Report on

Ex Situ Conservation

By:

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May 1, 1995

Interior Columbia Basin
Ecosystem Management Project
Science Integration Team - Terrestrial Staff

Preface

The following report was prepared by University scientists through cooperative agreement, project science staff, or contractors as part of the ongoing efforts of the Interior Columbia Basin Ecosystem Management Project, co-managed by the U.S. Forest Service and the Bureau of Land Management. It was prepared for the express purpose of compiling information, reviewing available literature, researching topics related to ecosystems within the Interior Columbia Basin, or exploring relationships among biophysical and economic/social resources.

This report has been reviewed by agency scientists as part of the ongoing ecosystem project. The report may be cited within the primary products produced by the project or it may have served its purposes by furthering our understanding of complex resource issues within the Basin. This report may become the basis for scientific journal articles or technical reports by the USDA Forest Service or USDI Bureau of Land Management. The attached report has not been through all the steps appropriate to final publishing as either a scientific journal article or a technical report.

The Berry Botanic Garden

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May 1, 1995

MAY 4

Lisa Croft
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Eastside Ecosystems Management Strategy Project
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Walla Walla, WA 99362

Dear Lisa:

Enclosed is the final corrected report on Ex Situ Conservation under your Purchase Order No. 40-OEOO-5-5230.

Both Ed Guerrant and I were pleased to be asked to prepare this report and will be glad to answer any questions you might have.

Yours sincerely,

Linda R. McMahan
Executive Director

Ex-Situ Conservation

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The Berry Botanic Garden
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Introduction

Ex situ or "off-site" conservation describes a range of activities more or less separated from "on-site" or *in situ* activities. Many interactions and overlaps exist between off-site and on-site activities; however, *ex situ* activities are usually considered to be such activities as seed storage, maintaining living collections at botanical gardens, or various research activities. In our view and that of many other writers on conservation, *ex situ* is not an alternative to *in situ*, but both are part of a larger, comprehensive conservation effort.

"The preference for *in situ* rather than *ex situ* conservation is sometimes posed as an 'either/or' frame, but should not be seen in terms of exclusive alternatives at all" is a statement that appeared early in the talks about *ex situ* conservation methods (Thompson, 1979). This early reference goes on to point out that sometimes *ex situ* intervention is the only hope for certain critically rare species. Many authorities on *ex situ* alternatives view them as mutually compatible if less desirable than *in situ* (Thompson, 1979), as a backup in case of catastrophe in the wild (New England Wild Flower Society, Inc., 1992), or as "complementary (e.g. Brown and Briggs, 1991). The Botanic Gardens Conservation Strategy (World Conservation Union, 1989), jointly produced by the World Conservation Union (IUCN), Botanic Garden Conservation International (BGCI: formerly Botanic Garden Conservation Secretariat, or BGCS), and the Worldwide Fund for Nature (WWF), states: "*In situ* and *ex situ* are the opposite ends of a spectrum and there is no absolute distinction between them." That document goes on to call for a "seamless blend" of *in situ* and *ex situ* conservation as the most effective way to conserve species and ecosystems.

From this and other concepts, we have drawn up a diagram of various activities considered to be conservation-related and placed them on just such a spectrum, with on-site and off-site on the horizontal axis. In addition, we have added another dimension (from low to high manipulation required) on the vertical axis (Figure 1). Habitat acquisition and preservation appear in the bottom left-hand corner as the most site related and least manipulative conservation alternative, however, simply locking up land will not necessarily promote conservation, without some active management. At the opposite extreme is an activity--gardening with native plants--that we consider to be rarely of conservation value. This is true whether that gardening be at someone's home

