SUPPLEMENTAL INFORMATION - CASE STUDY FULL TEXT

APPENDIX 2: CASE STUDY CONTRIBUTORS

We gratefully acknowledge the following individuals and organizations that provided case studies to support the North American Collections Assessment. The information and opinions presented below do not represent those of Botanic Gardens Conservation International.

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1. Conservation strategies at the Montreal Biodome for overharvested species.

By Andree Nault, the Montreal Biodome

Human activity creates enormous pressure on wild species: urbanization, industrialization and demographic growth are complex processes in which we can hardly interfere. For certain species, the threats are more readily identified and efficient conservation strategies can be developed. Since 1994, the research division of the Montreal Biodome has developed collaborative conservation strategies for two species which have encountered major population declines due to overharvesting: American ginseng (*Panax quinquefolius*) and wild leek (*Allium tricoccum*).

American ginseng is the most widely used medicinal plant in the world and despite cultivated sources being able to satisfy most of the demand, natural populations are still under great pressure from wild harvesting. In Canada, the species is restricted to the southern part of Ontario and Quebec where root harvesting, loss of habitat (urban and commercial development) and deer browsing are high. It is now 'endangered' and legally protected: harvest and commercial sales of wild roots are illegal. As a result, a conservation and restoration program has been developed at the Montreal Biodome in order to identify, monitor and promote the protection of key sites. A conservation strategy has been established among key actors: landowners, local conservation groups and provincial and federal conservation groups in order to meet a common goal: protect and secure at least 40 viable populations throughout Quebec. Site-specific conservation plans have been prepared and are now collaboratively implemented to reduce threats and ensure long term conservation of key sites (Figure 1). Improving our abilities toward better land protection (stewardship & landowner's awareness) and population viability (confidentiality, monitoring, camouflage, supplemental seeding) represent ongoing challenges!

Wild leek is sought for its edible bulbs and largely known as a spring delicacy in North East America. At the northern limit of its range, in Quebec, the species has been designated 'vulnerable' and legally protected since 1995. After surveying over 150 natural populations to evaluate the situation, the Montreal Biodome developed a comprehensive conservation strategy: 1- Protect remnant large colonies; 2- Educate the public to reduce harvesting pressures; and 3- Restore the species widely, through a public planting program. In collaboration with federal wildlife agents and interested landowners, a public planting program, SEM'AIL, was launched in 2000. Since then, a million wild leek seeds have been planted by 1,117 forest landowners throughout southern Quebec. Also, 440,000 ceased bulbs by wildlife agents were rescued and transplanted by SEM'AIL participants. We estimate that around 500 new colonies were created through this restoration program. Now, a scholar program for a younger audience, SEM'AILjr, is under development that will involve children, schools and local communities in restoration of the wild leek.



Figure 1: Supplemental seeding is applied to enhance recruitment in small populations of American ginseng (*Panax quinquefolius*). Photo courtesy of Andree Nault.

2. Community engagement in plant conservation.

By Kirsty Shaw, for El Charco del Ingenio Botanic Garden

The El Charco del Ingenio Botanic Garden (El Charco) and adjoining reserve borders the city of San Miguel de Allende, in Guanajuato, which faces continual urban expansion. El Charco was founded in 1991 as the outcome of a non-governmental project with an aim to protect the area from development and restore the degraded habitat. Following subsequent donations of neighboring land, the venture has expanded to cover over 100 hectares. In one section, a formal botanic garden presents native flora and environmental education opportunities to visitors.

Prior to the creation of El Charco, the land was freely accessed and used by local communities, particularly for the collection of fruits following the rainy season. Converting the land to an area no longer available for free access therefore presented a challenge for the pioneers of the project, who wanted to satisfy conservation priorities as well as provide benefits for the community. Eventually, the Garden decided to solve this problem by providing valued resources to the community, and promote a sense of inclusion as well as an appreciation of the natural environment. Interestingly, the official opening of El Charco, on July 11, 1991, coincided with a solar eclipse. On this day, local communities came to the garden offering their most prized possessions to their gods, which now lay buried under a cross at 'Cuatro vientos' (four winds), a focal point of the Garden. After that point, an annual ceremony has been established at El Charco, on the weekend which falls closest to July 11, when each community decorates offerings to their gods, using Dasylirion acrotriche, or 'cucharilla' (little spoon, Figure 1), a practice which lasts until the early hours of the morning. The plant holds, "huge cultural significance" to these important opportunities for communities to, "unite, work together and share,' says Don Polo who has taken part in the ceremonies since their commencement. The following day a procession passes through the Garden, where the offerings are presented to shrines representing their four gods. The ceremony is not advertised to the public, and although access is not restricted during the day, the evening ceremony is solely for the local communities. Recently, several celebrations like this run within the local communities from April to July each year. Prior to each celebration, a huge cross is erected, also decorated using D. acrotriche (Figure 2).

Due to the high demand of this plant for decoration, accompanied by habitat destruction, *D. acrotriche* is now listed as 'threatened' (A) under NOM-059-SEMARNAT-2010. Propagation of this species is thus a priority at El Charco and individuals are donated to communities to encourage domestic cultivation of the plant and reduce pressure on wild populations. Contrary to many cultural practices in Mexico, this tradition is strengthening, with more people learning the art of decoration and unique styles developing over time. "The plant itself is beautiful, but the most beautiful thing is how it brings communities together, what it represents," says Doña Chole, who has also been heavily involved in the ceremonies since their commencement. Its conservation is essential for the continuation of such important traditional practices.

This novel form of community involvement in the conservation of a diverse landscape was tailored specifically to the local population, whose role is vital to the functioning, maintenance and purpose of the garden and reserve. This has proven invaluable during the 20 years since the garden's founding, with proposals for a motorway and large hotel adjacent to the garden being overthrown due to opposition from the local communities and their Mayordomos (leaders), worried for the impact on the land within El Charco. Protecting the reserve has allowed for natural regeneration and enhanced ecological value, and recent reintroductions of D. acrotriche and some Agavaceae species have been carried out, which would not have been possible twenty years ago due to severe soil erosion on the site. This achievement, in an area where urban expansion is seemingly relentless, explicitly illustrates the importance of the strong community links.



Figure 1: *Dasylirion acrotriche*, 'threatened' (A) NOM-059-SEMARNAT-2010. Photo courtesy of Kirsty Shaw.



Figure 2: Ceremonial decoration using *Dasylirion acrotriche*. Photo courtesy of Kirsty Shaw.

3. Pedicularis furbishiae: an environmental icon.

By Jean Aucoin, New Brunswick Botanical Garden

Furbish's lousewort (*Pedicularis furbishiae*), a member of the snapdragon family, is named after Maine naturalist Kate Furbish who first recognized it as a new species in 1880. Furbish's Lousewort was thought to be extinct until it was rediscovered during environmental surveys conducted for the Dickey-Lincoln Dam, a massive hydro-electric project proposed for the upper St. John River in Maine in 1974. This prompted extensive field surveys in which Furbish's lousewort became an environmental icon and the plant and other factors resulted in the cancellation of the dam project. Today, it is limited to only in a few sites along a 225km stretch of the St. John River in northwestern New Brunswick and northern Maine, and this species in listed as endangered at the provincial, state and federal levels in both countries.

Furbish's lousewort has fern-like leaves in a basal cluster with small yellow flowers opening sequentially in a stubby cylindrical head on a 60-75 cm stem during July and August (Figure 1). It grows on moist, semi-shaded areas of the riverbanks subject to flooding, erosion and ice-scouring. Furbish's lousewort (now placed in the Broomrape or Orobanchaceae family) is an obligate root hemiparasite during its seedling stage (i.e., it requires a host to provide nutrients and water). The parasite extracts these solutions through a specialized structure (haustorium) connecting both root systems. Among the host plants observed in nature, exotic species usually found with Furbish's lousewort are clover (*Trifolium* sp.), cow vetch (*Vicia cracca*) and bird's-foot trefoil (*Lotus corniculatus*); while native species are Canadian tick-trefoil (*Desmodium canadense*) and alder (*Alnus* sp.).

New Brunswick's provincial recovery plan for Furbish's lousewort includes habitat protection, education and stewardship. As part of the strategy, the New Brunswick Botanical Garden has experimented with greenhouse propagation of the species since 2008. Funded by WWF-Canada, the project has been a collaboration between the New Brunswick Botanical Garden, Professor Jean-Yves Blanchette, Mr. Richard Fournier and Ms. Adélaïde DeBont of the Faculté de Foresterie de l'Université de Moncton, and the New Brunswick Department of Natural Resources.

