

Conserving Threatened Rare Plants: Some Nursery Strategies

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Abstract—The legacy of the 1992 Earth Summit and the mandates of the Convention on Biodiversity have influenced the propagation of threatened and endangered native plants. Minimum viable population guidelines, appropriate gene pool sampling, micropropagation techniques, and strategies for returning propagules to the environment guide the development of conservation plans. The rare Pacific Northwest endemics *Phlox idahonis*, *Douglasia idahoensis*, and *Hackelia venusta*; oceanic island megarrarities; and once-abundant but now uncommon plants were evaluated for both ex situ plant recovery programs and other propagation opportunities. Micropropagating seed conserved scarce germplasm of rare *Astragalus*, *Carex*, and *Lepidium* species. The strategy of first propagating the common species, *Astragalus aquilonius*, was successful in culturing the closely related rare species *A. amblytropis*. Nurserymen can participate in species recovery programs, propagate regional rarities to promote conservation education, and prevent further decline of once-abundant native plants.

INTRODUCTION

In the 1970's, botanists became aware that rare plant populations were declining in many parts of the world. Regions of high endemism particularly vulnerable to loss of diversity include remote oceanic islands, areas with Mediterranean climates such as South Africa and Western Australia, and parts of both the wet and dry tropics. Rising concern over species' decline resulted in global action which produced the Convention on Biodiversity at the Earth Summit,

sponsored by the United Nations Commission on Environment and Development, in Rio de Janeiro, Brazil in 1992. The Convention, ratified in 1993 and implemented in 1994, requires the signatory nations, including the United States of America, to develop National Conservation Strategies. Strategy goals include conducting national biological surveys to determine the life forms at risk, developing and executing appropriate in situ (on-site) and ex situ (off-site) conservation plans, and monitoring the biota. The Federal Native Plant

Conservation Committee (representing seven federal agencies) and the Center for Plant Conservation (CPC - a private consortium of botanic gardens) are cooperating to conserve the flora of the United States (CPC 1994).

Charles Darwin noted over 150 years ago that rarity precedes extinction (Darwin 1858). While not all rare plants are threatened, most threatened plants are almost always rare. Hence it is natural to ask whether rare species are threatened. Although many factors

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influence rarity (Harper 1981, Rabinowitz 1981), the greatest threats to small plant populations are human activity and random environmental events such as drought, seed predation or pollination failure (Menges 1992). Protecting habitat is the primary and necessary goal of conservation, and ex situ conservation cannot substitute for habitat protection. Ex situ action becomes appropriate, however, when habitat management alone cannot prevent species' decline. This paper focuses on the ex situ conservation of threatened flora and describes how nursery managers and plant propagators can apply new strategies to conserve rare plants.

EX SITU CONSERVATION METHODS

A multidisciplinary team (including taxonomists, ecologists, geneticists, population biologists, propagators, and habitat restorationists) identifies the risks, assesses the threats, and selects the criteria for species selection. Botanic Gardens Conservation International recommends working first with species directly threatened, followed by species in habitat under threat of disturbance, rare plants not under threat, wild relatives of crop plants, and other categories (BGCI 1993). The Center for Plant Conservation, at the Missouri Botanic Garden in St. Louis, lists in descending order of priority: highly endangered species, those rapidly declining from human causes, unique species, species with potential for

recovery, and useful genetic material (CPC 1991). After generating a consensus plan, team members capture the genetic variation with seed or vegetative parts, preserve the germplasm, and propagate plants for reintroduction or introductions to new sites.

For most species, a nearly complete sample of the genome can be captured. CPC guidelines recommend collecting seed from up to 5 populations per species, 50 individuals per population, and up to 20 seed per individual if that is less than 20% of the seed available from that individual (Falk 1992, Guerrant 1992). Vegetative propagation would require more individuals to be sampled to approach the genetic variation provided by seed.

The primary ex situ technologies involving nurseries are: storing germplasm from threatened habitat in seed or gene banks, growing living collections, and micropropagating rare plants in tissue culture laboratories. Seed banks store orthodox seed (seed which survives below freezing temperatures) of species primarily from the temperate zone and dry tropics. Viable seed dried to an equilibrium moisture content of about 5% and stored in low relative humidity (15%) at -20°C can potentially remain viable for up to 200 years.

Although requiring more space and more expensive to maintain than seed banks, living collections

preserve germplasm when seed cannot be stored below freezing (recalcitrant seed), as is the case with many species from the wet tropics and a few from the temperate zone. Nurseries in botanic gardens, arboreta, universities, and other plant research facilities maintain living collections for research and conservation education.

