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A successful partnership between seed banking and horticulture: the Kirstenbosch Threatened Species Programme

By Anthony Hitchcock, Nursery and Living Plant Collections Manager, South African National Biodiversity Institute (SANBI)

The Threatened Species Programme run by the Kirstenbosch National Botanical Garden in South Africa is an integrated programme involving a number of different players. Within the South African National Biodiversity Institute (SANBI), the Gardens, Research and Education directorates all work together on the programme. Our aim is to strengthen our capacity to help threatened biodiversity by further developing collaboration between these directorates and to extend this to the other SANBI gardens. Other partners and players include Cape Nature, South African National Parks, Table Mountain Park, conservation bodies run by the city of Cape Town, Working for Wetlands, Biodiversity and Wine Initiative, the Botanical Society of South Africa, the Millennium Seed Bank Partnership (MSBP), local area friends groups, horticultural clubs, private specialist nurseries and certain interested landowners.

The Kirstenbosch Gardens Threatened Species Programme originally tried to build up extensive *ex situ* living collections of threatened plant species, but found this to be too difficult. The main problem was a lack of both nursery space and horticulturists to manage the large number of threatened species. We could only keep a few pots of any particular species and this was not representative of the gene pool for that species. Many of the fynbos species are difficult to to grow, are disease prone and short-lived. We regularly lost species from our collections and had to re-collect from the ever-diminishing natural populations. We also focussed on attractive species irrespective of the state of their habitat. This meant that we would grow rare plants or narrow endemics from nature reserves rather than focussing our attention on species growing in threatened habitats. Kirstenbosch Gardens has therefore changed the programme in order to make a better and more effective contribution to conservation. The primary difference is twofold:

1. **Development of an *in situ* programme and integration of our *ex situ* and *in situ* conservation programmes**
2. **A change of focus from purely threatened species to include threatened habitats**

Our *in situ* programme focusses on the rehabilitation of threatened habitats on core conservation sites on the Cape Flats in and around Cape Town. We attempt to look at the threatened habitat holistically from a conservation viewpoint. This means that we concern ourselves with the proper, sustainable management of the conservation area including alien management, fire management, pollution of the site, sustainable plant and animal populations, pollinators and plant species complexes. We also work with, and depend upon, land managers who are critical to the success of the programme. In addition to habitat rehabilitation, we also undertake plant species restoration on these sites. Restoration includes the use of both seed and plants and involves supporting the local core conservation site managers through training their staff in seed collecting and horticultural techniques. Seed is used to restore species lost from the area or to increase the population size of a

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Threatened species stock beds with *Mimetes chrysanthus*
PHOTO: A. HITCHCOCK

Kew
ROYAL BOTANIC GARDENS

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threatened species and/or other species that make up the species complex of that area. Seed from the Millennium Seed Bank (MSB) plays an important role in this work. Only seed from the same habitat is used. Plants grown for reintroduction to conservation areas are often critically endangered, or even extinct in the wild species, that require special intervention to ensure successful reintroduction. These high profile plants sometimes serve as good 'flagship' species to promote the conservation programme. An example is *Erica verticillata*, which has been adopted as the flagship species for the Kenilworth Conservation Area, and is displayed on their logo.

The primary driver of the *ex situ* programme today is the MSB Partnership, which provides the best way to preserve the gene pool of a plant species. The MSBP South Africa primarily targets threatened species from threatened habitats, but also collects and stores seed from areas that are less threatened. We also continue to maintain a valuable *ex situ* collection in the garden, such as cycads and some trees. Other collections of threatened plants are maintained in pot collections, which are housed in our Millennium Collections Nursery. These include mainly succulents, bulbs and *Ericas*. The *Protea* family has a large number of threatened species, but they are not easy to maintain in pot collections or in the display garden. Therefore we have established threatened plant stock beds outside of the main display garden where threatened Proteaceae and some other threatened species are kept. The threatened plant stock beds are used to preserve species from extinction and as source material for restoration, seed banking and garden displays. The threatened plant stock beds have proved very successful in maintaining healthy collections of difficult to grow fynbos species. In addition, our *ex situ* programme includes back-up collections of certain threatened plants kept at other botanic gardens and, in a few cases, at specialist nurseries where they can be bulked up for restoration projects or special plant sales. We have also established a demonstration garden, the 'Garden of Extinction', to display threatened plant species. This garden has interpretive boards to educate and create awareness members of the public.

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Restoration work in Tokai park PHOTO: A. HITCHCOCK

***Erica verticillata* at Tokai park.** PHOTO: A. HITCHCOCK

A new role **for botanic garden horticulture**

By Victoria Wilman, Collections Coordinator, MSBP, SANBI

Traditionally, botanic gardens were the major collectors of plant species, focussing much of their resources on classification and identification and *ex situ* conservation of living collections. More recently though, botanic gardens have made great changes in priorities, as horticulture and taxonomic research are being supplemented and modified by the necessity of biodiversity conservation, public education, research and outreach. Through the Threatened Species Programme, and the MSBP, Kirstenbosch National Botanical Garden and other gardens in South Africa, are playing a more defined role as centres of both *ex situ* and *in situ* conservation action. The main function of gardens has been to maintain a wide range of species as part of their 'living collections'. Unfortunately it is not practical to grow and hold a large number of threatened species in living collections at one time, and seed banking provides an alternative and convenient way of storing and maintaining genetic diversity. In addition to safeguarding collections of seeds, propagation and cultivation techniques are developed for species which may never have been in cultivation, particularly those that are rare and threatened.

Seeds collected for storage are also germinated and grown on and are used for increasing the availability of seed, particularly of critically endangered species. At Kirstenbosch, stock beds, situated away from the rest of the garden in order to maintain genetic integrity, are home to some of the Cape's most rare and threatened species. The seed collections and plants grown are used for the re-establishment or to boost the population of threatened species, for habitat rehabilitation and restoration projects.