Propagation trials were set up using members of the Fabaceae family, as well as *Alnus* sp. as potential host plants, due to their ability to fix nitrogen, a vital element for developing lousewort seedlings. Seeds of the Furbish's lousewort were collected in early October 2008 at two selected sites. Originally three sites were selected but the third site lost most of the target plants in a summer flood in early August 2008. Following a pre-treatment, germinated seeds were planted close to the hosts and monitored for survival and growth over a four month period. Identifying emerging seedlings was a challenge since no literature description or photo images of the cotyledons were available. However, once the first leaves emerged the lousewort was easily identified. After four months some of the seedlings had leaves that were at least 5 cm long. Previous observations stated that one-year-old seedlings grown along the St. John River rarely had leaves more than 1.5 cm long. Our results suggest that it may be possible to shorten the normal three year period for flowering, and thus, fruit production for this rare species. Some of the seedlings have been planted in the New Brunswick Botanical Garden in St. Jacques in the summer of 2009. They will be maintained as a seed source and for further studies, and as an educational tool.



Figure 1: Furbish's lousewort (Pedicularis furbishiae)



Figure 2: Furbish's lousewort on display in the New Brunswick Botanical Garden as an educational tool. Photo courtesy of Jean Aucoin.

4. Robbins' cinquefoil: an endangered species success story.

By William Brumback, New England Wild Flower Society

After 23 years on the U.S. Endangered Species list, Robbins' cinquefoil (*Potentilla robbinsiana*) became the first plant to be delisted due to successful recovery efforts. This rare alpine plant (Figure 1), with a narrow distribution in the White Mountains of New Hampshire, was known from only one declining population when it was listed as endangered in 1980. Following listing, the New England Wild Flower Society, U.S. Forest Service, U.S. Fish and Wildlife Service, State of New Hampshire, Center for Plant Conservation and Appalachian Mountain Club worked collaboratively using *ex situ* and *in situ* conservation approaches to recover the species. Management actions *in situ* stabilized the last remaining population, and *ex situ* propagation and reintroduction work was successful in augmenting the existing population, and *ex situ* collections are maintained by the New England Wildflower Society as an ongoing insurance policy against extinction.



Figure 1: Robbins' cinquefoil (*Potentilla robbinsiana*). Photo courtesy of Doug Weihrauch.

5. Supporting integrated conservation of California native plants.

By Nancy Fraga, Rancho Santa Ana Botanical Garden

Rancho Santa Ana Botanic Garden (RSABG) is uniquely suited to implement *in situ* and *ex situ* measures to promote conservation of California's native flora. Our programs and facilities have a long and successful history in promoting conservation of California's natural heritage through research, education, and collections. Two of RSABG's core programs that aim to support conservation of California native plants are the Field Studies Program and the Seed Conservation Program.

The Field Studies program supports conservation efforts via field-based activities undertaken by skilled staff botanists. We conduct floristic inventories, monitor the status of rare plants, and work closely with partnering institutions to develop species management guides and conservation strategies. Floristic-based research is core to the program because knowledge of the California flora is vital to ongoing conservation efforts. Staff members work closely with local agencies and provide expertise on the California flora (especially southern California), and have a strong commitment to conserving California's native plants. The work conducted within the Field Studies Program is facilitated by the herbarium at RSABG; the largest and most active herbarium in southern California. The collection contains nearly 1.1 million specimens, of which more than 400,000 are from California. Among these, the 2,221 species of plants currently listed by the California Native Plant Society as rare, threatened, or endangered are abundantly represented. In fact, RSABG holds the world's largest collection of vascular plant specimens from the southern part of California.

The Seed Conservation Program curates more than 600 accessions that represent 120 state or federally listed rare, threatened, or endangered California plant species. Coupled with the living collection which maintains 62 accessions that represent 34 state or federally listed California plant species, the seed bank constitutes the largest *ex situ* conservation effort in California. As a charter member of the Center for Plant Conservation, RSABG is a national center for conservation of some of the most critically endangered plant species. Through a Memorandum of Understanding with the State of California Department of Fish and Game and the U.S. Fish and Wildlife Service, RSABG is authorized and regularly utilized as the principle repository for germplasm collections of rare, threatened, and endangered California native plant species (Figures 1 and 2).



Figure 1: In partnership with California State Parks and the U.S. Fish and Wildlife Service, RSABG developed a conservation strategy, a monitoring strategy and established a conservation seed bank for the federally endangered plant species, *Trichostema austromontanum* subsp. *compactum* (Lamiaceae). Photos courtesy of Michael Wall and John Macdonald.



Figure 2: In collaboration with the San Bernardino National Forest (SBNF), RSABG has participated in survey efforts for *Taraxacum californicum* (Asteraceae), a federally endangered species. Additionally, SBNF and RSABG are working to develop an *ex situ* conservation seed bank for this species. Photos courtesy of Michael Wall and John Macdonald.

6. In situ conservation inside our garden walls.

By Dave Ehrlinger, San Diego Botanical Garden

Preserving the existing natural areas in the San Diego Botanical Garden is very important. The local coastal sage scrub and southern maritime chaparral are some of the nation's most endangered vegetation types as they are restricted to coastal areas which are in high demand for human development.

Del Mar Manzanita (*Arctostaphylos glandulosa* ssp. *crassifolia*) is a federally endangered species that grows naturally in two natural areas on the Garden grounds. Over twenty shrubs grow in two small *in situ* populations in shallow soils on rugged sandstone outcrops.

Orcutt's hazardia (*Hazardia orcuttii*), a sub shrub in the Asteraceae family, grows in a small hilltop population only two miles from the Garden. This is the only naturally-occurring population in the U.S., while only a few disjunct populations are found in northern Baja California. In a reintroduction project supported by the California Fish and Game Commission several hundred plants were propagated and planted in local sites. In 1995 thirty plants were planted in the Garden in cooperation with state of California as well as the Center for Natural Lands Management. Most of the plants survive in the *ex situ* collection and several seedlings have been produced as well.

We have written interpretive signs about these plants and include conservation information about these species as well as other non-native species in educational classes, tours, and docent programs.

We also have a single specimen of Encinitas baccharis (*Baccharis vanessae*), grown from seed, which is growing at the edge of a natural area in the Garden. It is a small, dioecious shrub that is a federally listed threatened species. Apparently endemic to San Diego County, a small population grows in a natural area associated with Torrey pines, only a mile away from the Garden. We would like to get permits in the future, to acquire more germplasm to establish a larger *ex situ* population.



Figure 1: Del Mar Manzanita (*Arctostaphylos glandulosa* ssp. *crassifolia*) in the San Diego Botanical Garden. Photo courtesy of Dave Ehrlinger.

7. Common species threatened by exotic pests.

By Mark Widrlechner, NPGS (USDA-ARS)

Ash species (*Fraxinus* spp.) are found in urban and natural landscapes throughout North America, where they provide significant ecological, economical, and cultural value. Unfortunately, these species are now threatened with extinction after an exotic insect pest from Asia (Emerald Ash Borer, or EAB) was introduced to Michigan in the 1990s. The EAB kills nearly all native ash trees in a very short time, and has substantially expanded its range since its introduction. Millions of ash trees in urban and native landscapes throughout eastern North America have been killed and millions of dollars have already been spent trying to slow the spread of this pest and to replant trees. So far, it seems impossible to stop EAB, and the costs climb higher every year as an estimated eight billion ash trees are still in its path.

Before the introduction of EAB, most ash species in the United States were considered common and stable, and *ex situ* germplasm collections were very poorly developed. It was not until 2007 that a coordinated effort to build genetically diverse *ex situ* germplasm collections was begun to provide insurance against extinction for these species. This effort is led by the U.S. National Plant Germplasm System (NPGS), and numerous partners in the United States and Canada are now working to create genetically diverse ash germplasm collections for future research, education, and conservation use before it is too late.

Many EAB-response strategies are now being implemented to address short-term issues such as public awareness and monitoring the spread of EAB, as well as long-term questions such as genetic viability of remaining populations and the development of host-plant resistance. These efforts hold the promise of an eventual revival in planting ash as a landscape tree and of the re-introduction of ash populations to native forests. *Ex situ* germplasm collections, if well designed, can provide a wealth of genetic diversity, and supply known sources of clones and populations to support research. Fortunately, ash populations can be effectively conserved as seeds with orthodox storage characteristics, and clones cryogenically preserved as dormant buds. Field plantings can also compliment preserved seeds and buds. Our initial focus has been on the assembly of comprehensive seed collections. To accomplish this, we must ensure good initial seed quality, proper taxonomic identity, complete passport data, and effective sampling strategies.

