GENEBANKS OR GENEMORGUES?

THE NEED FOR A NATIONAL PLANT GERMPLASM PROGRAM

James A. Armstrong
Department of Conservation and Land Management, PO Box 104 Como Western Australia, 6152.

Abstract

Although conservation of genetic diversity is the central tenet of the World and Australian Conservation Strategies, extinction of species continues at an ever increasing rate. In the Australian flora, more than 100 vascular plant species are presumed extinct and some 3329 species are considered threatened with extinction, a figure without parallel on other continental land masses. Western Australia has 43% of Australia's threatened plant taxa, more than any other State on the continent and more than most other countries. Western Australia's South-West Botanical Province, is one of the most important centres of biotic diversity in the world. Yet deforestation, soil erosion, over-exploitation of natural resources and fungal disease are severely threatening the integrity of the regions biodiversity and its loss is an irreversible process of global concern. Dieback disease, caused by Phytophthora species, is out of control in some areas of the south west, especially in the species rich coastal heathlands, where many of the ecologically important and spectacular species are facing extinction. Since the existing in situ conservation reserves are also being decimated by Phytophthora fungi there is a pressing need to establish a well resourced ex situ preservation facility in Western Australia to secure the long term future of its conservation taxa.

The importance of germplasm preservation is now acknowledged in Australia and the management of threatened plants in ex situ collections is the current imperative faced by the major Botanic Gardens and many conservation agencies in this country. Despite the growing awareness of the importance of this work, the financial support necessary to research and develop these collections is not yet in evidence. Unfortunately, some holding institutions are fast becoming germplasm morgues as their stored seed loses viability and the rejuvenation and enhancement of their holdings lapse.

Sound management of sizeable plant germplasm collections requires a systems approach, with well-defined objectives, adequate facilities, competent staff, and sustained administrative and financial support. Components of management are interrelated and, for efficacy and cost effectiveness, should be integrated into a national plant germplasm system to reduce excessive duplication of effort and to facilitate a more efficient and effective collaboration amongst kindred holding facilities. It is timely that a National Plant Germplasm Program be developed in Australia to coordinate the Federal, State and private sector efforts to collect and preserve the germplasm of Australia's threatened plants. A proposed scheme is outlined in this paper and I would urge that its implementation be pursued with intensity.

Introduction

Australia's environment is facing profound problems. Currently, close to 20% of our vascular flora is listed as rare or threatened, a figure that parallels closely the percentage mentioned by Don Falk for the U.S. Only 5 per cent of the country has been set aside for nature conservation and only 11 per cent of Australia's rare or threatened plants are known to be amply represented in the nation's conservation reserve network (Benson 1990). Unfortunately, these reserves are no longer

bastions against extinction since virulent plant pathogens are now known to be dessimating Australia's last remaining centres of plant biodiversity.

"Wildflower dieback", caused by *Phytophthora* species, is out of control in those areas of Australia where floristic diversity is greatest. The impact of these pathogens is most obvious in the Southwest Botanical Province of Western Australia, where most of Australia's spectacular native plant species reside. *Phytophthora* disease poses a far greater threat to conservation and resource management in southern Australia than any of the more visible agents of land degredation, such as deforestation, salinity, soil erosion or land clearing for agriculture. The following short video, demonstrates graphically the conservation problems that we face:-

Video "Dieback on the South Coast" (9 minutes)
Produced by Tom Hill and Bryan Shearer, Dpt. Conservation and Land Management (CALM)

I cannot overstate the seriousness of the *Phytophthora* problem. Despite extensive research we are unable to halt it's onslaught and protected species, once thought secure in reserves, are now facing extinction in the wild as existing conservation reserves are being decimated by the fungi. The time has come for the establishment of well resourced germplasm preservation facilities to secure the long term future of our conservation taxa! Acceptance of this reality requires that we consider the most cost effective methods to preserve threatened plant germplasm.

Germplasm Preservation

Long-term cool storage of seed, under reduced humidity conditions, is the method used most frequently in Botanic Gardens to preserve the genetic variability of threatened plants. Indeed, in most holding institutions germplasm preservation is almost synonymous with seed storage.

Although the germination biology of Australian plant seeds is poorly known (Langkamp, 1987), what is know suggests that a large proportion of our plants possess seeds that are recalcitrant and/or of very short viability. I believe that the number of recalcitrant-seeded taxa in Australia is far greater than conventional wisdom suggests. Examples of the types of problems encountered in Australian plant species include:-

Heavy insect predation

Most known populations of *Lepidosperma scabrum (Cyperaceae)* have the seed heads damaged by insect predation (K. Dixon, pers. comm.) and it is not uncommon to find populations in which all fruits have been predated.