A **message** from Paul Smith

Millennium Seed Bank Partnership Leader

This issue of *Samara* showcases a number of ways in which seed banks are engaging with the horticultural sector. Seed banks have an essential role to play in supporting horticulture. The United Kingdom, with its mild climate and huge amateur gardening sector, supports an ornamental horticulture industry worth around £2 billion per annum. With a native flora of only about 1500 taxa, Britain's gardeners cultivate more than 70,000 taxa in their gardens. However, as any nursery manager or gardener will tell you, it's tough out there for plants – particularly if they are exotics needing constant care and attention. An early frost, an unexpected drought, pests and diseases all take their toll. So how can seed banks help? For the past five years we have been systematically backing up Kew's valuable horticultural collections as seed so that if they are lost in the nursery or garden, they can be replaced. This is not expensive to do because we 'black box' the seed, banking it as it comes in without the usual extensive testing that we do with wild material. We are now extending this service to other botanical gardens in the UK, most notably to the National Trust, the largest landowner in the country with more than 2500 km^2 of land holdings and over 200 gardens. Many of the Trust's horticultural collections are of great historic, aesthetic and cultural value making them literally irreplaceable – unless you have their seed, of course.

Seed scientists and **horticulturalists** working together to conserve threatened **Georgian species**.

by Clare Trivedi, International Projects Coordinator, MSBP

Over the past year, seed scientists and horticulturists from both Kew and the National Botanic Garden of Georgia (NBGG) have been working together to understand better how to grow 5 highly threatened plants: *Cyclamen colchicum, Galanthus kemulariae, Campanula kachetica, Pulsatilla georgica* and *Paeonia steveniana.*

The plants all occur in a list of 50 priority species of primary conservation concern for Georgia, which were identified during a project led by the IUCN, in cooperation with the Missouri Botanic Garden, to compile a regional red list of Caucasian endemic plant taxa. All the taxa are known from just a very few sites and are suffering declining populations.

Seed has been collected from all the taxa and this is conserved in both the Millennium Seed Bank and the seed bank at the NBGG. However the Georgian team have identified problems with growing plants from banked seed for these species. The objective of this project is to overcome these problems as the foundation for future activities to augment the surviving populations or re-introduce the species to historical sites.

Parallel studies at Kew and the NBGG to establish germination and propagation protocols for these taxa, have required the close collaboration of seed scientists in seed banks (whose expertise lies in germination) and horticulturalists in the gardens (whose expertise lies in propagatation). Germination protocols are first established by standard germination test procedures in the seed bank. These use seed grown on agar or filter paper in petri dishes under various light and temperature regimes. Germinated seedlings can then be transferred to the care of horticulturalists to raise in pots of compost in the glasshouses or gardens. We have also carried out direct sowing of seeds into compost. In this case the horticulturists have used the seed scientists' knowledge and understanding of germination requirements to try and achieve maximum emergence rates.

Germinated *Cyclamen* seed PHOTO: C. CLENNETT

Companula PHOTO: J. WENHAM

These studies are on-going and we already have healthy young plants of two of the species. The interactions between specialists are producing interesting outcomes. For example, the horticultural staff are confident at propagating several of these species from fresh seed but struggle with seed which has been dried and stored in the seed bank – a key challenge to be overcome if seed bank collections are to be used for re-introductions. For other species, seed bank staff have relied heavily on the specialist knowledge of horticultural staff to coax seeds into germination and growth. In practical terms, a challenging activity has been the establishment of experimental methods for comparing different treatments for the propagation trials and finding ways to measure and record the 'results' of these different treatments.

The current pilot activities will continue to run for several more months, and it is hoped that these will form the basis for a larger project in the coming years.

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From the **vault to the field**: seed production in the UK Native Seed Hub

By **Ted Chapman,** UK Native Seed Hub Coordinator, MSBP

***Campanula rotundifolia* in the UKNSH production site** PHOTO: RBG KEW

The UK Native Seed Hub (UKNSH) was launched in 2011 with the aim of increasing the quality, quantity and diversity of native plants and seed available for conservation and habitat restoration in the UK (see *Samara* Issue 20, May 2011).

Seed production is at the heart of the project, bringing together the scientific and technical expertise of the MSB with the practical skills and experience of the horticultural teams at Wakehurst Place. Focussing on species with limited availability in the commercial seed market, the UKNSH is working to provide high quality seed for restoration use and develop improved nursery and plant production techniques for native plants.

In July 2012 we were joined by partners from across the country to celebrate the opening of the new UKNSH production site, which provides a permanent home for our seed production beds and an inspiring place for visitors to learn about British wildflowers. Plants are grown in 24 long, narrow beds, creating striking blocks of colour amidst the meadows and woodlands of the Sussex High Weald. Willow sculptures of native flowers and seeds lead visitors around the site, with interpretation panels to introduce each species and explain how the project will work. The site is maintained by Vicki Foden and her colleagues in the nursery, but almost every team at Wakehurst Place has been involved in developing the site, whether by lifting turf and preparing the soil, fencing, hedge laying, installing bee hives or planting nearly 5000 plug plants in the wettest June on record. *Rumex acetosella* (sheep's sorrel) was the first seed collected, followed by *Campanula rotundifolia* (harebell) and *Salvia pratensis* (meadow clary). We expect heavy harvests of all 17 species planted so far from 2013 onwards, and production will be expanded with another eight beds this winter.