Since 2007, successful trips were conducted in New England, Missouri, Illinois, and Wisconsin. Trips to Minnesota and Wisconsin and to Kansas, southern Missouri, and northern Arkansas are planned for 2010. These collections and those of many collaborators are being incorporated into the NPGS and conserved at the National Center for Genetic Resources Preservation in Fort Collins, Colorado, with many of the best documented and representative samples being incorporated into the active collection at the North Central Regional Plant Introduction Station in Ames, Iowa, which I curate.

For more information see:

Widrlechner, M.P. 2010. The National Plant Germplasm System Ash Conservation Project. Available at www.ars.usda.gov/sp2UserFiles/Place/36251200/Ash_Project/HomePage.html



Figure 1: Natural ash stand lost to the emerald ash borer at Hidden Lake Gardens, Michigan. Photo courtesy of Andrew Gapinski.

8. Integrated plant conservation and collaboration to conserve Colorado's alpine plants.

By Michelle DePrenger-Levin and Jennifer Ramp Neale, Denver Botanic Gardens

Growing in Colorado are over 100 imperiled plants and 12 federally listed plant species. Since 1986 Denver Botanic Gardens has made a commitment to study, document and conserve rare species across the Southern Rocky Mountain region. We run labor intensive, long-term studies for several rare and listed threatened species, providing essential information to the Bureau of Land Management to aid in conserving rare flora. Genetic studies further inform conservation of listed or proposed species in the face of immediate threats.

We also conduct *ex situ* conservation through seed collection and long-term storage, in collaboration with the Center for Plant Conservation. Denver Botanic Gardens has collected rare and listed species over the past 20 years but many of these collections are now reaching 20 years of age. Seed viability can drop drastically over this time necessitating recollection in order to maintain viable, genetically diverse germplasm for future study or reintroduction. Seed of five federally listed species was collected in the early 1990s. In recent years, growing concerns over climate change and loss of habitat, have led to a collaborative effort by the U.S. Fish and Wildlife Service and Denver Botanic Gardens to collect seed of all 12 listed species found in Colorado. Now, as the sole organization in Colorado with a permit to collect all listed species in the state, Denver Botanic Gardens has set out to ensure the future of our rarest species by making new collections of these threatened species in the next few years (Figures 1 and 2). In 2010, we made great progress towards this goal by collecting seed of four of our listed species. Due to small populations and low reproductive rates, completing these collections may take multiple trips and several years. Even with a federal permit and the wealth of information housed at the Colorado Natural Heritage Program, this work will proceed only through continued collaboration with partners such as the Bureau of Indian Affairs, Forest Service and Bureau of Land Management. In addition to our formal partnerships, Denver Botanic Gardens relies heavily on many trained volunteers who donate countless hours to relocate sites of listed and rare species, and gather information about the phenology of the flora across the state, thus allowing Denver Botanic Gardens to more effectively preserve populations of rare and listed species.



Figure 1: *Gaura neomexicana* ssp. *coloradensis*, a federally listed threatened species, is monitored *in situ* and preserved in *ex situ* seed storage by Denver Botanic Gardens. Photo courtesy of Mary Goshorn.



Figure 2: *Gaura neomexicana* ssp. *coloradensis.* Photo courtesy of Scott Dressel-Martin ©.

9. The Center for Plant Conservation Network.

By Kathryn Kennedy, Center for Plant Conservation

Since 1984, the Center for Plant Conservation (CPC) and its member network of 36 botanic gardens have worked to establish *ex situ* conservation resources, conduct *in situ* conservation, and where feasible prepare and initiate reintroduction for the rarest plants in the United States. The cooperative CPC network maintains the National Collection of Endangered Plants, which contains genetically diverse and representative *ex situ* collections of more than 700 of America's most imperiled native plants. Live plant material is collected from nature following science-based, collaboratively developed CPC protocols designed to ensure a genetically representative sample of populations for maximum conservation value. They are carefully maintained, preferably as seed (or when needed as tissue culture, or cultivated plants) in CPC institutions and in the USDA National Center for Genetic Resources Preservation seed bank. Over the last 25 years, this network has banked nearly 22 million seeds destined for future reintroduction efforts. In 2009 the Center's National Collection contained material of 45% of endangered, threatened, and candidate species under the U.S. Endangered Species Act. Network institutions also conduct research on germination and clonal propagation as well as biology and ecology. Materials are carefully monitored so that imperiled plants can be more effectively grown and returned to natural habitats.

Many CPC institutions are also involved in *in situ* recovery and reintroduction, including on-the-ground fieldwork, data collection, habitat, and experimental reintroductions in appropriate secure habitat. In 2009 CPC initiated an online international reintroduction registry to facilitate information sharing, archive important data in an accessible manner, and support development of improved guidance, approaches and techniques for reintroduction. The registry currently includes 122 reintroduction projects from North America. Data from the registry was used as the basis for a symposium in 2009 that synthesized trends, challenges, and best practices in reintroduction. The proceedings of this symposium will be published in 2011, and will include updated reintroduction guidelines. Researchers and managers are encouraged to continue to register their reintroduction projects within the registry, whether considered successful or not, to enable monitoring of progress and best practices.

For more information about the reintroduction registry, visit the CPC website: www.centerforplantconservation.org/reintroduction/MN_ReintroductionEntrance.asp

10. An integrated approach to conserve Florida's Scrub Lupine.

By Cheryl Peterson, Bok Tower Gardens

For critically imperiled species, an integrated approach to conservation can often be effective in making a long-term impact on species preservation. Bok Tower Gardens is taking such an approach in conserving scrub lupine (*Lupinus aridorum*), integrating efforts that include biological research, *ex situ* germplasm storage, education, tissue culture micropropagation, plant rescues and population introductions.

Scrub lupine is a short-lived perennial that is found only along the Winter Haven Ridge and the Mt. Dora Ridge in two counties in central Florida, and only on the well-drained, sandy soils of scrub habitat. Being elevated and not prone to flooding, this habitat has experienced extensive loss over the years due to conversion into housing developments and orange groves. Prior to 2002, 45 populations of Scrub Lupine had been recorded and by 2009 only eight remained. Continuing losses are threatening this species with extinction.

With funding from the U. S. Fish and Wildlife Service and the State of Florida, Bok Tower Gardens began intensive efforts to both understand and preserve this species. Research efforts underway since 2008 have increased germination rates from 50% to nearly 100%, with nearly 100% seedling survival. Tissue culture micropropagation protocols have been developed which help produce plants from populations with little or no seed production. Plant rescues at future construction sites have preserved genetic diversity which would otherwise have been lost. *Ex situ* seed collections now contain germplasm from all remaining populations, and include seed from some populations which have been extirpated. Educational presentations and materials have cultivated an awareness of scrub lupine in local communities, resulting in several volunteers to help with conservation efforts for this species. With the help of these volunteers, Bok Tower Gardens has been able to introduce three new populations of scrub lupine onto protected land, increasing the number of populations to 13 by the end of 2010.

This integrated approach to conservation is only possible because of partnerships with agencies including the Center for Plant Conservation, U. S. Fish and Wildlife Service, Florida Fish and Wildlife Conservation Commission, Cincinnati Zoo, St. Thomas University, Ridge Rangers volunteer group, Florida Department of Environmental Protection and others. Together, these partners may be able to leave the legacy of successful species preservation, and keep scrub lupine alive for posterity.



Figure 1: Bok's Rare Plant Specialist, Juliet Rynear, next to a scrub lupine in the first introduced population established by Bok. The plant is 15 months old and was sown in the greenhouse at Bok, and planted out when it was a four-monthold seedling. This was the first flowering that occurred in an introduced population. Photo courtesy of Cindy Campbell.

Figure 2: A tissue culture propagated scrub lupine plant in a test tube at St. Thomas University in Miami Gardens. Photo courtesy of Dr. Pilar Maul.

11. Symbiotic reintroduction of a Mexican orchid.

By Kirsty Shaw for the Microcosmos Bioedáfico Laboratory, Department of Soil Science at the Institute of Geology, National Autonomous University of Mexico (UNAM)

Currently, 25 orchid species are native to the Pedregal Reserve of San Angel within Ciudad Universitaria, the main UNAM campus (Rangel-Villafranco and Ortega-Larrocea, 2007). Many orchids possess a high dependency on symbiotic fungi, thus conservation practices need to extend beyond the plant to conserve their associated fungi in order to be successful (symbiotic rather than asymbiotic) (Ortega-Larroceae and Gonzalez, 2008). Symbiotic reintroduction projects of orchids and their mycorrhizal fungi have the potential to lead to overall ecosystem enhancement as such fungi have also been shown to encourage the beneficial mineralization of organic matter in the soil. This counteracts decreases in soil biodiversity functions, a common symptom of degraded habitats (Rangel-Villafranco and Ortega-Larrocea, 2007).

