Introduction

This book contains the contributions of participants to an international workshop held in West Sussex, UK during July 2001, entitled 'Seed Conservation: Turning Science into Practice'[1]. The workshop was hosted by the Royal Botanic Gardens (RBG), Kew as part of its input into the Millennium Seed Bank Project (MSBP)[2]. The aim of the workshop was to review the science and technology that underpins seed conservation activities around the world, about which there have been few major reviews since Roberts in 1972 (though see, for instance, Dickie *et al.*, 1984). Therefore, it was felt important to re-establish a baseline position on seed conservation science and technology, against which future progress in the subject could be assessed. Towards this aim, many leading seed conservation scientists from around the world have contributed to this volume. Indeed, the book reflects the experiences of 103 contributors belonging to 46 organisations from 21 countries and six continents[3]. Although crop seed conservation is widely covered, there is a particular emphasis on the problems associated with non-domesticated seed conservation, for it is in this expanding area that many technical challenges remain.

The three main themes of the book are 'Planning and Collecting'; 'Processing and Testing'; and 'Storage and Utilisation'. These follow the general flow of seed bank operations (see Figure 1). Each of these themes is explored by means of reviews, research papers and case studies. In this way, the book should update current practitioners (including geneticists, plant breeders, seed biologists and taxonomists) and prove useful to the increasing number of organisations (and particularly botanic gardens) that are establishing seed banks. It will also be invaluable to postgraduate and undergraduate students and policy makers wishing to gain a full insight into the science of the important *ex situ* conservation technique of seed banking. While not strictly a handbook to seed conservation, it does contain significant practical guidance which, when combined with the case study experiences, should provide vital information for those wishing to embark on a programme of seed banking. Of necessity, the book draws on many other disciplines including biochemistry, biophysics, ecology, engineering, horticulture, information technology, law, and systematics. Due to its essentially practical content, this book goes some way towards updating the handbooks published by the International Board for Plant Genetic Resources, IBPGR, (now the International Plant Genetic

[1] See Box 1 for a definition of seed conservation, Box 2 for its context and Box 3 for the basic principles of seed banking.
[2] See Box 4.
[3] With such a large number of contributors, there is inevitably some variation in terminology used – see Box 5.

Planning and Collecting

Planning and permission seeking
↓
Field collection of labelled seed and voucher (pressed) specimens plus field data recording
↓
Rapid shipment of the seed to the bank
↓

~ Processing and Testing

Unpacking of consignment, check for problems, drying/mounting of vouchers
↓
Data record creation about each accession
↓
Assessment of likely seed storage characteristics (if unknown)
↓
Seed cleaning [1]
↓
X-ray analysis or cut-test
↓
Seed quantity determination
↓
Drying
↓
Determination of seed moisture status
↓
Initial germination test [2]
↓

~ Storage and Utilisation

Packaging and security duplication [3]
↓
Storage under cold conditions
↓
Characterisation and evaluation*
↓
Distribution of characterised sub-samples to users (through time)
↓
Germination re-tests (through time)
↓
Regeneration/multiplication of collections (when required) [4]

* As appropriate

Note

[1] Cleaning is sometimes preceded by a period of drying. Small samples may be multiplied at this stage with procedure after harvest as for field collected material.
[2] This step is sometimes left until after banking if confirmation is desired that seeds survive both desiccation and freezing. It then requires an easily re-sealable container.
[3] Duplication should be well away from the bank.
[4] Procedure after harvest is as for field-collected material.

Figure 1 Key elements of a generalised seed banking procedure

Box 1 What is seed conservation?

Seed conservation is the use of seed storage for *ex situ* (off-site) plant genetic resource conservation. Seeds are normally stored in seed gene banks (abbreviated to 'seed banks' throughout this book, though not to be confused with the same term used for seed persisting in the soil). Essentially, genetic diversity captured at the time of collection is usable at a later date, through seed storage. This time lapse between collection and use may vary from a few months to several centuries, depending on the nature of the material and the aims of the work. While there are several other methods of *ex situ* plant conservation (see for instance, Hawkes *et al.*, 2000), the balance between technical input, storage lives, amounts of diversity conserved and ease of obtaining gene products (including full-grown plants) make seed banking one of the most efficient (Linington and Pritchard, 2001).

Resources Institute, IPGRI), produced over a decade ago (Ellis *et al.*, 1985 a & b, Cromarty *et al.*, 1990). Furthermore, because this volume covers not only crop but also wider biodiversity conservation, its remit is broader than Schmidt (2000), which provides excellent guidance for the handling and storage of (primarily commercial) forestry seed.