Plants in the production site were grown from seed held in the MSB vault, and we are making new wild collections to expand the range of multi-provenance collections available to restoration practitioners. Great care

Saving Rapunzel

Phyteuma spicatum (spiked rampion), an unusual and beautiful member of Campanulaceae, survives in the UK in just a handful of sites in East Sussex, close to Wakehurst Place. The plant is associated with the fairytale Rapunzel, and is more commonly known as white rapunzel in continental Europe.

Spiked rampion requires damp, partly shaded conditions to thrive, and is thought to have declined as traditionally coppiced wet woodland has been lost. Remaining populations are small and fragmented, and restoration is complicated by a lack of seed, complex dormancy mechanisms and grazing by slugs and snails.

The UKNSH has joined a group of conservation organisations and land owners to expand existing populations and reintroduce the species to sites where it has been lost. Experiments by MSB and nursery staff have succeeded in breaking dormancy through prolonged chilling, with the first mature plants flowering at Wakehurst Place in summer 2012. Having developed a reliable propagation protocol, we will now be able to produce much larger numbers of plants for direct restoration to the wild and establish plants at the production site to secure a large, genetically-diverse supply of seed for the future.

***Phyteuma spicatum* in the Wakehurst Nursery** PHOTO: RBG KEW

is taken to secure the highest possible germination and establishment rates, minimising losses in genetic diversity between wild and regenerated collections. Seed is sown on trays of compost or agar plates, which are placed under optimal germination conditions in the MSB incubators. Seedlings are carefully acclimatised to nursery conditions before being pricked-out and grown in the glasshouses or polytunnel and planted out in the production beds. Detailed record keeping at each stage of the process helps identify problems and improve techniques, and will enable us to develop detailed propagation and cultivation protocols to accompany our seed.

It is this combination of MSB and horticultural expertise which places the UKNSH in a strong position to propagate and grow species currently considered too difficult for commercial production. *Hippocrepis comosa* (horseshoe vetch), for example, is much in demand for chalk grassland restoration but germinates poorly without chipping or scarification of the hard seed coat. Hot, damp conditions favour the germination of *Serratula tinctoria* (saw-wort), while the seedlings of *Pulsatilla vulgaris* (pasque flower) need careful protection from pests and disease. Other species may be less problematic to grow, but very difficult to harvest. The pale purple flowers of *Cardamine pratense* (cuckoo flower) are a common sight in spring, but the seed is dispersed in sudden, unpredictable bursts, making large-scale harvests impractical for most producers. Under the controlled conditions of the seed hub, however, we are able to make several collections a year, harvesting seed just before dispersal and using the ripening facilities of the MSB to ensure maximum viability with minimum loss of seed.

Some species remain a challenge. Our first attempts to propagate *Adonis annua* (pheasant eye), for example, produced just three seedlings and only a single flowering plant. Germination Specialist Rachael Davies is now experimenting with a range of after-ripening techniques and temperature regimes to understand and ultimately break dormancy, enabling us to bring this beautiful but nationally-scarce species into production. Research is also underway to compare different growing media and germination techniques for UK natives, and we are developing expertise in seed priming to make 'ready to grow' seeds available to clients and partners. All three projects combine laboratory techniques with practical horticulture, and are the fruit of cooperation between MSB students and horticultural staff.

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The UKNSH is funded by the Esmée Fairbairn Foundation.

Vicki Foden and Sian Wilson sowing seed in Autumn 2011 PHOTO: RBG KEW

Improving access to MSBP seed collections

One of the key priorities for MSBP is to facilitate better access to our collections so that they may be used by scientists and researchers worldwide. In particular we seek to enable innovation, adaptation and resilience in the fields of agriculture, horticulture, forestry and habitat restoration. As part of this work, we are happy to announce that the MSBP seed lists have been greatly improved for the benefit of all users.

Two seed lists are available at http://data.kew.org/seedlist/. One is for those organisations wishing to request seed under the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) for research, breeding or training related to food and fodder production. The other list is for all other requests for use in research, habitat restoration and species re-introductions.

A key change is that no prior registration is now required in order to browse the collections which are available. The search tools have been made much more user friendly and much of the guidance text has also been provided in Spanish and French.

Secondly, over 11,000 additional collections have been added to this release of the seed list, mainly representing collections which we cannot distribute from Kew, but which may be available from the partner seed bank in the country of origin. In this case the contact information currently held for the donor organisation is displayed. Collections are included in the seed list if there is enough seed available to allow distribution and if a germination protocol is available.

All seeds from Kew are supplied with data on collection location and a germination protocol.

The value of MSB seed collections for horticulture: Japanese knotweed case study

MSB seed collections can save research organisations significant time, money and effort, allowing them to get on with vital work to solve problems. For example, the UK research organisation CABI has been investigating potential biological control agents for the invasive plant Japanese knotweed (*Fallopia japonica*). Staff had identified a potential insect agent but wished to test its host specificity. The MSB provided seed from 48 species for this purpose and the resulting research concluded that the insect was indeed specific to the Japanese knotweed. Clearance of Japanese knotweed costs the UK alone £166 million per annum which gives an indication of the financial value of the MSB collections for research.

Kew's partnership with **Garden Organic**

by **Vanessa Sutcliffe,** Training Specialist, MSBP

Kew has recently formed a partnership with the UK's leading organic gardening charity, Garden Organic, to develop a community seed saving hub at Wakehurst Place. This will act as a focal point for learning about food diversity and plant conservation. Garden Organic runs the Heritage Seed Library, a collection of around 800 rare and endangered vegetable varieties, offering opportunities to members to make use of these seeds.

The range of vegetables and fruit grown by gardeners across the UK has declined over recent decades. This has affected our home-grown food diversity and created a culture of buying new seeds each year rather than using those produced by the previous year's crop. Research currently being undertaken by Kew scientists is demonstrating that heritage and traditional varieties actually have increased nutritional value. Open-pollinated, heritage varieties show a greater genetic diversity and are therefore better adapted to deal with pests and diseases and with the threats of climate change, than modern varieties which have increasingly narrow genetic bases.