A number of symbiotic orchid reintroductions have been performed in the Pedregal Reserve, with an aim to illustrate the importance of mycorrhizal fungi in achieving successful reintroduction projects over a long time scale. In 2000, 17 reintroduction sites for symbiotically reproduced *Bletia urbana* individuals were selected within the Reserve, with 10 individuals planted at each site. The seeds were from a germplasm store collected from the Pedregal Reserve in 1984 and the individuals were propagated in a tissue culture laboratory in UNAM. The associated symbiotic fungi were isolated in 1999, and planting took place in 2000. The viability of the seeds 16 years after collection is a noteworthy example of the value of *ex situ* collections, and the results of the project show strong evidence for the success of symbiotic reintroductions.

Reintroduction projects usually have a low survival rate, but at a global level, this case study illustrates one of the most successful reintroductions in terms of percent survival, with an average survival rate of 50% (Ortega-Larrocea and Gonzalez, 2008). As many as 9 of the 10 originally reintroduced individuals can be witnessed at some of the sites (visit with Ortega, 2010). Survival rates and plant size are influenced by micro-variations between the 17 locations, for example micro-climatic conditions and competition from other plants. In 2005, the first flowering of a reintroduced individual was recorded and seeds were collected for propagation. Since this date, further individuals have flowered and produced seed with some populations now naturally reproducing.

During the ten years since the reintroduction, periodic measurement of individual size, data on nitrogen levels, carbon dioxide levels, temperature, water content and soil samples have been recorded providing a detailed study of the conditions under which these individuals have survived. This will enable analysis and identification of the conditions that have proven most successful for reintroduction. Detailed results of this study are set to be published this year, with the final aim of producing a global model for symbiotic orchid reintroduction.

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Figure 1: *Bletia urbana* in flower five years after reintroduction into the Pedregal Reserve of San Angel. Photo courtesy of Maria Pilar Ortega.

12. Trillium conservation at Southern Illinois University Edwardsville.

By Kelly Barry and Doug Conley, Gardens at Southern Illinois University Edwardsville (SIUE)

Trilliums are beautiful, slow growing spring wildflowers found in the deciduous forest understory of North America. They are popular among wildflower enthusiasts, naturalists, and gardeners. Trillium populations are in decline, predominantly due to habitat disturbance but also due to collection from wild populations and the plants' very slow growth. SIUE student researchers, under the guidance of Biological Sciences faculty, are monitoring natural trillium populations on campus and developing tissue culture procedures for propagating trilliums. SIUE is a large campus, including The Gardens at SIUE, with extensive natural areas for pleasure, conservation, and conservation research. SIUE is currently in the process of formalizing the natural areas status of portions of the campus, including a region where *Trillium recurvatum* and *T. flexipes* populations exist (Figures 1 and 2). While neither trillium is threatened in Illinois, *T. recurvatum* is threatened in Michigan and *T. flexipes* is endangered in Maryland and New York.

Student researchers have determined how to germinate trillium seeds under sterile conditions and have produced clusters of mini-rhizomes from individual seeds. These clusters can be broken into smaller rhizome-like pieces for regenerating intact plants. These techniques have been applied to a rare trillium, *T. viride*. *T. viride* is found only along the Missouri River bluffs in Missouri and Illinois and it is classified as endangered in Illinois. The mini-rhizomes are also being used to initiate trillium liquid cultures. Embryos formed in the liquid cultures can be used for the production of synthetic seeds. Synthetic seeds offer the benefits of long term storage and easy distribution for conservation purposes as well as horticulture uses.

Trillium seeds undergo dormancy and germinate very slowly. Students are exposing seeds to varying concentrations of a plant growth regulator known to decrease dormancy in other plants. The goal is to couple a shortened dormancy requirement with procedures for producing several plants from single seeds.

Students have obtained rhizomes from *T. recurvatum* plants growing on campus and are using pieces of the rhizomes for multiplying trillium shoots by tissue culture. This procedure has the potential to generate large numbers of plants while returning portions of the original rhizome to its natural site.

Plants produced through tissue culture methods can be grown commercially and reduce the impact of collecting from wild populations (Figure 3). Tissue culture generated plants will also be planted in natural areas of The Gardens at SIUE, a 35 acre Signature Garden of the Missouri Botanical Garden. This will allow SIUE and The Gardens at SIUE to play a significant role in trillium conservation.



Figure 1: *T. flexipes* in Sweet William forest. Photo courtesy of Kelly Barry.

Figure 2: *T. recurvatum* rhizome collected from Sweet William forest. Photo courtesy of Kelly Barry.



Figure 3: Mini-rhizomes of *T. sessile*. Photo courtesy of Kelly Barry.

13. Dawn-redwood ex situ collection efforts at the Dawes Arboretum.

Mike Ecker, The Dawes Arboretum

Dawn-redwood (*Metasequoia glyptostroboides*), once believed extinct, was subsequently discovered living in central China in the mid-1940s. Seeds were collected and distributed worldwide. This species is now critically endangered in the wild according to the IUCN. Today, the Dawes Arboretum has one of the largest living genetic collections of documented, wild-origin dawn-redwoods outside of China.

In 1990, Rutgers germinated natively collected seeds, resulting in 48 genetic groups ("families"). In 1993, Dawes planted 344 trees from this venture; today, 320 individuals exist on eight acres, spaced 25' apart (Figures 1 and 2). This plantation of genetically unique trees will allow pollination between individuals that is normally not possible in China due to the geographical isolation of populations. Resultant seeds will be utilized for new plant production, seed banking, and ultimately restoration in the wild. This, in combination with *in situ* preservation of living plants, offers the best prospects for conservation.

The Dawes Arboretum is acquiring additional wild collected material from other gardens and seeking new collections from China, to obtain as complete an assemblage of genotypes as possible. The current collection consists of 96% genetic lines of wild origin. For more information refer to:

Ecker, Michael. 2009. A Little Piece of China – *Ex Situ* Style. *Public Garden*, Journal of the American Public Garden Association, 24:4, 26-27.

Payton, Greg. 2010. Conserving the Dawn Redwood: The *Ex Situ* Collection at the Dawes Arboretum. *Arnoldia*, Magazine of the Arnold Arboretum, 68:1, 26-33.



Figure 1: The Dawes Arboretum's genetic collection of dawnredwood. Photo courtesy of Greg Payton.



Figure 2: An example of the diversity in habit represented in The Dawes Arboretum's dawn-redwood collection. Photo courtesy of Greg Payton.

14. Management plan for threatened cactus species.

Kirsty Shaw for the Botanic Garden of Cadereyta

The management plan of the Regional Botanic Garden of Cadereyta outlines a clear objective: to create a better scientific knowledge of the region's flora, and highlight and illustrate its importance to the local community and beyond thereby supporting its conservation (Sanchez and Sanaphre, 2009). The state of Queretaro is located at the southernmost tip of the Chihuahuan Desert. Although a relatively small state, it is biologically rich with high endemicity levels and a high number of threatened plant species due to a history of damaging anthropogenic activities in the area, particularly during the last 30 years.

A particular focus of Cadereyta Botanic Garden is Cactaceae. A total of 169 species are held in the garden's collection, all of which are listed under CITES, which illustrates a high level of commercial trade, as outlined by Barcenas (2003). Many of the species are included in propagation programs. A large stretch of a locally and internationally important species, *Echinocactus grusonii*, creates a striking visual in the garden. The development of the Zimapan hydro-electric dam, from 1990 to 1995, affected half of the known natural habitat of *E. grusonii*. Many individuals were flooded, but 856 individuals were rescued, 492 of which entered into botanic gardens, 321 were reintroduced into the adjoining area, and 43 died during transplantation (Ortega, 2004 in Sanchez et al., 2010). Population numbers are still low and thus the species is listed as 'in danger of extinction' (P) under NOM-059-SEMARNAT-2010 and 'critically endangered' (CR) under the IUCN Red List. It is estimated that 2-3000 individuals from 95 rescued individuals. To celebrate such a success, *E. grusonii* has been adopted as the symbol of the garden, representing the important conservation work carried out.