The book is structured within the context of the flow of seed banking procedures (see Figure 1) as follows:

Planning and Collecting (Chapters 1–14)

The Convention on Biological Diversity has radically influenced the legal and ethical framework for seed conservation activities. This influence is explored in Chapter **1**. The next two chapters (**2** and **3**) review the factors that need to be considered when planning a programme of plant genetic conservation, including ecogeographical studies of target species. Such planning is increasingly assisted by the use of Geographical Information Systems (**4**) and requires knowledge of the genetics of the species (**5**). Prior to collecting, an understanding of the effect that seed maturity will have on the storability of the seeds is essential (**6**). This is further illustrated by specific research examples relating to seed development (**7** and **8**). Chapter **9** provides practical guidance on the collection of seed from non-domesticated species, and complements the extensive information provided by Guarino *et al.* (1995), particularly on the collection of crop genetic resources. The following five chapters are case studies on collecting. Of these, Chapter **10** shows how seed collections of temperate forages are made from field-collected vegetative samples. Chapters **11** and **12** outline how targeting is organised in projects within Namibia and Mexico, respectively. The last two chapters in this section (**13** and **14**) illustrate the development of the MSBP conservation programme in South Africa and forest seed collecting in Burkina Faso.

Processing and Testing (Chapters 15–32)

Efficient processing and viability testing of seed collections requires an understanding of seed and fruit structure (**15**). The general principles of seed processing for storage are described in Chapter **16**, with an emphasis on forest species. An illustration is then given of how similar work is carried out for the extremely diverse MSB collections (**17**) and how the associated data is recorded and synthesised, and disseminated electronically (**18**).

Unfavourable ambient conditions can cause loss in seed viability before collections reach the safe conditions of the seed bank. Practical guidelines to minimise this risk and a description of seed drying methods are covered in Chapter **19**. Accurate monitoring of seed moisture is essential at several stages in the process of seed conservation. Chapter **20** describes new, non-destructive approaches. Chapters **21** and **22** cover aspects of desiccation tolerance and sensitivity and recommend how desiccation-sensitive seeds should be handled. The following chapter (**23**) describes research into the seed biology of an important medicinal plant of South Africa in relation to its ecology and conservation.

Viability testing of conserved seed samples can be a major technical difficulty in seed conservation. The various methods in common use, as well as the plethora of terms and definitions, are reviewed in Chapter **24**. Specific approaches including the use of *in vitro* techniques for the recovery of stored embryos, and use of FDA staining, are discussed in Chapters **25** and **26**. One of the main causes of difficulty is that seed dormancy can be particularly pronounced in wild species. Chapter **27** considers the concept of molecular testing of seed germination that would require fewer seeds than conventional tests. Chapter **28** provides a predictive classification of seed dormancy in relation to biogeography and phylogeny. The next two chapters (**29** and **30**) consider dormancy-breaking treatments for selected Australian species. The effectiveness of one approach adopted – the use of smoke treatments – is reviewed in Chapter **31**. A research paper (**32**) on the effects of temperature on Mexican cactus seeds completes the section.

Storage and Utilisation (Chapters 33–54)

Although many crop seed banks became established during the last half of the 20th century, facilities for wild germplasm are still being developed. Consequently, Chapter **33** provides an update on the principles of seed bank design. A constraint to achieving effective dry storage is the availability of truly moisture-proof containers. Examples of trials for quality assurance of containers used by the MSBP are given in Chapter **34**.

The improved seed viability equation developed by Ellis and Roberts (1980) was an important milestone in the quantification of storage longevity for orthodox seeds; Chapter **35** considers the use and abuse of this equation. The

Box 2 **What is the context of seed conservation?**

In 1992, the Rio Earth Summit addressed concerns over the security and exploitation of the world's biological diversity. The Convention on Biological Diversity (CBD) arising from this summit establishes a framework, in which scientists and policy makers can develop measures to limit future losses of diversity and ensure that benefits arising from the use of this diversity are shared equitably. The reasons for loss of plant diversity are many and varied. Most can be attributed to the pressures and changes imposed on plant populations, regardless of whether they are crops or wild populations, by a burgeoning human population and its understandable desire for improved living standards. Particularly over the last century, low yielding though genetically diverse and dynamic crop assemblages, resulting from thousands of years of selection, have been replaced in farmer's fields, across much of the world, by high yielding but genetically uniform varieties, bred to meet the demands of intensive agriculture. Ironically, the modern generation of varieties was initially bred from the displaced diversity and has then drawn upon it as opportunities have arisen. Their uniformity makes them ephemeral. As disease resistance breaks down, or minor improvements are made, they are replaced. The role of crop seed banks has been to conserve the displaced diversity and to feed it into plant breeding programmes. The coming decades may see the role of such seed banks change, as genetic engineering breaks the limitation that a crop's gene-pool (its source for genetic material) extends only to closely related species. For more information on the work of crop seed banks, readers are referred to Plucknett *et al.* (1987) and FAO (1996). Links to a wealth of other related information can be obtained by reference to the websites of both the Food and Agriculture Organisation of the United Nations (FAO, access via www.fao.org) and the International Plant Genetic Resources Institute (IPGRI, www.ipgri.cgiar.org).