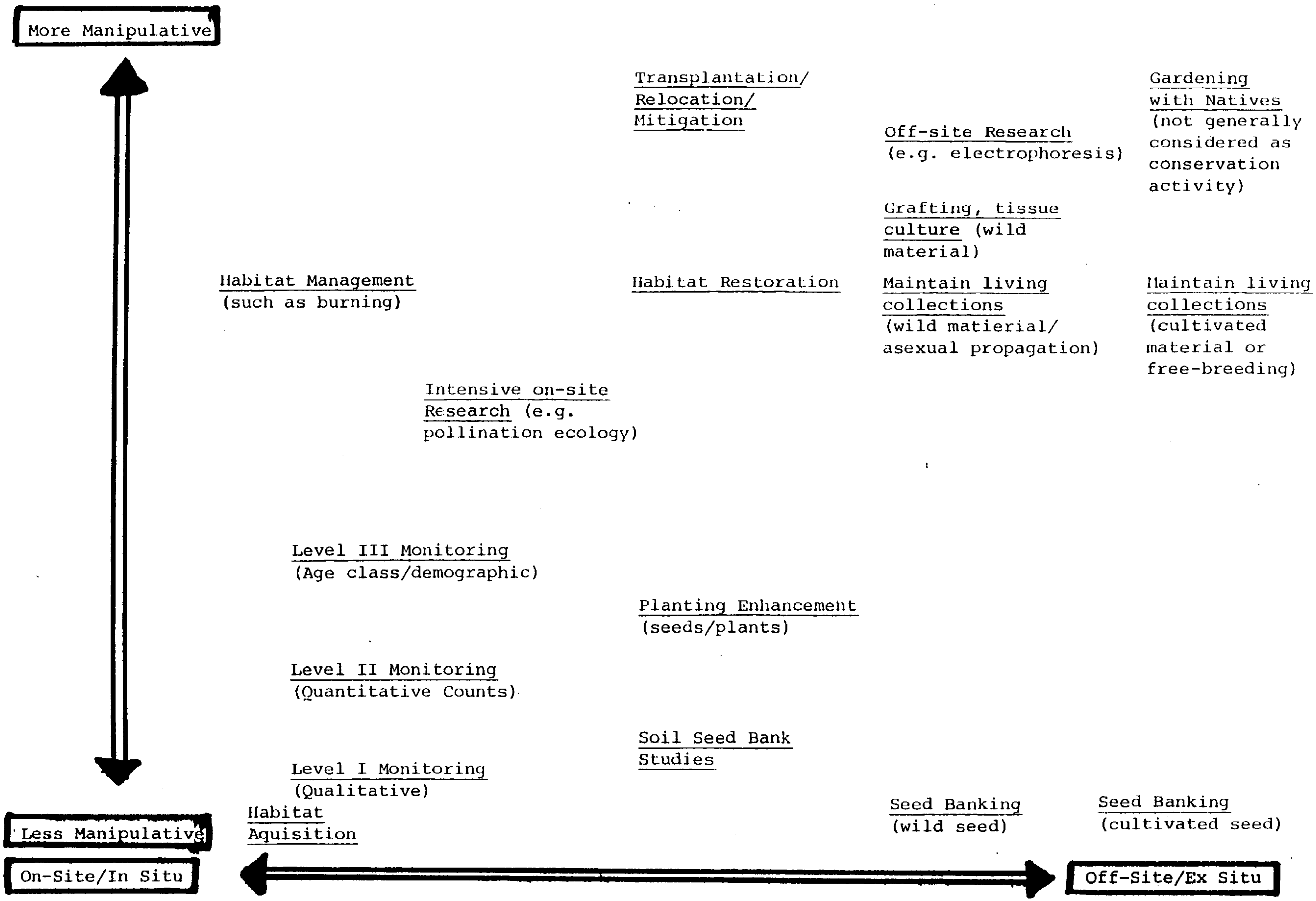

Figure 1. Spectrum of different conservation activities on scales of On-site vs. Off-site and Manupulative verses less manipulative.

or in a botanic garden when the primary purpose is for display to the public, but does not include propagation connected with learning about the life history characteristics of plant species. Gardening *per se* is both distinctly separated from wild sites and is highly manipulative, although some information gained from these gardening activities can help us learn more about the survival of wild plants. Other activities, such as habitat restoration, research, seed banks, and habitat management, are distributed somewhere between these two extremes In our view, none of these activities is inherently more or less "good" than any other activity, but some may relate more directly than others to conservation of species and habitats. Any tool is best judged in the context of its use.

Methods of Off-Site Conservation

Ex situ or off-site conservation is generally considered to consist of germplasm storage methods such as seed banks, maintaining living collections, tissue storage (such as in tissue culture, or pollen). Also relevant are many activities that take place, at least in part, away from wild sites and habitats, but that rely heavily on material from these sites, including efforts at replanting, restoration, and transplantation, studies of soil seed banks, and laboratory research (e.g. taxonomic, life history). Off-site storage of seeds is not an end in itself, but one means among many that contribute to the end of conservation. "New populations can arise phoenix-like out of the ashes of extinction only if collections exist off-site. It is also critical to note that *ex situ* collections are not an end in themselves. Their ultimate value will be derived from how they are used and their effect, if any, on the long-term prospects for survival of rare plant species" (Guerrant, 1992).