A project entitled 'Propagation of Threatened Species of the Cactaceae family in the Queretaro Semi-desert and Preliminary Ecological Assessment within their Habitat', led by the Garden, aimed to identify Cactaceae species with problematic survival in the area in order to include them in propagation programs, and propose basic guidelines for advancing their *in situ* conservation. Within a study area of 2,500km², 50% of the Cactaceae species recorded for the whole state of Queretaro (11,684km²) were found, 13 of which were listed under a risk category of NOM-059-SEMARNAT-2001, along with a further six species identified as possessing characteristics which would merit their inclusion on the NOM-059-SEMARNAT-2001 (Sanchez et al., 2006). Seeds were collected from wild individuals of these 19 priority Cactaceae species in three consecutive years and supplemented by seeds from individuals within the botanic garden. The species were propagated employing a three step method of germination, initial development and final development. Survival rates were high for all species, averaging between 80 and 90%. Precise and comprehensive guidelines have been published, providing transparency of practices, enabling other propagation facilities to employ the same techniques (Sanchez et al., 2006).

References:

- Barcenas RT, 2003. Los Cactos del Desierto Chihuahuense en Mexico: Una evaluacion del comercio, la administracion y las prioridades de conservacion. WWF, Washington.
- Sanchez Martinez E; Hernandez Martinez MM; Chavez Martinex RJ and Hernandez Oria JG, 2006. Especies de Cactaceae Prioritarias para la Conservacion en la Zona Arida Queretano Hidalguense. CONCYTEQ, Cadereyta Bot. Gard., Queretaro.
- Sanchez Martinez E and Sanaphre Villanueva L, 2009. El Jardin Botanico de Cadereyta 'Ing. Manuel Gonzalez de Cosio' Plan de Manejo. CONCYTEQ, Queretaro.
- Sanchez Martinez E; Hernandez Oria JG; Hernandez Martinez MM and Torres Galeana LE, 2010. La Barranca del Infiernillo en Cadereyta, Queretaro.



Figure 1: Collecting seeds of Mammillaria herrerae (NOM-059-SEMARNAT-2010 P, IUCN CR). Photo courtesy of Kirsty Shaw.



Figure 2: Initial stages of propagation at Cadereyta Botanic Garden. Photo courtesy of Kirsty Shaw.



Figure 3: Propagation of *Echinocactus grusonii* (NOM-059-SEMARNAT-2010 P, IUCN CR) at Cadereyta Botanic Garden. Photo courtesy of Kirsty Shaw.

15. Reintroduction of Echeveria laui in the Biosphere Reserve of Tehuacán-Cuicatlan.

By Kirsty Shaw for the National Autonomous University of Mexico (UNAM) Botanic Garden

The strong conservation ethic of the UNAM Botanic Garden and its large academic network have caused the establishment of conservation projects beyond the confines of the Garden. Work has taken a particular focus in the Tehuacan-Cuicatlan Valley, where 180 plant families, 891 genera and 2621 species have been located, 13.9% of which are endemic to the region (Davila et al., 2002). High local endemicity levels have resulted in naturally low population numbers, exacerbated by the negative impact of human activities such as deforestation, agriculture, cattle grazing and illegal harvesting of plants.

In 2002, Vivero La Iberia, a nursery in the town on San Juan Bautista Cuicatlan, was founded with the aim to drive the project 'Recovery of Cacti and other endemic plants of the region of Cuicatlan, Oaxaca'. The nursery's principal activity was the propagation of native plants for reintroduction, with a particular focus to mitigate damage caused by the construction of a network of electricity pylons in the area. The nursery is an officially registered UMA (Unidad de Manejo y Aprovechamiento), which provides legal permission for the propagation and commercialisation of endemic plants. The project originally involved seven biologists from UNAM and funding from the Mexican Cactology Society and the Federal Electricity Commission (CFE). Through UMA, successful cultivation programs have developed propagation protocols, provided workshops and published manuals. Also, Vivero La Iberia was used as a case study for establishing a nursery for propagation of cacti and other succulents in a manual published by CONAFOR, the National Forestry Commission (Reyes, 2010).

In 2005 the UMA nursery dedicated a particular focus to support conservation of *Echeveria laui* (In danger of extinction, P, NOM-059-SEMARNAT-2010), an endemic succulent plant in the Crassulaceae family. The species was propagated with the aim of producing individuals for sale and reintroduction to the wild. In 1992, when the species was barely studied, UNAM recorded just 250 individuals in the wild and it was thought to be at the point of extinction. Through UNAM research on *E. laui* metabolism, germination and population dynamics, the species progressed from being practically unknown, to one of the most studied species of *Echeveria*.

Since initiation of *E. laui* reintroduction efforts, more than 25,000 individuals have been propagated vegetatively and through seed. Based on population dynamics research, plants were propagated at the nursery (Figure 1), then after one and a half years placed outside in a six-month intermediate stage to reduce susceptibility, before reintroduction of well developed two year old plants to the wild. At the onset of the project, exact coordinates of the natural populations were recorded and reintroductions were carried out within these natural ranges; selecting isolated locations to limit human intervention and planting next to larger plants to provide further protection (Figure 2). The reintroduction was carried out in 2008, selected individuals were marked and their progress is being monitored (Figure 3). Survival rate is visibly high and individuals are successfully reproducing in the wild.

The reintroduction site is within a reserve and is continually monitored. Rather than excluding visitors from the reintroduction zone, Reserve authorities have now created a trail through this remote area, providing an opportunity to educate visitors about threatened species endemic to the region, such as *E. laui* and *Mammillaria huitzilopochtlii* (Subject to special protection, Pr, NOM-059-SEMARNAT-2010). Additional reintroductions of *E. laui* individuals are planned, with the hope that *E. laui* could one day be assigned a lesser threat status.

References

Dávila P; Arizmendi M; Valiente-Banuet A; Villaseñor JL; Casas A and Lira R, 2002. Biological diversity in the Tehuacan-Cuicatlan Valley, Mexico. Biodiversity and Conservation 11: 421-442

Reyes J, 2010. Conservación y Restauración de Cactáceas y otras Plantas Suculentas Mexicanas: Manual Práctico. Comisión Nacional Forestal. Tlalpan, México.



Figure 1: *Echeveria laui* propagation in Vivero la Iberia. Photo courtesy of Kirsty Shaw.



Figure 2: Reintroduction of *Echeveria laui* individuals. Photo courtesy of Jeronimo Reyes.



Figure 3: Reintroduced *Echeveria laui* tagged for monitoring purposes. Photo courtesy of Kirsty Shaw.

16. San Francisco Manzanita: From Extinct to Endangered.

By David Kruse-Pickler, San Francisco Botanic Garden

The Franciscana Manzanita (*Arctostaphylos franciscana*) was discovered during road construction in San Francisco in 2009. Thought to have been extinct for over 70 years, the plant caught the eye of Daniel Glusenkamp, director of habitat protection and restoration at Audubon Canyon Ranch, as he was driving by and spotted the low growing, but quite large manzanita (Figure 1). The manzanita thrived in a large isolated triangular patch of land surrounded on all three sides by major highways created when the Golden Gate Bridge and its many connectors were built. This isolation, combined with a perfect patch of serpentine rock habitat, provided an ideal environment for this lone plant to survive.

To provide legal protection for the re-discovered species, three organizations-Wild Equity Institute, the Center for Biological Diversity, and the California Native Plant Society filed an emergency petition for Endangered Species Act protection. Tom Parker and Mike Vasey, manzanita researchers from San Francisco State University, analyzed the DNA from this plant to confirm its identity. With guidance from experts including the Presidio Park nurseries director, Betty Young, and the UC-Berkeley Botanic Garden curator, Holly Forbes, many cuttings were taken and distributed to suitable botanical gardens and conservation nurseries. San Francisco Botanical Garden (SFBG) curator and nursery manager, Don Mahoney along with California Native gardener, Terry Seefeld, provided critical guidance on how to successfully transplant and re-locate the 25,000 pound swath of soil holding the manzanita to a new and appropriate site (Figure 2). Currently, from the cuttings received at SFBG, 230 individuals were planted. The first dozen were potted up after three months, with the majority taking five months, with all of them taking root. Some have already been planted out in SFBG's California Native garden. The lone plant that started this amazing process is thriving in its new, secret location in San Francisco's Presidio Park, a part of the Golden Gate National Recreation Area.

Everyone involved in this discovery worked quickly and passionately to save and protect the now last known Franciscana manzanita to exist in the wild. Scientists, transportation and government officials, horticulturalists, volunteers and many others were all involved in the process. This collaboration is probably summed up best by Gluesenkamp when he writes, "...this is really a story about how the system worked!"



Figure 1: *Arctostaphylos franciscana* in flower. Biologist Dan Gluesenkamp discovered the single surviving wild specimen as he passed by on Doyle Drive in the Presidio. Photo courtesy of Daniel Gluesenkamp.