Seed saving and exchange is an essential part of practical public engagement in safeguarding food diversity and botanical inheritance and for building resilience and sustainability for home food growing. The community seed saving hub will raise awareness of food diversity and provide an educational forum for seed saving, as well as an annual event for seed swapping.

Project outputs

- Technical training workshops in heritage vegetable growing and seed saving, for specialists and home growers, provided through Kew's established and popular adult education programme.
- A centre of learning to inspire and educate young people in the origins of food, understanding inheritance, vegetable growing, harvesting and seed saving. Families will be engaged through vegetable garden artwork and heritage vegetable name trails.
- A project website with information resources for exchange and use of seeds.
- A managed display area for heritage vegetable varieties at Wakehurst Place, open to the general public.
- An annual, community seed swap event at Wakehurst focussing on seed saving, seed exchange and delivering technical knowledge, reaching over 500 people each year.
- Duplicate storage and curation of 800 Heritage Seed Library accessions at Kew's Millennium Seed Bank amounting to 43 different species.

Rachael Davies demonstrating seed cleaning techniques at the Great Seed Swap, 2011. PHOTO: J. HOLDEN

The Great Seed Swap, Wakehurst Place 2011. PHOTO: J HOLDEN

The Great Seed Swap at Wakehurst Place

The first seed swap was held in September 2011 as an inaugural event for the launch of this project, delivered jointly between Kew and Garden Organic. It was held in the Millennium Seed Bank at Wakehurst Place. The day was attended by over 300 members of the public, who were able to exchange seeds and gather information from like-minded gardeners, heritage seed suppliers and experts.

The focus of the event was a seed swapping area, where visitors could bring saved seeds (or a monetary donation) and swap them for other seeds available. There were presentations from Matthew Biggs, giving a Gardeners' Question Time session and James Wong who spoke about under-utilised food plants. Representatives from Garden Organic and Kew staff also gave talks. Organisations including specialist seed suppliers, local food producers and artisans and community groups staffed stalls at the event.

The second seed swap is being held on the 20th October 2012 and we are hoping to build on the success of last year. Once again, the focus of the event will be the seed swapping area. There will be a presentation from James Wong, as well as cookery demonstrations using heritage vegetable varieties by two of Raymond Blanc's chefs. There will also be a 'seed bomb' making workshop run by Josie Jeffrey. Representatives from Garden Organic and Kew will give talks covering a range of topics from environmental stewardship to preserving varietal purity and the science of seed saving. A new feature this year will be the National Fruit Show, showcasing and offering tastings of British grown varieties of apples and pears. There will also, for the first time, be limited tours of the Millennium Seed Bank vault for members of the general public. Hopefully this year's event will be even more popular than 2011.

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Kew's horticulturalists **pollinating for the future**

by **Noelia Alvarez,** Seed Collection Officer RBG, Kew and **Michele Sanchez**, Botanical Horticulturalist, RBG Kew

In 2006, RBG Kew established a project to ensure the long-term seed storage of the living collections held at Kew Gardens and Wakehurst Place. Noelia Alvarez was appointed to lead this effort. She works in the Great Glasshouse and Training Section at Kew, which includes the tropical nursery and tropical and temperate conservatories. For the past 6 years Noelia has been busy setting up and carrying on this significant task. Assistance from the horticultural team of the nursery was essential for working on a target list of more than 5,000 accessions and 200 different plant families. Extra help came in 2009 when Michele Sanchez joined the project. So far, seed from more than 700 living plant accessions have been banked in the MSB; more than half of them coming from natural source material and circa a third red listed in the IUCN Red List Database.

Priority accessions for seed banking were identified by preliminary work entering and updating Kew's Living Collections Database (LCD) with IUCN ratings. The prioritised plants were then tagged with red dots, enabling identification and awareness of these important collections. Seeds from natural source material and accessions of historical and horticultural importance are also collected. Apart from the contribution to Kew's Breathing Planet Programme and the MSBP objectives, this project also focusses on delivering training on pollination biology, breeding systems, the isolation of flowers and the handling and storage of seeds to staff, trainees, students and volunteers. It has been a useful way of developing skills and methods for hand pollinations and flower isolation in order to further our knowledge on plant reproductive biology.

Isolation is necessary in most cases to avoid hybridization between accessions. The methods of isolation we use before flowers open include the physical separation of plants and placing barriers to pollinators or pollen transfer, such as paper/polyester bags, horticultural fleece and pollen proof bags.

Noelia Alvarez pollinating *Petunia exserta* PHOTO: RBG KEW

Michele Sanchez isolating the flowers of *Opuntia boldinhii*
PHOTO: RBG KEW

Herbarium vouchers, verifications and taxonomic updates of collections are carried out at the Herbarium at Kew, and data is fed back to the MSB and LCD; this is crucial for keeping records up to date. Photographs of the flowers are taken and stored on the pollination database. The tools used for hand pollinations include natural hair brushes, sewing head pins, toothpicks and tweezers. Contamination with unwanted pollen is avoided by cleaning the tools with absolute alcohol between pollinations. Pollinated plants are tagged and information on pollination date, type (self, cross, and so on) and number of flowers pollinated, is recorded on the tag and on the pollination database. When the fruits show signs of maturity, they are collected in paper bags (dried fruits) or glass jars/fabric bags (fleshy fruits) and sent to the MSB, along with a data collection form.