The Botanic Garden Conservation Strategy (1989) states the role of off-site conservation is to "provide protective custody" of plants, and advocates processes like seed and pollen banks, maintaining vegetative propagules, or tissue and cell cultures. The authors of this document clearly prefer seed storage, but offer the alternative of "field genebanks"-- maintenance of living collections-- as a short-term alternatives. Newer and less tested alternatives, such as tissue culture, are less often considered appropriate, however, they have found prevalence in certain circumstances. Brown and Briggs (1991) picture the types of activities for *ex situ* conservation generally as in Figure 2, where material (usually seeds) from wild habitats are taken into an off-site setting for seed storage or growing into stock plants. These can be returned to the wild through processes such as replanting or vegetation enhancement.

Seed storage and living collections maintenance must follow strict guidelines to be at all effective (McMahan and Guerrant, 1991). Done incorrectly or casually, they may cause considerable harm if the material in storage is actually needed for any purpose. Poor or incomplete genetic representation can hamper scientific research or efforts to revegetate

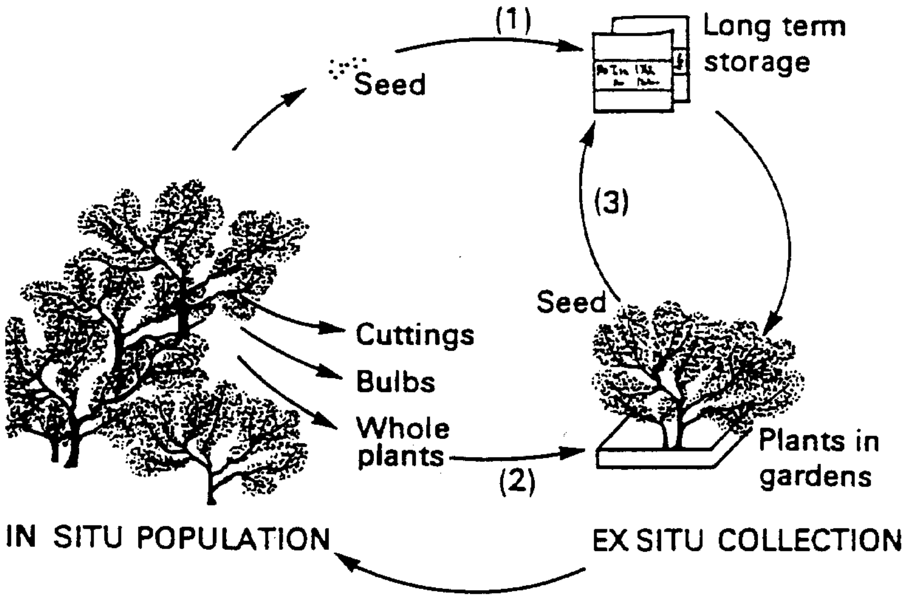

Figure 2. Three basic routes by which an ex situ collection is established, and its feedback to the source in situ. (Source: Brown and Briggs, 1991).

or restore habitats. They can also lead to a "false security." Indiscriminate breeding can lead to loss of genetic diversity in an amazingly short time, as was shown recently in one concrete example: *Amsinckia grandiflora*, an endangered annual species from California. Pavlik et al (1993) found that of the available stored seeds, those that had been grown out just two generations had lost a significant amount of the original genetic diversity. Any attempts to grow out stored seed will almost necessarily lead to reduction in genetic diversity, or at least a change in the genetic composition from the wild -- the selective pressures in a garden setting will always be different than in the wild.

Seed Banks

A seed bank is a facility to store genetically representative seed collection under conditions designed to ensure the long-term viability of these seeds. Seed storage is generally considered to be a good alternative for seeds that are orthodox (desiccation tolerant) (Eberhart et. al. 1991). Desiccation intolerant seeds--termed recalcitrant--a category that includes 20% of the world's seeds (The World Conservation Union, 1989), cannot be stored for more than a few weeks or months, and cannot be stored at below freezing temperatures at all. Fortunately, most of the seeds of temperate regions are orthodox, with the exception of seeds of some aquatic species, and some plants with large, wet seeds such as oaks (Eberhart et. al., 1991).

