminating pollen sources. In most countries several stands were planted at one site, typically close to research- or gene conservation stations. To group stands at one site provided advantages in terms of protection and management, but made it difficult to secure appropriate isolation.

Flowering and seed setting was generally very poor. The poor environmental match of many provenances may have reduced their reproductive potential. Secondly, stands were rarely thinned

which restricted crown development and thereby flowering.

Poor isolation from contaminating pollen sources and limited cone setting restricted the use of the stands as seed sources. Therefore, regeneration of the stands seems doubtful. The costs involved and the poor prospects for recovering part of the expenses by seed sales will hamper the interest of institutions to regenerate the stands at the end of their rotation (Theilade *et al*. 2001).

3.3 Regeneration, the bottleneck for *ex situ* conservation stands

Comparing the experience from the Indonesian *ex situ* stands with that of *ex situ* conservation stands of tropical pines established some 30 years later, it is interesting to note astonishing similarities in what went well and what failed.

For both dipterocarps and tropical pines it proved possible to establish stands over a wide range of conditions and to some degree maintain the populations over several decades though samples must be duplicated at different sites to prevent losses. In both programmes it was difficult to ensure that data of stands was maintained. Most importantly, the two programmes both pointed to the uncertainty of regeneration as the Achilles' heel of *ex situ* conservation stands. The question of regeneration of many *ex situ* conservation stands still remains to be properly addressed (Cohen *et al*. 1991). Regeneration protocols have to be developed and practices implemented as older collections mature. Otherwise, there will be no security for their genetic resources, and *ex situ* conservation stands may become *ex situ* morgues.

Photo 3. Assessing the success of *ex situ* conservation of tropical pines. Photo: Allan Breum Larsen

4. Suggestions in developing *ex situ* gene populations

4.1 Considerations on establishment of *ex situ* conservation stands

Tree species have an extraordinary diversity of reproductive systems. These differences are considered to be major determinants of genetic patterns within populations. The kind of reproductive system influences the minimal viable population sizes needed for conservation (Wilcox and Murphy 1985). In brief, self-pollinating and vegetatively reproducing species will vary more genetically between than within breeding populations (Allard 1960). But among outbreeders, especially dioecious species, the reverse will hold true though differences in gene frequency between reproductively isolated populations will increase over time. In self-pollinating species, representative samples of a wide range of breeding populations should be sampled, but individual samples need be represented by comparatively few individuals. For outbreeders, individual populations should be well sampled, but fewer representatives of different populations will generally be necessary (Ashton 1988).

It is generally agreed that most genes do not vary at the population, or even species, level (Ashton 1988). Most variable alleles are sufficiently abundant to be adequately sampled and conserved without danger of chance extinction through random drift in artificial populations as small as 50 (Marshall and Brown 1975) to 100 (Frankel and Soulé 1981) randomly selected individuals. However, such small sample sizes will not sample rare alleles and the importance of these in the long-term survival of species or populations is unknown.

Burdon (1988, 1995) discusses issues involved in developing *ex situ* populations. The number of mother trees collected from and the final size of the *ex situ* stand will of course depend on the objective of the stand and funds available. For example Johnson *et al.* (2001) suggests that a minimum of 50 unrelated individuals per population be used to establish a gene resource population. This would ensure the capture of genes with frequencies of 0.1 and greater. In order to ensure adequate conservation of the sampled material each sampled mother tree needs to be represented by a number of progeny in the conservation stand. For example 50 unrelated individuals could be established in stands of 30 progeny per mother tree, that is 1500 stems per stand. It should be noted that it is the final number of stems at the time of rotation that will be important rather than the number planted. Therefore natural loss and thinning should be factored in at the time of planning population size.

In addition to the above genetic considerations it must be ensured that stand sizes are kept at a manageable level and that the burden of future management and regeneration is within the capacity of the institution in charge.

Multiple stands should be established to spread the risk of losing a population (cf. Table 1). If regeneration plans depend on wind pollination rather than controlled pollination, which is most often the case, larger stands should generally be considered to ensure that the pollen component in the next generation is from the appropriate population. FAO (1992) provides general guidelines for establishment of *ex situ* conservation stands using common plantation practices.

The number of mating individuals varies greatly from one flowering episode to another. In species with mass fruiting, such as in the Malesian dipterocarp forest, adequate sampling for *ex situ* collections requires collection of seeds in the years when the maximum number of individuals participate in the reproductive episode.

Attention must be given to matching of seed sources to planting site to optimise chances for survival and a good seed set but also to preserve the specific features of the gene pool. Movement to ecological different sites will trigger selection away from the specific feature of the sampled gene pool.

Finally, *ex situ* stands should be established in ways facilitating a long rotation cycle. The longer the life of a stand, the longer it will be before one has to collect seed and re-establish new stands.