Working with so many different plant families presents a range of challenges including the presence of self-incompatibilities, reduced number of plants or cloning by vegetative propagation in the accessions and lack of male or female plants in dioecious systems. The work is easier when there are several distinct individuals of the same accession and plants were originally grown from seed stock, but that is rarely the case when dealing with threatened plants in botanic garden collections. Some significant successful seed collections have included *Abutilon menziesii* Seem. (criticially endangered, Hawaii), *Wahlenbergia angustifolia* (Roxb.) A.DC. (endangered, St. Helena), *Cylindrocline commersonii* Cass. (critically endangered, Mauritius) and *Rhaphithamnus venustus* (Phil.) B.L.Rob. (vulnerable, Juan Fernández Island). A new challenge is attempting the successful fertilisation of *Metastelma anegadense* Britton, an Apocynaceae climber endemic to the British Virgin Islands with minute flowers, complex floral morphology and a possible self-incompatibility system.

Despite the challenges, it is very enjoyable and rewarding to be able to share our experiences and knowledge with a wide range of people and work with so many interesting plants whilst contributing to safeguarding them for future generations.

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In situ and *ex situ* conservation of orchids in Jordan

By **Khaled Abulaila**, National Centre for Agricultural Research and Extension (NCARE), Jordan.

Orchis papilionacea PHOTO: K. ABULAILA

Orchids are one of the most popular ornamental plants worldwide, but in their natural habitat, many species are highly threatened due to climate change, habitat degradation, loss of pollinators and over-collection for their horticultural and medicinal value. The forests of Jordan, a critically endangered and fragile ecosystem, host 26 species of Orchidaceae, many of which are threatened. To advance both *in situ* and *ex situ* conservation of these plants, I recently completed a PhD on the conservation of *Orchis papilionacea* and *O. sancta*, two terrestrial orchids native to Jordan. The degree was funded by the MSBP project in Jordan, under the supervision of Dr Tim Marks (MSBP) and Professor Dawud Al-Eisawi (University of Jordan).

The decline of orchid species in Jordan is attributed to several factors, including habitat destruction and severe drought conditions. Drought has a critical effect on the mycorrhizal association which is essential for germination and seedling establishment (Al-Eisawi, 1986; 1996). However, little is known about these mycorrhizal associations, as most of the information on the symbiotic fungi colonisation process derives from *in vitro* experiments rather than from the field (Peterson and Farquhar, 1994). The identification of the true mycorrhizal symbionts is of critical importance in the understanding of orchid ecology and to advance conservation efforts. Using scanning electron microscopy, I described the spatial and temporal fungal association in relation to seed germination. I found that germination reached the protocorm stage in less than 25 days and development was positively proportional to fungal penetration. With colleagues in Kew's Jodrell laboratory we were also able to identify *Tulasnella calospora* as the dominant symbiont.

In order to develop effective *ex situ* conservation to fit the specific requirements of Mediterranean terrestrial orchids, there is an urgent need to establish seed banking programmes and detailed studies on orchid seed storage physiology. Although considered orthodox (desiccation tolerant) in storage behaviour, some orchid seeds can quickly lose viability if stored under conventional seed bank conditions (-20 °C and 5% moisture content) (Pritchard and Seaton, 1993). However, for seeds of *O. papilionacea* and *O. sancta* I found that viability was not dramatically affected by low seed moisture content, making them superior to other terrestrial orchids in desiccation tolerance. Seed acquisition of desiccation tolerance occurred about one week before dehiscence in *O. papilionacea* and *O. sancta*: an important developmental stage for us to identify, in order to help successful long-term conservation of the germplasm of Orchidaceae species. I also observed species specificity to suitable storage conditions, often with optimal temperatures different to those used for conventional banking temperature; a likely result of adaptation to the natural habitat.

The results of this study have enhanced our understanding of conserving orchids in both natural and *ex situ* environments. This will contribute towards making long-term strategies to protect such a precious and rare floral component of Jordanian local habitats.

For further information please contact Dr Tim Marks (t.marks@kew.org) or Dr Khaled Abulaila (kabulaila@gmail.com)

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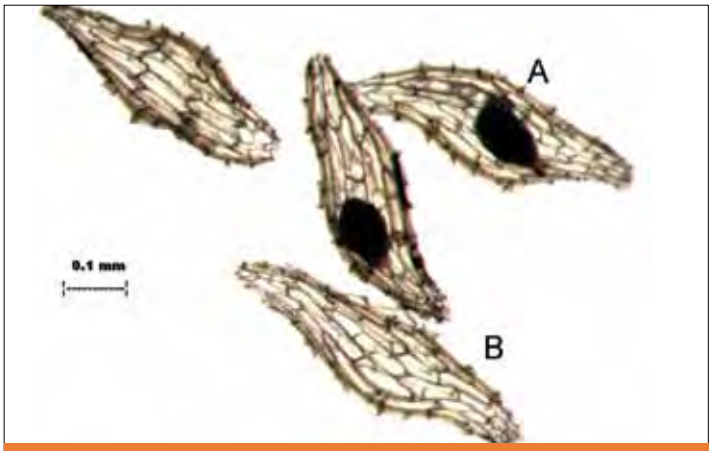
Khalid Abulaila working in the field PHOTO: K. ABULAILA

Viability assessment in seeds of *Orchis papilionacea* (viable seeds are stained red) PHOTO: K. ABULAILA

Nutritional requirements for *in vitro* seed **germination** in 12 terrestrial, lithophytic and epiphytic **tropical orchids**

by **Jayanthi Nadarajan** (Cryobiologist) and **Tim Marks** (Biotechnologist), MSBP.

The Orchidaceae is the second largest plant family with an estimated 25,000 species world-wide. Many are of notable horticultural value and occur as different life forms in diverse habitats. Seed storage is being successfully applied for both conservation and horticultural use. Although association with their natural symbiotic fungus can be critical to some re-introductions, the use of asymbiotic germination is often used in the regeneration of seedlings, in monitoring their longevity in seed banks, and in producing flowering plants.