Micropropagating rare plants in vitro, by both seed and vegetative means, is increasingly used to conserve rare species.

Micropropagation requires only small amounts of material and therefore minimizes damage to the species. A useful strategy to avoid wasting valuable rare material is to develop a propagation protocol for a close relative and then apply it to the threatened species. Seed is the preferred choice of plant material since the sterile environment of in vitro seed culture can often enhance germination success over conventional propagation (Fay 1992). When seed is unavailable, in short supply, or cannot be germinated readily (unviable, dormant, damaged by disease or insect, etc.), vegetative micropropagation techniques are appropriate (Hartmann et al. 1990). The potential explant (starting material) for a propagation trial could consist of a shoot tip, shoot segment, leaf, petiole, flower, or embryo. Culture involves destroying surface microorganisms and placing the decontaminated explant on a sterile gel medium containing mineral nutrients, vita-

mins, and carbohydrates. To increase plant numbers, plant growth regulators supplement the medium to induce multiple bud development on the explants. The microshoots elongate after transfer to a hormone-free medium and, after excision from the explant, may root in the sterile laboratory environment or in a high-humidity propagation area. Finally, plantlets (micropropagated plants) are hardened in a greenhouse.

To persist, a species must have a minimum viable population large enough to minimize the chance of extinction from random catastrophe for a long time (Menges 1992). A rule of thumb suggests a plant species with 5 or fewer distinct populations or less than 1,000 individuals in total may become extinct if a chance event causes a population crash (Falk 1992). Nursery-grown plants can be reintroduced to re-establish the species where it has declined or been extirpated (Maunder 1992). Planting stock, however, should be free of disease and pests to prevent introduction of pathogens to the sites. Although of increasing importance in North America, reintroduction techniques are still experimental.

APPLICATIONS AND DISCUSSION

We now examine how species of varying degrees of rarity (moderately rare endemics of the Pacific Northwest, extreme rarities on

oceanic islands, and once-abundant but now uncommon plants) could be considered for ex situ plant recovery programs and other propagation opportunities. We also cite examples where micropropagating seed and vegetative parts conserves scarce germplasm.

Pacific Northwest rarities

Idaho phlox (*Phlox idahonis*), one of the state's rarest vascular plants, is restricted to a small region of northern Idaho and is therefore considered a narrow endemic species (Moseley and Crawford 1993). Recent population monitoring of Idaho phlox revealed eight occurrences in four metapopulations, with several hundred to many thousand individuals per occurrence, and a total of about 10,000 individuals. Since the populations appear stable and Potlatch Timber Corporation (which owns most of the phlox habitat) plans to prevent decline of this species, ex situ conservation is unnecessary.

Douglasia idahoensis was described as a new Idaho endemic species in 1981 (Henderson 1981). About as abundant as Idaho phlox, *D. idahoensis* numbers about 9,000 individuals but is more widely distributed in 24 known populations, some containing fewer than 50 individuals (USDAFS 1993). Although these demographics indicate no immediate threat, climate warming could result in a rapid decline of this

Pleistocene relict since it is confined to northeasterly aspects on subalpine peaks and therefore lacks upslope refugia. The species should be carefully monitored and ex situ action taken quickly if population declines are observed.

Hackelia venusta or showy stickseed (Boraginaceae), an herbaceous biennial endemic to the Washington Cascades (Hitchcock and Cronquist 1973), exists as one population with fewer than 500 individuals in habitat degraded by human activity. In cooperation with the Forestry Sciences Laboratory at the Pacific Northwest Experiment Station and the Wenatchee National Forest, we micropropagated 10 clones of *H. venusta* from shoot tips. The explants multiplied rapidly producing an average of 3 new microshoots per explant per month on MS medium (Murashige and Skoog 1962) containing low levels of the cytokinin benzyladenine (BA) from 0.05 to 0.1 mg/l. Microshoots of all 10 clones rooted, at rates from 90-100%, on a culture medium containing the natural auxin indoleacetic acid (IAA) at 0.5 mg/l (Fig. 1). The rooted microshoots were acclimatized and grown in Roottrainer containers for ease of plug extraction (Fig. 2). Pest and disease-free plantlets will be introduced at four suitable sites with initial plantings of 500 plantlets per site.

Megararities

Human activities have pushed some naturally rare plants to extreme

rarity. If the genetic base of a very rare plant is narrow, a species recovery program may not be useful. Nurseries, however, can play other important conservation roles.