Figure 2: *Arctostaphylos franciscana* transplanting: Workers use a crane to move the only Franciscan manzanita known in the wild. Photo courtesy of Daniel Gluesenkamp.

17. Endangered species traveling exhibit.

By Anamari Dorgan, The Morton Arboretum

Trees from around the world are facing threats that jeopardize their survival and our quality of life. As a worldrenowned leader in tree science and natural history education, The Morton Arboretum is uniquely suited to promote the environmental, health, and aesthetic benefits of trees and the importance of conserving threatened species. Working in collaboration with Botanic Gardens Conservation International (BGCI), The Morton Arboretum is developing an outdoor traveling exhibit designed to educate people about the status of globally endangered trees and the important role they play in sustaining livable communities.

This traveling package, funded in part by the Institute for Museum and Library Services, Museums for America Grant Program, offers arboreta, botanical gardens, and related organizations across the United States an exhibit consisting of interpretive panels and supporting resources including an installation manual; a pod cast; resource lists for obtaining living specimens of featured trees and relevant publications; suggested hands-on activities for families; a teacher's guide; a guide for science-attentive adults; a guide for creating a walking tour; and a media kit. Exhibit visitors will experience compelling stories about the earth's most vulnerable trees and learn about efforts to save them from extinction.

All exhibit components are based on sound science and take account of the needs of local communities that depend on a tree's resources for their survival. Visitors will explore the often complex circumstances that pose threats to trees, often pitting immediate human needs against long-term sustainability. The exhibit will also feature tree heroes in the form of individuals and organizations. For example, most Americans are familiar with the magnolia tree, but Chinese magnolia (*Magnolia sinica*) is one of the most endangered magnolias in the world. Known only from a single population of less than 10 mature individuals on a forested mountain slope in China, its story illustrates real efforts and tangible results as part of the Global Trees Campaign. Conservation of this rare species includes 200 nursery-grown saplings planted in a nature reserve, providing a critical lifeline for this tree.

Target audiences for this exhibition are visitors to the more than 500 institutions who are current members of the American Public Gardens Association (APGA). Across the globe, arboreta and botanical gardens are leading players in the effort to connect people with plants and nature. Through a wide variety of programs, they provide the best combination of resources – beautiful natural landscapes, trained scientists and educators, and credibility within our communities.

This exhibit provides an opportunity to focus the attention of a broad cross-section of this national audience on the important role trees play in providing sustainable resources and contributing to a healthy environment. Our goal is to empower arboretum and botanical garden visitors across the United States with a new understanding of why we must protect endangered trees and to equip them to act as champions of globally endangered trees.



Figure 1: Fewer than 100 mature individuals of the critically endangered Wollemi pine (*Wollemia nobilis*) remain in the wild, making this tree one of the rarest in the world. Photo courtesy of Jaype, Wikimedia Commons.

18. Walpole Island community plant conservation efforts.

By Jane Bowles, Sherwood Fox Arboretum

The Walpole Island First Nation is located on a large freshwater delta at the mouth of the St. Clair River in the heart of the Great Lakes. It boasts a mixture of prairie, savannah, forest, wetland and coastal waters and provides habitat for some eighteen plants that are federally listed species at risk, some of them found nowhere else in Canada. In 2003, the Sherwood Fox Arboretum (SFA) of the University of Western Ontario and the Natural Heritage Program of the Walpole Island Heritage Centre (WIHC) began to cultivate collaborations relating to indigenous culture, public information, research, training and capacity building to conserve and restore natural ecosystems and the species they contain.

One initiative that arose from this partnership is that seeds collected from Walpole Island are germinated in greenhouses and nurseries by SFA and the plants transferred back to Walpole Island for use in community planting and conservation projects (Figure 1). For example, Walpole Island is one of the few places in Canada where wild Kentucky coffeetree (*Gymnocladus dioica*) produces viable seeds, because the species is so rare in Canada that male and female plants of this dioecious species are rarely found together. Through our collaborative efforts to collect and grow seed of these trees, about 50 small Kentucky coffeetrees were returned and planted on Walpole Island as part of Earth Day celebrations in 2008.

Additional components of this partnership help increase awareness of threatened species and the importance of biodiversity preservation. Research by students using both *ex situ* and *in situ* populations of plant species at risk at SFA have filled in knowledge gaps about the biology and ecology of rare plants at Walpole Island (Figures 2 and 3). Educational and outreach material developed at the SFA and the WIHC highlights threatened plant species on everything from calendars to decks of playing cards, T-shirts and placemats for the local restaurant. These materials have been used to increase awareness of species at risk on Walpole Island and engage the community in conservation projects.



Figure 1: Seeds collected from Walpole Island are grown by SFA and transferred back to Walpole Island for use in conservation projects. Photo courtesy of Jane Bowles.



Figure 2: *Ex situ* seed preservation efforts of community members and students have helped SFA increased knowledge of the biology and ecology of rare plants at Walpole Island. Photo courtesy of Jane Bowles.

Figure 3: In situ species monitoring on Walpole Island involving community members and students. Photo courtesy of Jane Bowles.

19. Berry Botanic Garden Seed Bank for rare and endangered plants of the Pacific Northwest.

By Ed Guerrant, Berry Botanic Garden Seed Bank

Seed banks provide an economical way to conserve substantial numbers and great diversity of plants over long periods of time. Established in 1983, the Berry Botanic Garden Seed Bank had, by late 2010, over 18,000 separate seed samples (accessions) of more than 350 of the Pacific Northwest's rarest plant taxa (Figure 1). Seed and other living plant material stored safely outside of natural areas (*ex situ*, or off-site) amount to an insurance policy against the wild population becoming irrecoverably lost. *Ex situ* plant conservation is a means to an end (supporting species survival in the wild), and a part of a larger whole (integrated conservation strategies).



Figure 1: Note how the number of taxa (species, subspecies or varieties) increased most rapidly in the early years, and has slowed in recent years, while the number of accessions increased relatively slowly early on, and has risen dramatically in later years. This pattern is the result of a conscious strategy to get at least one population sample of the most endangered species as quickly as possible, and then over time collect samples from more populations. Early on, we averaged fewer than 2 accessions per taxon. This number has steadily increased over time, and is approaching 50 samples per taxon. The purpose of this is to obtain a representative sample of a species' genetic diversity across its entire range.

Off-site samples can take many different forms, including seeds, growing plants, slow growth tissue culture, cryogenically preserved vegetative tissue, and so on. Each of these techniques has its strengths and limitations, and not all will necessarily work with any given taxon. One significant advantage for taxa having orthodox seeds (those that can withstand desiccation and freezing temperatures) is that in both absolute and relative terms, very large numbers of individuals can be stored as seed at low cost for long periods of time. Another is that cross incompatible species, or genetically distinct and possibly differentially adapted populations, can be stored in close proximity to another without fear of hybridization.

The Berry Botanic Garden has worked to provide a full range of *ex situ* plant conservation services, including seed collection, long term storage and, if and when necessary, the reintroduction of stored material to the wild. The Garden has actively sought to work in partnership with the major land management and regulatory agencies, as well as private land owners, in the Pacific Northwest. The Berry Botanic Garden has been directly or indirectly involved in a large number of reintroduction attempts, including efforts to restore *Stephanomeria malhuerensis* to its native site after it had become extinct in the wild.

Sadly, the Berry Botanic Garden was closed to the public in October 2010, and the property has been offered for sale. While the fate of the growing collections is uncertain, the Conservation Program and Seed Bank are moving to Portland State University which offers a long-term commitment to provide a home base for the Seed Bank. Seed banks are intergenerational endeavors which universities often support, so the match is complementary.

20. Cincinnati Zoo & Botanical Garden: Providing critical tools for conservation.

By Valerie Pence, Cincinnati Zoo & Botanical Garden

Propagating Plants for Recovery

Propagation is a key element in providing plants for recovery projects. For many endangered species, traditional propagation by seed or cuttings can meet propagation needs, but for species with few or no seeds or few individuals, plant tissue culture can be used to supplement these methods. The Plant Research Division of the Center for Conservation and Research of Endangered Wildlife (CREW) at the Cincinnati Zoo & Botanical Garden focuses on developing and using tissue culture (in vitro) methods for propagating U.S. species of conservation concern. In collaboration with the botanical garden network of the Center for Plant Conservation and with support from grants from the Institute of Museum and Library Services, CREW has developed in vitro propagation protocols for over 40 of the nation's most imperiled species. By partnering with botanical gardens, governmental agencies and other non-profit organizations, the work at CREW is being integrated into conservation efforts for species recovery and demonstrating that tissue culture propagation can be an important tool for conservation and restoration.