Seeds of *Cattleya maxima* with typical structure for orchids; fusiform in shape with a ovoid embryo made of only a few hundred cells, no endosperm, and a testa that is one cell layer thick (A); Empty seed (B). (Magnification bar = 0.1 mm). PHOTO: J. NADARAJAN

Although numerous media have been developed to evaluate both seed germination and further seedling development after storage, few studies have attempted to relate nutritional requirements to life history traits. For a range of terrestrial, lithophytic and tropical epiphytic orchid species, we made comparisons of germination on various media to represent variations in available nitrogen. Tested media included Knudson C, with and without activated charcoal or banana powder, Norstog and Phytamax™ media. Germination varied, with maxima ranging from just 9% for *Prosthechea cochleata, Platanthera* sp. and *Spathoglottis paulinae*, to 95% for *Phragmapedium longifolium.*

Along with *Paphiopedilum delenatii, Paphiopedilum philippinense* and the epiphyte *Guarianthe bowringiana, Phragmapedium longifolium* germinated well on most media. Germination was significantly higher on Norstog than the other media for four of the six epiphytes tested, and germination was maximal on Knudson C medium with activated charcoal for four of the six terrestrial/lithophyte species. The results indicate a greater preference for nitrogen from amino acids, rather than ammonium or nitrate salts, in seeds of tropical epiphytes compared to some terrestrial orchid species.

Further reading

J. Nadarajan, S. Wood, T. R. Marks, P. T. Seaton & H. W. Pritchard (2011) Nutritional requirements for in vitro seed germination in twelve terrestrial, lithophyte and epiphytic tropical orchids. *Journal of Tropical Forest Science* 23 (2): 204-212.

For further information please contact Dr Jayanthi Nadarajan (j.nadarajan@kew.org).

Kew and Chinese delegates at the OSSSU-China workshop

OSSSU in China

By **Tim Marks**, Biotechnologist, MSBP

In April, Hugh Pritchard, Phil Seaton and Tim Marks met colleagues both old and new from botanic gardens, research institutes and universities at the National Orchid Conservation Center, Shenzhen, China. Our agenda was to discuss the development of an in-country OSSSU programme for China that would support the conservation of their 1,350 orchid species, and benefit from the easier exchange of material and expertise in-country.

Key science publications

(April – September 2012)

1 Drummond, C. S., **Eastwood, R.**, Miotto, R. J. & Hughes, C. E. (2012) Multiple continental radiations and correlates of diversification in *Lupinus* (Leguminosae): testing for key innovation with incomplete taxon sampling. *Systematic Biology* 61 (3): 443-460.

2 Vandelook, F., Janssens, S. B., & **Probert, R. J**. (2012). Relative embryo length as an adaptation to habitat and life cycle in Apiaceae. *New Phytologist* 195 (2): 479-487.

3 Hu, D., Ma, G., Wang, Q., Yao, J., Wang, Y., **Pritchard, H. W**., & Wang, X. (2012) Spatial and temporal nature of reactive oxygen species production and programmed cell death in elm (*Ulmus pumila* L.) seeds during controlled deterioration. *Plant, Cell & Environment*. 35 (11): 2045-2059.

4 Mondoni, A., Rossi, G., Orsenigo, S. & **Probert, R. J.** (2012) Climate warming could shift the timing of seed germination in alpine plants. *Annals of Botany* 110 (1): 155-164.

5 Popova, E. V., Kim, D. H., Han, S. H. & **Pritchard, H. W.** (2012) Narrowing of the critical hydration window for cryopreservation of *Salix caprea* seeds following ageing and a reduction in vigour. *CryoLetters* 33 (3): 219-230.

Non-invasive techniques for **monitoring seed quality**

By **Louise Colville**, Biochemist, MSBP

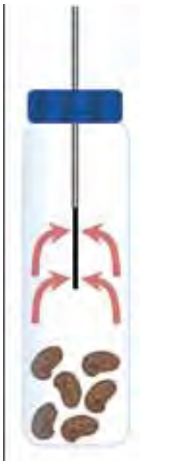
The use of seeds in agriculture, horticulture and in conservation programmes depends on high quality seed, which germinates and produces healthy seedlings. Seed viability is routinely assessed in seed banks by germination testing, but low seed numbers (germination is a destructive process) or the time and expense associated with slow-to–germinate seeds, mean that germination testing is not always feasible. In such cases the ideal solution would be to diagnose seed quality (viability and vigour) rapidly, without destruction of seeds. One such non-invasive test that is currently under investigation is the use of gas chromatography-mass spectrometry (GC-MS) to analyse the volatile compounds that are released from seeds, as quality decreases during the ageing process.

Under seed bank conditions of low relative humidity (RH) and -20 °C, the seed ageing process is slowed, but not halted, and all seeds gradually deteriorate. The ageing process involves damage to cellular components such as membranes and macromolecules (lipids, nucleic acids and proteins) caused by reactive oxygen species which are formed through auto-oxidation reactions. These can still occur in dry seeds where the cellular cytoplasm is in a glassy state. Damage to lipids gives rise to a diverse range of peroxidation products including aldehydes, alcohols and ketones, many of which are volatile. These can be detected within the sealed storage container, and accumulate with storage time.