4.2 How to maintain genetic diversity in gene field banks?

For both *in situ* and *ex situ* conservation the aim is to maintain the genetic diversity. How do the conservation officers maintain genetic diversity in *ex situ* populations? Managers of breeding populations face the same question and valuable experience is available from here. Breeding programmes aim to manage breeding populations to better hold on to genetic variation while still obtaining genetic gain. Making wise decisions in early generations are crucial in maintaining the genetic variation later (Johnson *et al.* 2001).

One way to maintain genetic variation is to substructure the gene resource population in subpopulations. Used in multiple population breeding it refers to having many subpopulations of relatively small size (20-50) designed to maintain genetic diversity. While any one population may lose specific alleles to random drift, as effect of sampling or selection, each population will lose different alleles. As a result, each population may end up with a different set of genes.

Box 1

Experiments with mixing several succession phase species in *ex situ* plantings

The São Paolo Forest Institute has been conserving native Brazilian forest trees *ex situ* in its breeding programme since 1979. Initially, all species were established in pure stands. However, starting in 1990, with the discovery of the importance of respecting succession stages for better adaptation and growth for the majority of native forest species, experiments were set up in mixed plantings, combining several succession phase species such as pioneers, initial secondary, late secondary and climax species. One such example is the experiment with *Guazuma ulmifolia* (pioneer), *Genipa americana* (secondary), combined with *Peltophorum dubium* (initial secondary), *Myracrodruon urundeuva* (late secondary), and *Esenbeckia leiocarpa* (climax).

Conservation is in the form of plantations in experimental designs, which allows for the study of silvicultural behaviour, heritability of traits, population structure and monitoring of genetic variability. Another advantage is the possibility of transforming the experiments into seed orchards, which permits the recombination of material and perpetuation of populations by using their seeds for reforesting altered and degraded areas. The use of low intensity selection within the *ex situ* field banks will allow the production of improved seeds with high genetic variability.

From:

Ex situ conservation of tree species at the São Paulo Forest institute, Brazil. Sebenn *et al.* In print.

Many breeding programmes are using their first generation selections (Kang *et al.* 1998) or progeny tests (Purwanto 2001) as gene resource populations. Because progeny tests will not survive indefinitely, methods are discussed to regenerate stands to maintain populations in the long term as multiple populations, as suggested for *Pinus taeda* by Namkoong *et al.* (1997).

Depending on the initial design of the conservation stand it might have to be thinned one or several times before rotation age. The aim of thinning is to obtain healthy trees with good crown development to ensure sufficient flowering and fruiting. In seed production stands and in plantations selective thinning favours superior individuals. For conservation stands, systematic thinning has been advocated to maintain original gene frequencies. Systematic thinning is relevant in the cases where there are few progeny per mother tree and in consequence there is a chance of loosing entire families in the thinning operation. It might be an idea to keep track of families within the conservation population. In any case, following a rigid programme of systematic thinning may be problematic as poorly adapted or unhealthy trees remain in the stand while superior phenotypes potentially valuable for local breeding programmes may be lost.

While it is unlikely that *ex situ* conservation stands maintain over time the exact gene frequencies that would be maintained *in situ* it is important to note that decisions related to management can influence the level of deviation from the original material sampled. If the conservation stand is to serve rehabilitation and reintroductions into an original habitat, it might be wise to maintain original gene frequencies as far as possible. If the conservation stand serves as a gene resource population for breeding programmes it may be desirable to enhance heritable tributes related to production.

4.3 Pure conservation stands or mixed?

So far, conservation of forest trees *ex situ* has mainly been restricted to pioneer species, for which seed are readily available, stored, and which are easy to propagate and grow. The design of *ex situ* stands has followed plantation practices implying mono-cultures and even-aged stand structure. Plantation forestry is the management of intentionally unstable systems and requires timely interventions to prevent the collapse of the system (Palmer 1991). Plantation design is feasible provided that resources are available for intensive management and regeneration. Using this design, the conservation officer will, after relatively few years, have to initiate thinning and start considering how to make the generation turnover, i.e. collect seed and establish a new stand. If not combined with utilisation of the trees, this is a costly procedure (Kjaer *et al.* 2001).

Furthermore, the plantation design may not be suitable for many tropical forest tree species of subsequent succession stages (Box 1). Species of late succession usually occur in mixed and un-even aged stands and are often shade tolerant. For conservation of these species to succeed *ex situ*, issues such as nurse crops, mixtures of trees, reproductive ecology including maintenance of pollinators and the collection and handling of recalcitrant seed need to be addressed.

Thus, if conservation of trees *ex situ* is to play a role in the conservation of the numerous threatened tropical tree species, and not just for a few pioneer species, it may be necessary to think in larger areas with a mixture of species. This will favour natural regeneration and long term stability of established *ex situ* populations.

4.4 International cooperation and donor concern regarding *ex situ* conservation

Most threatened tree species are found in developing countries where funds for conservation programmes are limited. Conservation of forest genetic resources *ex situ* might in some instances be the only option but it is also a long-term activity with a large initial investment and continuing cost. Donor agencies have increasingly incorporated environmental considerations in international development activities but support is generally provided for protection of plants *in situ* because of the urgent need to protect ecosystems in face of imminent change. Furthermore, *ex situ* conservation presents few immediately tangible benefits except for employment.