Key threats to plant diversity in the wild have come from the conversion of land from its natural or semi-natural state, as a result of building, agriculture, mining and logging. Due to loss of habitat, plant populations have been fragmented to the point where they are no longer viable. When this genetic erosion operates across the entire range of a species, the risk of its extinction looms and some have predicted that as many as half of the world's species may reach this state by the end of this century (Wilson, 2002). Potentially exacerbating the effect of land conversion is the as yet unquantified effect of climate change. More verifiable are the erosion of fuel-wood, medicinal and other valued species through over-exploitation, and the threats posed by invasive exotic biota. While *in situ* (on-site) conservation of habitats and the plant diversity they hold must always be the paramount aim of botanical conservation, the pressures described above mean that this approach is fallible. For this reason and as a means to provide material for research, many botanical organisations are taking out an added insurance policy against wild plant population loss by carrying out *ex situ* conservation. Increasingly, this takes the form of seed banking.

Box 3 **What are the basic principles of seed banking?**

At present, seed banking can only be used for species with desiccation-tolerant (orthodox) seed. These appear to be produced by the majority of Spermatophyte species (see the MSBP Seed Information Database, www.rbgkew.org.uk/data/sid/). The banking procedure is shown in Figure 1. On arrival at the bank, seed-lots (samples with a common history following harvest) are cleaned, dried and packaged before maintenance at a low, usually sub-zero temperature. Viability is monitored through time, usually by germination tests on sub-samples of each seed-lot. When seed quantity is low, or when viability reaches a fixed value (usually a high value to minimise genetic change resulting from ageing, and often 85%, see FAO/IPGRI, 1994), the seed-lot may be grown out and, following flowering and seed-set, a fresh seed-lot, genetically equivalent to the parental plants, is obtained. This process is termed ‘regeneration’, though ‘multiplication’ is often used when seed number increase is the sole activity. There is the risk of selection or loss if this process is not carried out with great care.

Maximising seed-lot longevity is at the heart of an efficient and effective operation. Even if seed is used long before its viability goes into rapid decline, the higher the seeds’ viability, the greater its ability to germinate under field conditions (see for instance, Ellis and Roberts, 1981). The collector has a large influence on the seed-lot’s viability at the start of storage. Rather surprisingly, the seed bank manager or curator has rather less control over seed longevity than might be first imagined. A key strength of seed banking is the universality of the conditions that can be applied to a wide range of orthodox species. A consequence of this is that seed drying methodology tends to be standardised within seed banks based on the recommendations of the seed research community. Similarly, technology and practicality mean that subsequent seed storage utilises low temperatures, either in the approximate range -20 to +10°C (conventional refrigeration) or under liquid nitrogen conditions -196 up to -160°C (cryopreservation). Once the storage conditions have been applied, the longevity of a given species within the bank has substantially been determined. Where the manager does have a role in maximising seed longevity is through ensuring that collections are processed quickly into the bank and then that unnecessary losses are avoided by proper monitoring of the storage conditions, seed viability and moisture status.

Although the conservation role of seed banks must never be underplayed, banks will be most effective when the seeds are also made available for use (e.g., plant breeding, research or re-introduction). Demonstration of utility tends to strengthen funding security and may have a beneficial impact on the ‘*in situ*’ conservation of species. The usefulness of material will be greatest when the sample is distributed with good collection (passport) data, germination instructions and some characterisation data. With crop material, this data has traditionally been obtained by recording strongly genetically determined characteristics under field or glasshouse trial conditions. Increasingly, such character assessment is carried out in the laboratory. With wild material, characterisation is usually the verification of species or sub-species identity using representative voucher specimens (for methodology, see Bridson and Forman, 1998) collected at the same time as the seeds.

two chapters (**36** and **37**) that follow then consider optimal long-term storage conditions in the light of biophysics and particularly intracellular glassy states. The suggestion is made that optimum longevity at sub-zero temperatures requires water (moisture) contents that are higher than those predicted by the seed viability equation. Chapter **38** is a research paper that elucidates the optimal storage conditions for the important economic tree species, neem (*Azadirachta indica* A.Juss.). The next chapter (**39**) reviews conventional tree seed storage conditions.