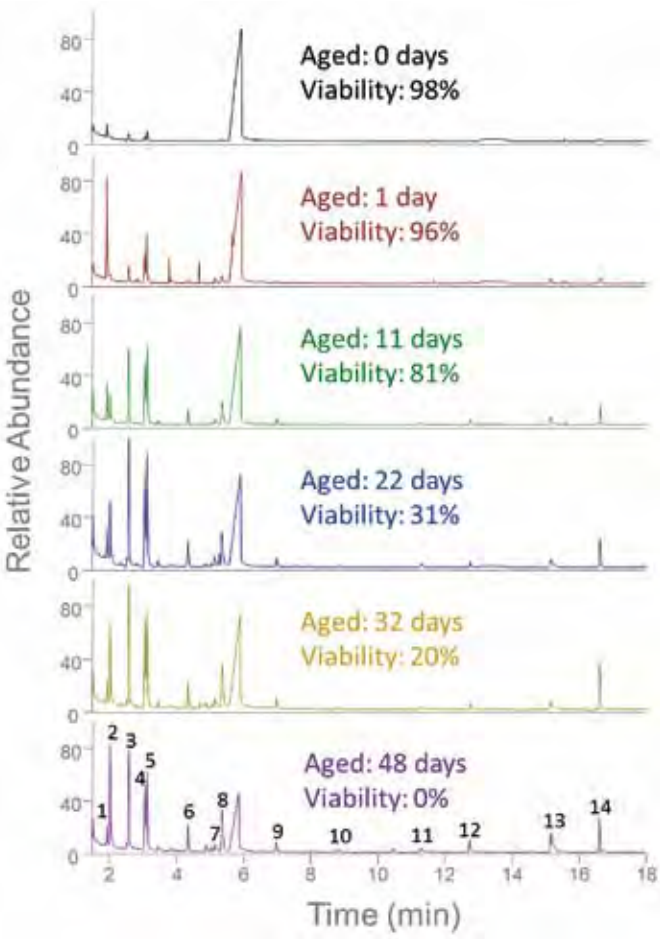
We have been studying the release of volatile compounds using seeds subjected to artificial ageing. This accelerates the ageing process by storing seeds at higher RH and temperature, so that viability loss occurs within weeks, rather than the decades that it would take under seed bank conditions. One of the key findings of these studies is that seeds produce ageing-related volatile compounds prior to any sign of viability loss. This means that volatile analysis could enable us to detect the early signs of ageing before seeds start to die, allowing intervention to take place; for example, the regeneration of collections or collection of new seeds.

It was hoped that an individual compound would be a marker for seed viability, but the biochemical analysis reveals a more complicated situation. For example, pea seeds released 24 different volatile compounds during artificial ageing, of which 12 correlated with seed viability. However, only two of these (methanol and acetic acid) also correlated with viability in another legume species, *Lathyrus pratensis*. Neither methanol nor acetic acid correlated with viability of *Cytisus scoparius* seeds. Further studies are ongoing, therefore, to gain an understanding of the influence of seed characteristics such as structure and chemical composition in terms of storage reserves, as well as the effect of moisture content and storage temperature on the volatiles released by seeds during ageing. These studies will provide valuable information on the ageing process in seeds, and enable us to fully assess the potential of volatile analysis as a non-invasive technique for diagnosing seed quality.

For further information please contact Dr Louise Colville (l.colville@kew.org).



Solid phase microextraction (SPME) is used to trap the volatile compounds present in the headspace above the seeds in a sealed vial. The autosampler syringe needle pierces the vial cap and exposes an adsorbent fibre to the air within the vial. Volatiles are adsorbed onto the fibre which is then transferred to the injection port of the GC, which is held at high temperature to desorb the volatiles from the fibre. Compounds are then separated by gas chromatography and detected using mass spectrometry.



Gas chromatograms of the volatile compounds present in sealed vials containing *Robinia pseudoacacia* seeds subjected to artificial ageing at 62% RH and 50°C for 0, 1, 11, 22, 32 and 48 days. Seed viability was assessed by germination testing. The peaks correspond to: 1, acetone; 2, methyl acetate; 3, methanol; 4, 2-propanol; 5, ethanol; 6, acetonitrile; 7, 2-butanol; 8, 1-propanol; 9, 2-methyl-1-propanol; 10, 1-butanol; 11, 2-methyl-1-butanol; 12, 1-pentanol; 13, n-hexylformate; 14, acetic acid.

Seed collecting in Mediterranean islands

By **Teresa Gil**, European Partnership Officer, MSBP

The flora of Mediterranean islands is known for its outstanding biodiversity and endemic richness. Many of the endemic species are restricted to individual islands. This flora is critically threatened by human development and climate change and requires urgent conservation measures to safeguard it. The main goal of the "Ensuring the survival of endangered plants in the Mediterranean" project is to ensure the survival of 900 plant species in six Mediterranean islands though *ex situ* conservation measures.

The project started on the 1st October 2011 and will run for three years. It is an initiative led by seven conservation organisations from Crete, Corsica, Cyprus, Mallorca, Sardinia, Sicily and the UK and is funded mainly by the MAVA Foundation with the support of other co-funders including "Obra Social Sa Nostra, Caixa Balears" and the University of Cagliari.

Vendicari juniperetum PHOTO: P. MINISSALE

A joint seed collecting trip in Crete PHOTO: G.P. GUISSO

The first seed collecting expeditions have been successfully carried out in the six islands and high quality seed material from about 300 plant taxa has been collected in accordance with national and international regulations and standards. Most of taxa are endemic, rare, threatened or protected. The seeds will be stored in local seed bank facilities in the six islands with a back up in a second facility such as the Millennium Seed Bank.

During the project several joint seed collecting trips will be done and will allow the partners to exchange information, experience and skills and encourage team building.

The project website http://www.medislandplant.eu/ is online and we invite you to follow the activities and results of this Mediterranean project.