Tecomanthe speciosa (*Bignoniaceae*), a woody vine species, exists as a single genotype in subtropical forest on a remote offshore island in northern New Zealand (Wilson and Given 1989). Introduced goats which damaged the island vegetation have been removed but no seedling regeneration has occurred. Fortunately, cuttings taken from the wild plant have produced many plants which produce viable seed in cultivation on the mainland. Increasing popularity of this species as a garden ornamental has at least delayed its extinction, and

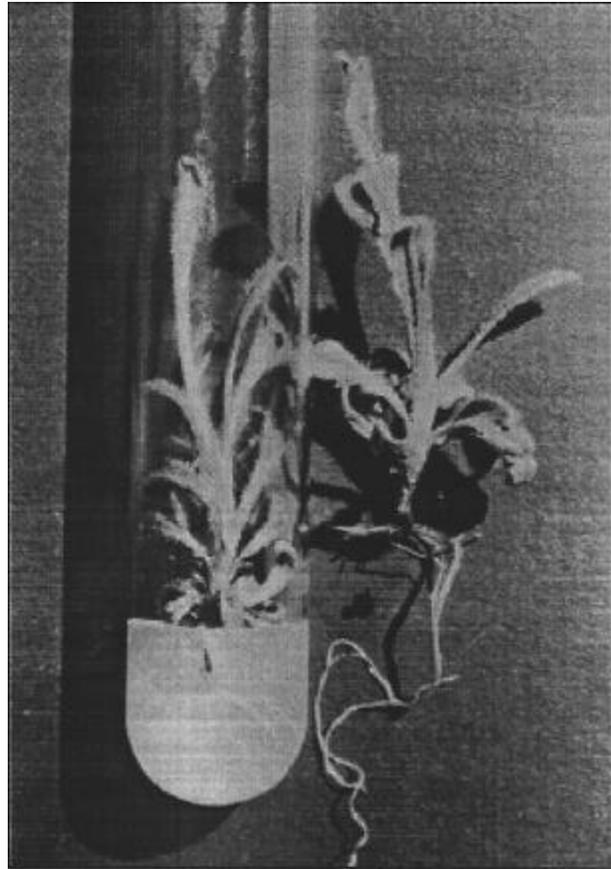


Figure 1. In vitro rooted plantlets of *Hackelia venusta* ready for acclimatization.



Figure 2. Fully-hardened plantlets of *Hackelia venusta* for introduction to a forest site.

propagation of this megarearity has developed public interest in conserving native flora of the region. It is presently unknown whether there is sufficient variation in the population of cultivated plants to allow recovery of this species, and there is a risk of contaminating the wild site with the garden fungus *Phytophthora cinnamoni* on reintroduced nursery plants (Maunder 1992).

Sophora toromiro, a leguminous tree, was once found in volcanic craters on Easter Island. Overcutting reduced the population

to 1 tree by 1917 which was last seen by the Heyerdahl expedition in 1955 (Christensen and Schlatzer 1993). Mature specimens were found growing in an arboretum on mainland Chile, and seedlings were reintroduced by the Chilean Forestry and Conservation Agency (Maunder 1992). In addition, Jacobsen and Dohmen (1990) have attempted to increase genetic variation artificially by inducing somaclonal variation in *toro miro* micropropagules. Equally important for the species survival, the Easter Islanders are likely to protect the reintroductions since *toro miro* wood was used for wood carving associated with the mythology of the statues (Heyerdahl 1958). Many other countries also propagate "flagship" rarities for both economic and cultural reasons.

Although there are no known megararities in Idaho, the rarest of our plants do provide an opportunity for nurseries to promote conservation in the Interior Northwest. The Idaho phlox grows readily from seed in local gardens, and *D. idahoensis* has grown successfully in our Moscow greenhouse.

ONCE-ABUNDANT PLANTS IN DECLINE

At the other end of the rarity spectrum are once-abundant endemics now in decline. Collection of showy natives is a major problem worldwide. The international trade in cut flowers threatens *Banksia coccinea* (Proteaceae)

endemic to Western Australia. Plant families widely threatened include orchids in the wet tropics and temperate forests and bogs, cacti in the Americas, and carnivorous plants in the southeastern USA. Venus Flytrap (*Dionaea muscipula*), a unique carnivorous species (Ayensu 1981) once relatively abundant, is now threatened by loss of habitat and trade in poached plants (Culotta 1994). The Convention on International Trade in Endangered Species (CITES) controls export of the Flytrap, but the plant is still being dug and traded within the US. The nursery industry could both increase mass propagation to deter digging and support government action to ensure sustainable use of this and other resources.