In Rutaceae, up to 5% of harvested seeds are predated by Hymenopteran larvae and, in genera such as *Phebalium* (Armstrong, in Ms.), less than 50% of harvested seed was found to be viable.

Seed deterioration is another serious problem

In *Astroloma* spp. (Epacridaceae), viable seeds are not germinable due to poorly understood dormancy factors. What is known is that approx. 50% of harvested seed is of poor viability (K. Dixon, pers. comm.). Of the viable seeds, 15% will have lost their viability within 12 months of normal storage and the proportion of seeds becoming inviable increases exponentially beyond that time.

Caustis dioica (Cyperaceae) produces normal looking seeds but the embryos are generally shrivelled. Less than 1% of intact seeds are viable and all viable seeds are recalcitrant!

Seeds of *Geleznowia verrucosa* (Rutaceae) are unable to be germinated despite intensive research on it's dormancy mechanism (K. Dixon, pers. comm.). Rapid viability decline occurs in the first year of storage with less than 40% viability remaining by the end of the second year. Its hartening to note that in this species as well as in numerous other species of Rutaceae, embryo extrusion is easily achieved and freshly excised embryo's germinate readily *in vitro.*

Despite extensive research to devise new methodologies for the long-term storage of recalcitrant seeds, we are unable to preserve such seeds for long periods in genebanks. I find it remarkable that despite the knowledge that many Australian plant taxa possess seeds that are impossible to store in conventional seed facilities, conservation agencies in this country continue to rely on conventional seed storage as their prime *ex-situ* preservation strategy! Unfortunately, some holding institutions in Australia are fast becoming germplasm morgues as their stored seed lose viability and the rejuvenation and enhancement of their holdings lapse. Of course Australia is not alone in its race to establish the largest germplasm morgues. A recent investigation of the world's largest gene bank, the US National Seed Storage Laboratory in Fort Collins *, revealed that only 28% of its 230,000 seed samples were healthy.

For germplasm storage to be efficient and effective, systems of storage must be utilized that have minimum maintenance requirements and that preserve the genetic integrety of the material being stored (Reed, 1989). In my view, we need in Australia to integrate our existing seed storage facilities with the most effective new technologies available. *In vitro* techniques hold the greatest promise for the storage of recalcitrant seeded species, and for long-term storage, cryopreservation

---

*Associated Press investigation, reported in *Seedling*, 6(5):2, October 1989

is the method recommended (Reed, 1989). Cryostorage requires that tissue or cell cultures be pre-treated with a cryoprotectant (e.g. Dimethylsulphoxide) to lower the freezing point of the cultured cells and thereby reduce the damage caused by ice crystal formation. Once protected, slow freezing promotes cellular dehydration to minimise further the amount of intracellular ice formation. Long-term storage is then achieved by cryopreserving the cultures under liquid nitrogen.

In Western Australia we are fortunate in having a world class *in vitro* research facility at Kings Park, headed by Kingsley Dixon. CALM's Conservation Research Program at the Western Australian Herbarium, are working closely with the Kings Park Laboratory to develop long-term *in vitro* storage methods for Western Australia's conservation taxa and the successes to date have been outstanding:-

Rare and threatened taxa of Rutaceae are now successfully cultured *in vitro.* Flowers of the endangered *Diplolaena andrewsii* have been induced to form callus tissue from cells at the base of the ovary. Subcultures of this callus undergo spontaneous embryogenesis to produce embryoids. Embryoids, ideal for cryostorage, have also been produced from whole leaf material.

Research on the best tissues to use in cryopreservation experiments is well advanced and adventitious shoots have been induced *in vitro* for a range of endangered taxa in the Proteaceae .

Microspore technology now allows the pollen grains of endangered species to be cultured and, once re-diploidized, ex plants can be produced for long-term storage.

The remarkable technology of zygote capture, enables the salvage of recently fertilized ovules and their subsequent germination *in vitro.* This technique enables the recovery of early aborted zygotes from multi-seeded fruits.

Although it is well known that seeds of Australian orchids can be stored for considerable periods and germinated *in vitro* in symbiotic cultures, it is not well appreciated that the fungal symbioants cannot be conventionally stored. Recent evidence indicates that stored fungal cultures develop soma-clonal variants that are parasytic on the orchid seed. Cryostorage of the fungal cultures in liquid nitrogen, has been very successful since such cultures are amenable to ultra low temperature storage and are readily revitalized when rapidly thawed.