For more information please contact: Jonas Mueller (j.mueller@kew.org) or Teresa Gil (teresagilgil@kew.org)

Project partners:

- Royal Botanic Gardens, Kew, London, United Kingdom (Project Co-ordinator)
- Jardí Botánic de Sóller, Sóller, Malloca, Spain
- Mediterranean Agronomic Institute Chania, Chania, Crete, Greece
- Conservatoire Botanique National de la Corse, Corte, Corsica, France
- The Agricultural Research Institute, Nicosia, Cyprus
- Centro Conservazione Biodiversitá, Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Sardinia, Italy
- Dipartimento di Scienze Biologiche, Geologiche e Ambientali, University of Catania, Catania, Sicily, Italy

Producing collecting guides using **BRAHMS reports**

By **Ruth Harker,** Collecting Guide Compiler, MSBP

The Crop Wild Relatives Project has been developing a new method of producing seed collecting guides using the visual reporting tool in BRAHMS (Botanical Research and Herbarium Management database).

Data for each target species is recorded in an RDE (rapid data entry) file, including a species description, habitat, distribution, phenology, conservation status, best collecting method, and how to distinguish the target species from others with which it may be easily confused. High quality images of the plant in the field and distribution maps are stored as jpegs in the RDE.

The visual reporting tool within BRAHMS can be used to design a template for the layout of the species profile pages which integrates the botanical information, images, and maps, which are stored in the RDE with graphics and labels. The visual report may also be used to specify the font, colour and size of text as well as the layout. This tool has proved to be extremely useful and efficient in compiling collecting guides, as the species profiles which are produced do not need any additional editing and can be exported straight to a PDF.

The resulting species profiles will be used in collecting guides to provide collectors in the field with key information for identification and collection of target species.

Contact: r.harker@kew.org for more details.

Kew magazine

The latest issue of *Kew* magazine was published at the end of September and includes a wide variety of articles and features on Kew's work and collections. *Kew* magazine specialises in going behind the scenes at Kew and meeting the people who make it tick and revealing the most important projects Kew is involved in. The MSBP, and other international partnership projects, have featured in many issues of the magazine. The latest issue meets the team behind the Fuelwood Project in the caatinga of Brazil, and talks to leading wood anatomist Dr Peter Gasson.

If you would like to read *Kew* magazine, you can subscribe to the print edition or to an online-only edition (which also gives you access to issues dating back to winter 2008), which offers much better value and is a greener option too! You can look at some past and current features for free on the *Kew* magazine web pages – go to www.kew.org/kewmagazine and click on 'archive' to browse features. You can also find out much more about how to get your copy here.

Seed collecting training in Mozambique

A three day seed collecting training course and field trip in April 2012 launched the recently-signed partnership with Mozambique. Fourteen participants from the Instituto de Investigação Agrária de Moçambique (IIAM), Eduardo Mondlane University and the Ministry of Agriculture's seed testing laboratory heard about the global MSB Partnership and plans for the collection, conservation and sustainable use of priority species in Mozambique. We then focussed on the essential theory and practice needed to ensure that seeds of Mozambique's wild plant species are safely conserved for present and future generations. A field trip to Michafutene provided a good opportunity for tree seed collectors to experience the different requirements of collecting seeds of herbs and grasses, and for everyone to practice their skills in preparing herbarium vouchers.

PhD awards

Many congratulations to three PhD students funded by the MSBP who recently defended their PhD research: Dr Rosemary Newton (Germination Specialist, MSBP), was awarded a PhD by the University of Reading for her work on the 'Development, dormancy, germination and *ex situ* survival of seeds of selected Amaryllidaceae species'. Rosemary's supervisors were Prof. Richard Ellis (University of Reading) and Dr Fiona Hay (MSBP, now at IRRI in the Philippines). Dr Susanne Claessens was awarded a PhD by Wageningen University, the Netherlands, for her research on 'Dormancy cycling in seeds: mechanisms and regulation'. Susanne's project was supervised by Dr Peter Toorop (MSBP). Dr Khaled Abulaila, University of Jordan, was awarded a PhD by the University of Jordan for his research on the 'Ecology and *ex situ* conservation of *Orchis sancta* (L.) and *Orchis papilionacea* (L.) (Orchidaceae) in Jordan'. Khaled's project (see article on page 8) was jointly supervised by Dr Tim Marks (MSBP) and Prof. Dawud Al-Eisawi (University of Jordan).

Millennium Seed Bank Collection Figures December 2012

Total collections	61520
Number of species	31945
Number of genera	5348
Number of families	333

New MSB agreements			
Country	**Start date 2012**	**Duration (years)**	**Counterpart name**
South Korea	17 July	5	National Arboretum of the Korean Forest Service
Portugal	5 August	5	Univerdidade de Lisboa
New Zealand	12 April	5	New Zealand Plant Conservation Network
Mozambique	14 August	5	Micaia Foundation
Romania	26 June	5	Institute of Biology
Kyrgyzstan	30 May	2	Institute of Biotechnology
Bulgaria	2 July	3	Institute of Biodiversity and Ecosystem Research
Kenya	15 August	5	Kenya Forestry Research Institute

Participants on the SCT course, 2012 PHOTO: W.STUPPY

Seed Conservation Techniques training course

Exactly ten years and one day after the very first Seed Conservation Techniques (SCT) course, 13 participants from 12 countries gathered in the seminar room of the Millennium Seed Bank for the first day of the 2012 course. The course followed the tried and tested formula of previous courses, with lectures about the theories underpinning the MSBP's technical protocols interspersed with opportunities to practice seed collecting, cleaning and viability testing. RBG Kew's new Director, Richard Deverell, observed the team work and enthusiasm as participants made collections from the grasslands surrounding the MSB. Participants 'strongly agreed' that the course had provided them with improved technical skills and knowledge, and all returned home with action plans for things they wish to achieve in their respective institutes. MSB staff will be working with them over the coming months to help implement these plans.

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Samara aims to provide information and inspiration for MSBP partners and a flavour of the successes of the Partnership for other interested recipients and is available as a PDF from the MSBP website at: **www.kew.org/samara**

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