In order to overcome the problems with funding, *ex situ* genetic conservation programmes may be successfully carried out by multiple organisations working cooperatively. Examples include the provenance studies carried out by IUFRO in the past and the current efforts of the Central America and Mexico Coniferous Resources Cooperative (CAMCORE). CAMCORE is a cooperative working to establish *ex situ* gene resource populations of tropical species of which many are threatened. Presently, 24 organisations are members of the cooperative. *Ex situ* populations have been established for 22 conifer and 13 hardwood species (CAMCORE 2000).

Ex situ conservation is also attended to by international agencies like IUFRO, IPGRI and FAO that have been instrumental in drawing global attention to the need for collection and conservation of forest genetic resources. *Ex situ* programmes coordinated through multilateral organisations usually have a reasonable time horizon for funding because of the commitment from member governments. However, secure long-term funding is rarely available because donors continuously reassess priorities and redirect limited funds. Therefore, international centres cannot carry the conservation responsibility alone (Plucknett *et al.* 1987). In addition, many forest trees are outside the mandate of international organisations and are responsibilities of national programmes, many in the developing world.

5. Discussion - the role of *ex situ* conservation stands

Ex situ conservation of tropical forest trees is hampered by the very large number of taxa that require protection, the large area needed for the cultivation of trees, and the lack of adequate methods for long-term storage of seeds of many species. Moreover, once in cultivation, continual propagation by seed would be limited in many species because pollinators may not be abundant or even present at the new site. On the positive side, the long life cycles of many tropical trees should ensure survival of the original material for many years (Bawa and Ashton 1991).

The most important function of *ex situ* conservation stands is to provide material for planting and breeding programmes. *Ex situ* stands of experimental design may be transformed into seed orchards, which achieve two objectives simultaneously, conservation of genetic resources and seed production. An example of this is the *ex situ* stands of dipterocarps in Indonesia (Table 1) and Brasilian species at São Paolo (Box 1). On the other hand, if interest in maintaining *ex situ* stands is tied too intimately with seed sales this may endanger the maintenance and regeneration of the stands once demand is low as was the case with the *ex situ* stands of tropical pines (Theilade *et al.* 2001).

Often we assume that the traits of interest to tree breeders will remain constant. We do not foresee breeding programmes losing interest in improving rate of growth, but history shows that new traits are often desired. Examples of traits added to breeding programmes include wood density, pulping characteristics, and disease and insect resistance. Therefore, an important function of gene resource populations, whether *in situ* or *ex situ*, is to maintain variation so new traits can be incorporated into breeding populations in the future. In Europe, gene conservation programmes have been proposed that use both *in situ* and *ex situ* populations in different multiple populations (Eriksson 2001). Use of multiple populations conserves genetic variation better than a single population of the same size as the sum of the multiple populations (Namkoong 1984).

Ex situ conservation is extremely important when an organisation is breeding exotics and the species is in jeopardy within its native range. One example is the collections and plantings made by Australia and New Zealand of *Pinus radiata.* The natural distribution of *P. radiata* is limited to five relatively small populations in California. Collections of these populations are planted in large blocks in Australia and New Zealand with management plans in place.

Besides providing material for planting and breeding programmes, the accessibility of plants in cultivation presents research opportunities not possible with remote and dispersed wild populations as well as opportunities for education and for increasing public awareness that would not otherwise exist. *Ex situ* stands generate knowledge on biology and silviculture. This role is vital if we are to have knowledge about plant populations on the edge of extinction that provide a sufficient basis for their management *in situ*.

So far, *ex situ* stands have mainly served to provide material for plantations and breeding programmes but material conserved *ex situ* is of great relevance to rehabilitation of *in situ* sites too. The United Nations Environment Programme (UNEP) has requested support to establish centres for *ex situ* conservation, particularly to conserve samples for restoration of ecosystems (UNEP 1990). There is no doubt that the role of *ex situ* stands in providing material for rehabilitation of altered and impoverished forest areas will increase in the decades to come. For example, huge tracts of national parks and other protected areas have been degraded from forest to grassland or shrubs. These areas are obvious candidates for rehabilitation efforts, which could draw on planting material from *ex situ* plantings.

It is believed that it is preferable to conserve both species and ecosystems *in situ*. However, it is obvious that this will not be an option for many species or provenances where habitat destruction is total. Where *in situ* and *ex situ* techniques for conservation come together most closely is in re-introductions and recreation of habitat for rare and endangered species (Prance 1997). As more habitats are lost and some tree species are conserved only *ex situ*, it will be necessary to restore suitable habitats. We recommend that future *ex situ* conservation efforts on tropical trees should focus more on creating habitats to move the species back into *in situ* like situations. By mimicking *in situ* conditions it may be possible to facilitate natural regeneration. Such strategy will broaden the range of species which can be considered for *ex situ* conservation in living stands, and may very well be a regular component of large-scale reforestation and rehabilitation programmes already undertaken in many countries today.

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