Idaho's Pacific dogwood (*Cornus nuttallii*) is a disjunct of the widespread coastal population. Now infected with dogwood anthracnose (*Discula destructiva*), this outlier population has produced little seed since 1990, and with fewer than 700 trees remaining the population may be extirpated. Edson et al. (in press) multiplied in vitro-derived shoot tips at an average rate of 2.9 microshoots per explant on an agar-based medium containing 1 mg/l BA, and rooted 50% of the microshoots after a basal talc dip of 4.5% IBA. Since no disease resistance has been found, reintroduction would likely fail. Seed banking the genome and storing all the clones in tissue culture are presently the only alternatives to conserve the species.

SEED MICROPROPAGATION

In vitro germination avoids damping off and other pathogens of conventional propagation often resulting in higher survival of germinants in culture. *Astragalus columbianus* (Fabaceae), once thought to be extinct was rediscovered in 1976 (Sauer and Mastrogioseppe 1979). Untreated seed germinated at 13% on a propagation bench. Three weeks after scarifying with sand paper, in vitro seed germinated at 92% on MS medium supplemented with 10% agar, versus 64% germination of the scarified ex vitro seed ($p < 0.001$). *Carex hystricina* (Cyperaceae), a sedge rare in the Pacific Northwest, germinated ex vitro at 27% with the seed enclosed in the perigynium, the papery covering often left intact when propagating common sedge species (Hurd and Shaw 1992). We compared germination rates, with and without the perigynium present, both in culture and on a propagation bench. Within three weeks of removing the perigynia, the in vitro germination rate of 94% surpassed that of the conventionally sown seed by 32% ($p < 0.001$) (Fig. 3). *Lepidium papilliferum* (Brassicaceae), an Idaho endemic, germinated at 14% in vitro versus 0% ex vitro. After seed dissection, however, 88% of the embryos produced seedlings.

VEGETATIVE MICROPROPAGATION

The strategy of first propagating

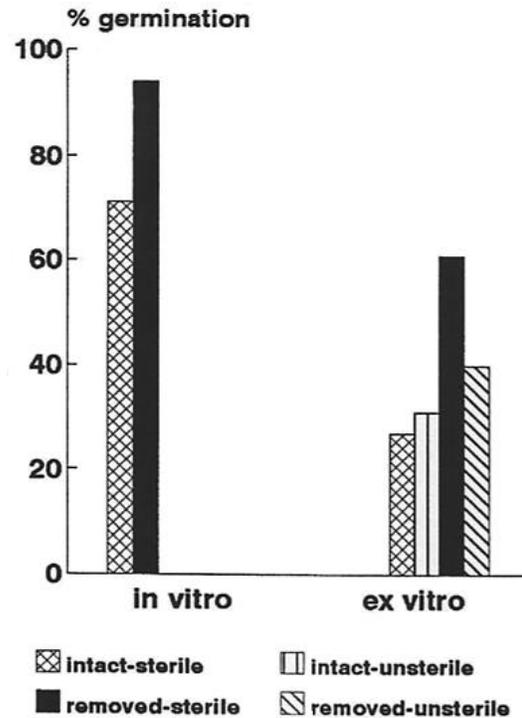
the common species, *Astragalus aquilonius*, was successful in culturing the closely related rare species *A. amblytropis*. The two taxa produced an average 1.4 and 1.8 microshoots per explant per culture period (3 weeks) on MS medium supplemented with 0.1 mg/l BA, rooted at 73% and 57% on hormone-free medium, and survived at 92% and 91% respectively. Since this protocol also produced similar results with the rare *A. columbianus*, some of the other more than 150 threatened species of *Astragalus* in the United States (Falk 1993) may also be propagated using this procedure.

Clonal variation can affect shoot growth and rooting response. The rooting of some *A. columbianus* clones was inhibited at moderate to high levels of IAA. Minimizing the concentrations of plant growth regulators as media supplements may lower the expression of differences between clones, deter callus development, and reduce the chance of genetic change.

CONCLUSIONS

Using efficient micropropagation strategies, nurseries can better face the challenges of propagating rare plants. In the coming years, as the national biologic survey progresses, we will likely find increased opportunities to participate in plant recovery programs to help avoid extinctions. We can also propagate regional rarities and other threatened

Figure 3. In vitro versus ex vitro germination of *Carex hystricina* with perigynia intact or removed.



endemics for a variety of uses. The positive net effect of these activities will be to promote conservation of our native flora.

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