Future Research

Although much progress has been made in the development of techniques for the *in vitro* storage of germplasm, we are not yet at the point in Australia where such

procedures are being used routinely for germplasm preservation. Not only do we need to develop new *(in) vitro* protocols, but we require a more complete understanding of the genetic stability of tissue and cell culture, in general, and of preserved cultures, in particular. It is the conservation research laboratories in Australia that should be conducting this research and it is timely that these facilities turn their attention to cryostorage research. Despite the growing awareness of the importance of this type of research, the financial support necessary to develop appropriate methodologies for Australian taxa, is not yet in evidence.

A National Germplasm Program

Sound management of sizeable plant germplasm collections requires a systems approach, with well-defined objectives, adequate facilities, competent staff, and sustained administrative and financial support. Components of management are interrelated and, for efficacy and cost effectiveness, should be integrated into a national system to reduce excessive duplication of effort and to facilitate a more efficient and effective collaboration amongst kindred holding facilities. It is timely that a National Plant Germplasm Program be developed in Australia to coordinate the Federal, State and private sector efforts to collect and preserve the germplasm of Australia's threatened plants.

CONCOM and the ANPWS would need to become closely involved with the program, since national systems require administrative support and a source of funding. The program would need to network the various participating agencies and should be structured so as to co-ordinate operational activities and evaluate the priorities for storage.

The scheme proposed in Fig. 1 attempts to establish the networks that are needed. It is but one of many possibilities and is presented merely as a thought provoker - it needs to be discussed more fully and I would hope that we have an opportunity to do this in the workshop sessions in this Conference.

The key elements of the proposal include:-

- Firstly, the establishment of an Australian Germplasm Council to advise the federal, state and industry organizations of the operational activities of the National Program. The Council would need to lobby federal and state governments for the funding to administer and operate the National program. Council membership would need to be drawn from the appropriate federal, state and non-government organizations.

- Secondly, we would need to establish a National Germplasm Committee to co-ordinate the operational activities of the National Program and to

evaluate the priorities for storage. This expert panel would represent the interests of all the holding agencies, including botanic gardens, statutory and non-statutory seed centres, germplasm laboratories and non-government holdings. The Committee would need to ensure that it co-ordinated it's program of storage with the conservation programs already established by the ANPWS - particularly the Recovery Plan Program of the Endangered Species Unit. Since there is no scheme in Australia for prioritizing germplasm acquisitions, an important and immediate role for the Committee would be to devise a nationally accepted multi factorial coding scheme for threatened taxa to prioritise germplasm collecting. The Scheme that is currently being refined in my own Department (CALM) could serve as the template for a national scheme. CALM's scheme is derived from the New Zealand system outlined in a previous session by David Given.

- And finally, we would need to establish a Germplasm Information Network. This network would become the central database for information on the threatened germplasm being stored under the National Program. In the past, much of Australia's conservation data have been collected with little regard for coordination and data sharing. We could avoid these problems, by establishing our new computerized network on a distributed database platform. Participating agencies would be the custodians of their data and would be responsible for maintaining the integrety of their data. National exchange protocols would need to be established to develop standards for data collection and to facilitate the exchange of core data for summary purposes. The Network would need to source the point source data available through ERIN and the various specimen databases held in Herbaria, as well as the various taxon databases such as ROTAP and CAVP.

Acknowledgements

I am grateful for the assistance of Kingsley Dixon and his research team at Kings Park for providing many of the examples used in this presentation and for the helpful discussions about the issues presented in this paper.

References

Benson, J. 1990. Australia's threatened plants. In M. Kennedy (ed.) "A Complete reference to Australia's Endangered Species". Simon Schuster, Australia. pp. 145 - 185.

Langkamp, P.J. (ed.) 1987. "Germination of Australian Native Plant Seed". Inkata Press, Melbourne. 236pp.

Mowder, J.D.; Stoner, A.K. 1989. Plant germplasm information systems. In L. Knutson and A.K. Stoner (eds.). "Biotic Diversity and Germplasm

Preservation, Global Imperatives", Kluwer Academic Publishers, Dordrecht. pp.419 -426.

Reed, S.M. 1989. In Vitro conservation of germplasm. In H.T. Stalker and C. Chapman (eds.) "Scientific Management of Germplasm: Characterization, Evaluation and Enhancement", IBPGR Training Courses: Lecture Series 2. IBPGR, Rome. pp. 23 - 30.

National Germplasm Program

Australian Germplasm Council

CONCOM; ANPWS/ANBG; CSIRO; WWF

Australian Germplasm Information Network

Point Source Data: ERIN/GIS; Herbaria Databases

Taxon Data: ROTAP; CAVP

National Germplasm Committee