The effect of drying seeds to low moisture contents is explored through a research paper on *Nothofagus* seed that finds that the optimal moisture content is as low as 2.7% (Chapter **40**). This is followed by a review of the effects of ultra-low drying on a range of orthodox seeds (**41**). Chapter **42** considers seed storage of Western Australian species, whilst Chapter **43** examines the effect of drying *Mammillaria* seeds, using silica gel, on storability. The hydration conditions required to successfully cryopreserve the non-orthodox, oily seeds of nine species of coffee (*Coffea*) is reported in Chapter **44**.

This section of the book is completed by 10 case studies (Chapters **45–54**) that provide outlines on aspects of seed conservation in Ethiopia, Spain, Greece, USA (Oregon), The Netherlands, the Nordic countries, Jordan, India, Morocco and USA (Hawaii).

The book concludes with two chapters. The first (**55**) is a perspective on seed conservation by the editors. Drawing upon publications that have arisen since the main chapters were written, this perspective acts as a quick guide to key points highlighted within the book and incorporates elements of the discussions that took place at the end of each session of the 2001 workshop. The final chapter looks at the future of seed banking (**56**).

It will be apparent to readers that a number of relevant subjects have not received a detailed review within this book. Notably absent are specific reviews on seed regeneration, the use and value of seed collections in seed banks, and the relationship between *ex situ* and *in situ* conservation. These subjects were addressed at the 2001 workshop and have been covered to some extent in certain chapters. However, the reader is directed to texts that cover these subjects in detail, for example, Sackville Hamilton and Chorlton (1997), ten Kate and Laird (1999) and Maxted (2000). For an introduction to aspects of germplasm health, readers are referred to Frison and Jackson (1995). There are many further subjects, such as the evaluation and characterisation of germplasm, that are also relevant to seed conservation, particularly for crops. Readers interested in such topics are referred to the publications listed on the website of the International Plant Genetic Resources Institute (www.ipgri.cgiar.org).

As will become apparent on reading this book, much remains to be done, if seed conservation is to meet the challenges of the 21st Century. This book aims to set the baseline so that science and technology of seed conservation can be progressed in an innovative and practical way in the coming years.

Box 4 **What is the Millennium Seed Bank Project?**

The MSBP is an international conservation project managed by RBG Kew's Seed Conservation Department. Its major financial sponsor is the Millennium Commission, one of the UK's lottery fund distributors. The project also receives substantial support from the Wellcome Trust, a leading research-funding charity, from the telecommunications company, Orange plc, and from RBG Kew (which in turn receives grant-in-aid from the UK's Department of the Environment, Food & Rural Affairs, Defra). This ambitious project has already conserved seed from most native UK Spermatophytes (Alton and Linington, 2002). Through partnerships with organisations in many countries, it aims to conserve population samples of the seed from some 24,000 (particularly dryland) species during the years 2001 – 2009 (see Smith *et al.*, 1998). The work involves programmes of research, technology improvement, capacity building, and information dissemination including via its website (www.rbkew.org.uk/msbp/). The Wellcome Trust Millennium Building, located at RBG Kew's garden of Wakehurst Place in West Sussex, UK, acts as a focus for the project, containing the Millennium Seed Bank, processing and research laboratories, lecture facilities, scientific visitor accommodation and a public education area.

Box 5 **Terminology and plant names used within this book**

With so many contributors from so many organisations, it is not surprising that some authors prefer the use of some terms not used by others. Consequently, percentage water content (w/w) and percentage moisture content (dry weight basis), $m.c._{d.b.}$, can both be found in this book. They are interchangeable. It is possible to convert such data to percentage moisture content (wet weight or fresh weight basis), $m.c._{w.b.}$, using a simple equation (see Cromarty *et al.*, 1990):

$$m.c._{w.b.} = m.c._{d.b.} \times 100/(100 + m.c._{d.b.}).$$

The definitions of 'Orthodox', 'Intermediate' and 'Recalcitrant' seed storage behaviour vary between authors as does the use of the term 'desiccation sensitivity'. Such inconsistency represents the development of concepts and their natural evolution as seed conservation develops.

Scientific names of plants used in the book have been checked against the International Plant Names Index (IPNI) and Tropicos (at Missouri Botanic Gardens), and changed accordingly. In some cases, such as with *Cactaceae* and the conifers, specialist literature has been consulted in an attempt to unify the taxonomy used by authors. All scientific names are shown in italics. The species index shows the taxonomic authorities; it has not been practical to insert these authorities throughout the chapters.

Where possible, S.I. units have been used. However, for clarity with respect to day-to-day usage, units such as litres have been left.

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