The Cumberland sandwort (*Minuartia cumberlandensis*) is a small plant that grows only in the sandstone rockhouse habitat in the Daniel Boone National Forest of southern Kentucky and northern Tennessee. In order to demonstrate the feasibility of using tissue culture propagated plants for the recovery of this species, an experimental outplanting was made at a site in Kentucky in collaboration with the U.S. Forest Service (Figure 1). Over the course of five years, these plants have grown and reproduced well at the site, indicating the viability of the methods.

The CryoBioBank®--A Resource for the Future

The CryoBioBank (CBB) is a unique collection of frozen seeds, spores, and tissues at the Cincinnati Zoo & Botanical Garden's Center for Conservation and Research of Endangered Wildlife (CREW), creating a true Frozen Garden (Figure 2). The CBB houses 150 plant species all stored in liquid nitrogen (-196°C; -320°F). The collections in the CBB's Frozen Garden include a Regional Seed Bank made up of seeds and spores of state and federally endangered species from Kentucky, Indiana, and Ohio; a Pteridophyte Bank of spores and tissues of ferns, a Bryophyte Bank of spores and tissues of mosses; and an Endangered Plant Tissue Bank. Material is continually added to the collections, and some samples are over 20 years old.

Perhaps one of the most important uses of the CBB is to preserve tissues from endangered species that produce few or no seeds or that produce seeds which are sensitive to drying (recalcitrant seeds) and are thus not adaptable to the conditions used in traditional seed banks. For these "exceptional" species, banking tissues such as shoot tips or embryos in liquid nitrogen can provide an alternative method for long-term germplasm storage. Species such as the four-petal pawpaw (*Asimina tetramera*), beautiful pawpaw (*Deeringothamnus pulchellus*), and Rugel's pawpaw (*Deeringothamnus rugelii*), recalcitrant species from Florida, and Avon Park harebells (*Crotalaria avonensis*) and Todsen's pennyroyal (*Hedeoma todsenii*), species with few or no seeds from Florida and New Mexico, have been banked in projects funded in part by grants from the Institute of Museum and Library Services and U.S. Fish & Wildlife Service (Figure 3). By collaborating in the field and using molecular genetic analysis, multiple lines have been collected and banked, thus helping to preserve the genetic diversity of each of these threatened species.



Figure 1: Cumberland sandwort (*Minuartia cumberlandensis*) in vitro to support reintroduction efforts of this threatened species. Photo courtesy of Valerie Pence.



Figure 2: CREW's CryoBioBank can be used as part of an overall strategy for long-term *ex situ* conservation of rare plant species as a resource for the future. Photo courtesy of CBB-CZBG.



Figure 3: Avon Park harebells (*Crotalaria avonensis*), one of the threatened species preserved in the CryoBioBank. Photo courtesy of Monica Pence.

21. A community approach to seed banking.

By Flo Oxley, Michael Eason, Damon Waitt, Lady Bird Johnson Wildflower Center

The Lady Bird Johnson Wildflower Center is one of six nongovernmental partners of the Seeds of Success (SOS) program. Together with private landowners, volunteer organizations, state and federal agencies, and other groups, the Wildflower Center is working to collect and preserve the seeds of the Texas native flora.

Using protocols developed by the SOS program, species needed to stabilize, rehabilitate, and restore damaged and degraded habitats in Texas are being collected (Figure 1). Endemic species, wild relatives of economically important species, close relatives of invasive or potentially invasive species and species that have wildlife value, have also been targeted for collection. To date, the seeds of more than 500 species have been collected as part of the program. Collections are stored at the Royal Botanic Gardens, Kew Millennium Seed Bank, the National Center for Genetic Resource Preservation, Mercer Arboretum and Botanic Gardens and the Wildflower Center.

This work would not be possible without the collaboration and assistance of private landowners throughout the state, a dedicated corps of trained volunteers from Master Naturalist and Master Gardener chapters, members of the Native Plant Society of Texas, and the Wildflower Center's own volunteers. Private landowners have provided access to more than 300,000 acres of private property and, in many cases, the landowners themselves have become project volunteers and are monitoring and collecting targeted species identified on their land. More than 125 trained volunteers help the Center's seed collectors locate, monitor, and collect targeted species. Collections are brought back to the Center where volunteers clean, count, split, and package the collections for storage. Volunteers also input data into collection databases, mount and label herbarium voucher specimens, and maintain the collections.

The Wildflower Center's seed banking program is an excellent example of private citizens, volunteers, state and federal agencies, and nonprofit organizations with common goals working together to make a difference.



Figure 1: More than 125 trained volunteers help the Center locate, monitor, and collect targeted species. Photo courtesy of Christine Murrey, University of Texas.

22. Conserving oaks in North American plant collections: a collaborative approach.

By Emily Griswold, Quercus NAPCC Multi-site Collection

Plant collection networks have a special role to play in the conservation of plant diversity in public gardens, especially for large plant groups. In 2007, the North American Plant Collections Consortium (NAPCC) inducted the first multisite collection into their program with a focus on the genus *Quercus*. Oaks dominate many North American landscapes, and with approximately 500 species worldwide from a broad diversity of climate and soil conditions, oaks were a natural choice for the first multisite collection.

The NAPCC Multisite *Quercus* Collection started with 15 member gardens, which were selected to represent a range of climate zones across the U.S. and for holding a broad diversity of oaks. Each garden has committed to maintain its oak collection to high curatorial and horticultural standards, and to contribute to group goals to expand and improve the collection. A *Quercus* curatorial group composed of staff representatives from each garden meets annually and communicates regularly through a discussion group to share information and ideas. Group collaboration is a fundamental premise of a multisite collection. From sharing data to sharing acorns to collaborating on collecting expeditions, member gardens work together to help the collection grow. The member gardens agree to use a common reference for oak nomenclature and to share plant inventories. The shared inventory is key for tracking and analyzing the combined collection and facilitating the identification of gaps in the collection. The inventory is publicly available online for researchers and other oak enthusiasts to download from the APGA website.

The NAPCC Multisite *Quercus* Collection is by no means complete. Many oak species are still unrepresented in the group's combined holdings or are represented by just a few individuals. Group efforts to expand the collection are focused first on North American taxa followed by Asian and European species. Acquiring species of conservation concern is, of necessity, a higher priority. Many unrepresented North American oak species are from areas where there are currently no NAPCC gardens. Prioritizing the recruitment of new NAPCC gardens in the southeastern and southwestern United States as well as Mexico has been recognized as the most efficient method of increasing representation of taxa from these regions. Two new gardens from the southeast have applied to join the multisite collection since 2007, and negotiations are underway with gardens in the southwest U.S. and Mexico to encourage their participation as well.

As of October 2010, the collection has grown from 15 to 17 member gardens, with two more pending applications in review (Figure 1). The current holdings of the multisite collection include 417 taxa recognized by PlantSearch, including 97 native North American species, subspecies, and varieties (Figure 2). Of the 21 North American oak taxa recognized as highly threatened, 9 occur in the NAPCC multisite collection. A comparison using PlantSearch with the holdings of other gardens around the world reveals that 43% of the oak taxa in the multisite collection are represented in 3 or fewer collections worldwide. The combined resources of a collection network are essential to take on the daunting task of making robust, genetically diverse *ex situ* collections of all North American *Quercus* a reality.



Figure 1: Current NAPCC *Quercus* multi-site collection locations (17 in blue), and pending applicant locations (2 in yellow). Map courtesy of Google Maps and Emily Griswold.



Figure 2: The endangered Brandegee oak (*Quercus brandegeei*) from Baja California, Mexico grows at the UC Davis Arboretum. Photo courtesy of Emily Griswold.

23. Cycad collection and plant records management.

By Kirsty Shaw for Francisco Javier Clavijero Botanic Garden

The Francisco Javier Clavijero Botanic Garden (Clavijero) holds both the national collection of cycads (of the Zamiaceae family) and the national collection of bamboo (Poaceae). Species held in the garden are predominantly from Mexico and particularly Veracruz, with non-native species included in some collections to illustrate species diversity. Efforts are made to represent threatened taxa, with high representation of Zamiaceae, Aracaceae and Orchidaceae.

National collection of Cycads: Dr. Andrew Vovides, curator and founder of the garden, is an internationally renowned cycad specialist who has described various species of the Zamiaceae family, of the order Cycadales. He has dedicated many years to their collection, study and conservation, publishing prolifically on the family. Clavijero holds a collection of 43 taxa of the Zamiaceae family (Figure 1). In April 2010, the garden held 1354 individuals of the Zamiaceae family, with many of the species involved in onsite propagation programs.

Twenty years ago, a community-based project was implemented by Clavijero to propagate an endangered cycad species, *Dioon edule*, listed under NOM-059-SEMARNAT-2010, P. Despite difficulties with SEMARNAT permits, and infrequent sales, one member of the community has maintained the project, cultivating *D. edule* in a simple nursery next to his house (Figure 2). When sales are made they supplement his normal wage from cultivating lemons. The local municipality bought a large number of specimens, planting them in prominent locations within Xalapa and both recognition and appreciation of the native endangered species has visibly increased, with more local people planting the species in their own gardens.

Management of records and data sharing: When acquisition of plants for Clavijero began before its official opening in 1979, a strong foundation was laid for the maintenance of accurate and detailed accession data, which has continued through the Garden's history. Every specimen brought to the Garden has detailed accession records maintained in a plant records database as well as on plant labels. Work is currently underway to provide plants within Clavijero that pre-existed its founding with a numerical reference and associated information. Interestingly, the second plant accessioned by the Garden remains in the collection, *Dioon edule* collected by Dr. Vovides, in April 1975 at Tuzamapan, 500m ASL.

Plant records techniques and technology have advanced greatly through Dr. Vovides's career. The garden now uses BG-Base, complimented by BG-Map, with much of the Garden's collection geo-referenced. Clavijero is well-known for having the best plant records management of all gardens in Mexico. The records are kept current, with bi-annual audits of the Garden's collection, to take measurements and record any plants that have been damaged, died, or disappeared from the collection. As a final step, updated inventory reports are produced for staff members, allowing comparison through time and easy access to current collections information. Clavijero is currently adding microchips to their plant records tools, an innovative way to monitor and geo-reference individuals within a collection (Figure 3).



Figure 1: A portion of the cycad collection at the Francisco Javier Clavijero Botanic Garden. Photo courtesy of Andrew Vovides.

Figure 2: Community cultivation of *Dioon edule* (NOM-059-SEMARNAT-2010 P). Photo courtesy of Kirsty Shaw.

Figure 3: New microchip method for plant records management at the Francisco Javier Clavijero Botanic Garden. Photo courtesy of Kirsty Shaw.

24. Backing Up Living Conservation Collections.

By Chad Husby, Montgomery Botanical Center

The strategy of backing up ones work is well known in many areas of human endeavor. Ex situ conservation is itself often a form of "back up" for naturally occurring populations either in gardens or other remote sites. With so many locally endemic species imperiled around the globe, vulnerable to habitat destruction and degradation, maintaining populations elsewhere is especially important. Backing up of ex situ conservation collections is also crucial because collections in any garden are vulnerable to contingencies that cause loss of what may be irreplaceable plants. As the old gardening adage goes: "the best way to keep a plant is to give it away". Plants species, such as the giant palm *Corypha taliera*, that survive only in cultivation, or have a highly restricted natural distribution, such as *Microcycas calocoma*, are perfect examples of the importance of ex situ conservation at multiple locations.

Montgomery Botanical Center (MBC), a research and conservation garden, in Miami, Florida uses several strategies to ensure that its key collections are backed up. Palms and cycads constitute the focus of MBC's conservation efforts, with a secondary emphasis on tropical conifers. Montgomery organizes multiple expeditions each year to obtain representative samples of palm and cycad seeds from wild populations. These seed collections are backed up initially by sharing with botanical institutions in the country of origin. In addition, when permits allow, seeds are shared with appropriate botanical institutions in the US to ensure that precious germplasm is grown at multiple sites.

Once sufficient numbers of seedlings from each population are planted in the landscape, remaining plants are distributed to other gardens in the US or abroad that have appropriate growing conditions. This helps to ensure a second degree of backup for the collections.

After conservation plants are grown to maturity in the landscape at MBC, efforts are made to pollinate them and collect seeds. Many of these seeds are shared with other gardens and the horticultural community through MBC's seedbank program. This program distributes nearly one million seeds annually.

Recently, MBC has been exploring other means of backing up its conservation collections, especially those collections that do not lend themselves to seed propagation. In partnership with the Georgia Institute of Technology and the Atlanta Botanical Garden, MBC is exploring tissue culture propagation of its rarest cycads to ensure that they can be backed up. In addition, MBC, the Atlanta Botanical Garden, and the Royal Botanic Garden Edinburgh have been working to propagate rare tropical conifers by cuttings and to back them up at multiple institutions, so that loss of precious germplasm is not risked at any one site.

Backing up of conservation collections has been facilitated by new means of efficient information exchange over the Internet. The BG-Base Multisite Search and BGCI's PlantSearch provide important means for gardens to learn about each other's collections and inquire about propagation and sharing of those collections.



Figure 1: *Microcycas calocoma*, critically endangered and endemic to a small area of Cuba. Intensive propagation efforts at MBC have resulted in distribution of over 6,000 seeds of this species. Photo courtesy of Michael Calonje.



Figure 2: *Corypha taliera*, extinct in the wild, all surviving individuals are cultivated in gardens. MBC has 13 of the approximately 20 in cultivation. Photo courtesy of Patrick Griffith.

25. A 25-year perspective of ex situ collection maintenance of Torreya taxifolia.

By Abby Hird, Michael Dosmann, Arnold Arboretum of Harvard University; Jennifer Cruse-Sanders, Michael Wenzel, Atlanta Botanical Garden.

Torreya taxifolia, once a towering giant in the forests of Georgia and Florida, has been diminished to twig-like sprouts by an obscure fungal disease over the past century, and is now one of the most threatened conifers in the world. Several ongoing conservation efforts strive to understand the pathology of the disease, and find effective management and reintroduction strategies. Several *ex situ* collections of the species have been aimed at conserving the narrowing genetic diversity of extant wild populations, as well as producing seeds and cuttings for research.

One such *ex situ* effort began in the mid-1980s by Rob Nicholson and Mark Schwartz, funded by the Center for Plant Conservation and the Arnold Arboretum of Harvard University. The two men undertook a massive collection and distribution of wild-origin *T. taxifolia* rooted cuttings to 10 institutions in North America and Europe. As part of the Center for Plant Conservation National Collection of Endangered Plants, *T. taxifolia* had been at the Arnold Arboretum since 1985. By analyzing the Arnold Arboretum's plant records, inventory records among the 10 collection holders from 1992 and 1996, and an international *ex situ* inventory we conducted in 2009, we were able to identify trends and lessons learned from this long-term *ex situ* effort and provide guidance for a CPC National Collection transfer of *T. taxifolia* from the Arnold Arboretum to Atlanta Botanical Garden in 2010. By studying the long-term *ex situ* conservation efforts of *T. taxifolia*, we have also determined some effective practices that can be applied to similar efforts (Hird and Dosmann, 2010).

Results

- Significant loss of unique lineages at the Arnold Arboretum observed through time, attributed to incompatible climate, poor adaptability to nursery conditions and human error (staff changeover, labeling errors, etc.)
- The number of unique lineages at single institutions increased through time, while the overall lineages preserved declined only slightly.
- As a result of the 2009 *ex situ* inventory, redistribution of germplasm has occurred among collection holders to preserve and back-up *ex situ* maternal lines at more than one institution.

Applications

- Appropriate ex situ site selection is critical to lineage and specimen preservation of rare taxa.
- Curatorial oversight is critical for successful *ex situ* conservation. All collection holders required some sort of records update, corrections, or inventory of *T. taxifolia* holdings during the 2009 *ex situ* inventory, and several specimens had been disassociated from their collection records, and less valuable for conservation purposes.
- Distribution of germplasm to other institutions has prevented overall germplasm loss as responsibility was shared among institutions, and redistribution of germplasm recovered most losses at individual institutions.
- Living collections can contribute to the collective conservation power of public gardens. The 2009 *ex situ* inventory of *T. taxifolia* demonstrates successful preservation when a group of collection holders work together.
- Gardens can increase access to their collections via online resources such as BGCI's PlantSearch Database. For example, PlantSearch reported *Torreya taxifolia* holdings at 24 institutions worldwide in early 2010, and 34 institutions following the North American Collections Assessment.

References:

Hird, A. and M. Dosmann. 2010. Breaking the barriers to successful *ex situ* plant conservation. The 6th Annual Harvard Plant Biology Symposium. The Arnold Arboretum of Harvard University, Cambridge, MA.



Figure 1: A portion of the *Torreya taxifolia ex situ* collection maintained by the Atlanta Botanical Garden. Photo courtesy of Michael Wenzel.