Seed banking is generally considered to be the favored off-site germplasm conservation method when it is available (New England Wild Flower Society, 1992; The World Conservation Union, 1989; Hawkes, 1990; U.S. Congress, 1987). Seed banks can accomplish certain conservation goals, as long as their limitations are understood. Botanists at the USDA National Seed Storage Laboratory recommend storage at a minimum of -20 degrees C for seeds that have low seed moisture, that is, have been desiccated to a certain level (Eberhart et al, 1991). Generally, seed experts at the Royal Botanic Gardens at Kew and the National Seed Storage Laboratory consider that letting seeds equilibrate to an atmosphere at 15 degrees C and about 19-20% Relative Humidity, will produce an appropriate moisture content (personal communications with Ed Guerrant). Seed longevity under these conditions is estimated to be in the order of 100-200 years, with some loss of viability during that time. Higher temperatures, even those below freezing, increase the gradual loss of viability. For longer term storage, Eberhart et. al. (1991) recommend cryogenic storage below -130 degrees C. Storage under these conditions, usually employing liquid nitrogen, may greatly extend the viable life of seeds which can withstand this degree of freezing. However, these extremely low temperatures may cause/be associated with structural damage to the seed/developing embryos - for example, some plants, legumes come to mine, may lose their cotyledons, and even though the tissue may be alive, the embryonic shoot axis is cut off from its source of nutrition. So there are down sides to cryogenic storage (personal

Living Collections -- Field Genebanks

Maintaining living collections, or field genebanks, is a less desirable alternative to seed storage (The World Conservation Union, 1989; New England Wild Flower Society, 1992). In central Florida, where a large percentage of the flora has recalcitrant seeds, Bok Tower Gardens had to devoted over five acres to field genebanks (see Wallace, 1990). In each plot, 50 or more individual plants are planted out in a grid pattern and carefully labelled. One or two backup plots, made from cuttings of the original plants, are also maintained. Despite this very labor and resource intensive work, germplasm is inevitably lost as certain genotypes respond better to cultivated conditions than do others. In addition to loss of genetic diversity by random genetic drift--due to the statistical 'laws' of probability alone, which is unavoidable in small populations--it is practically impossible not to subject plants in cultivation to selective pressures different than they would experience in field. The result of this is an unconscious tendency to 'domesticate' any stock grown off-site, making it less well adapted to life in its native habitats.

Elias (1987) reconstructed long-term survival of several species in cultivation at the Rancho Santa Ana Botanic Garden in Claremont, CA. For example, he found that of more than 100 seedlings of *Carpenteria californica* (a very showy woody shrub, listed by state of California as Threatened and listed as a federal C1 species), established from an original seed collection in July 1935, only 22 still remained in March of 1951. By the spring of 1972, 16 remained, to be reduced to only 4 in June 1982. Admittedly, loss of these species was by natural attrition with no effort at ongoing propagation, but the examples do illustrate the false security that one can get from establishing "captive populations."

Alternative Culture

Other possible techniques such as grafting, tissue storage, cell culture, and embryo culture are beginning to be mentioned as alternatives for *ex situ* conservation, but we have generally less experience with these methods that others. Guerrant (in press) refers to some ongoing studies, including grafting of some extremely rare Hawaiian species and micropropagation. Bramwell (1990) describes micopropagation techniques for rare island endemics in the Canary Islands. Micropropagation or tissue culture was designed to reproduced large numbers of genetically identical individuals (Guerrant, in press). This is a technique used for revegetation of mine tailings with many plants described by Palacios and Ruiz (1990). However, it is also used extensively as a method to propagate species such as orchids (see e.g. Fay and Muir, 1990 for European terrestrial orchids) and lilies, greatly speeding up plant germination and growth. It is also used to maintain callus tissue of trees, and for storage and reproduction of species. The tissue communication between Ed Guerrant and Simon Linnington at the Royal Botanic Gardens, Kew). In general, specific procedures for seed banks are well worked out, principally because of efforts to save seeds of crop species worldwide. The general references and additional information can be found in Ellis et. al. (1985a and 1985b), Cromarty et. al. (1982), and Hanson (1985).

The Berry Botanic Garden has the only seed bank for rare and endangered species in the Pacific Northwest. It consists of an 8 by 10 ft room, encased in 8-inch thick concrete walls for fire protection. Humidity is maintained at 20%, which is a good humidity level for short term seed storage and for initial desiccation of collected seeds. Temperature is maintained at 59 degrees F (15 degrees C). Inside the room is a standard chest freezer maintained at about -20 degrees C, in which seeds are stored after they have been weighed, counted, and sealed in water vapor proof metal-foil packets. Computer and manual tracking systems account for material in storage.

Cryogenic storage is available through an agreement between the National Seed Storage Laboratory and the Center for Plant Conservation, of which The Berry Botanic Garden is a participating member.

Seed banks do not work alone, even in off-site situations. To function properly, scientists and horticulturists must be able to grow new plants from the seeds. See, for example Hawkes (1990), who suggests specific protocols for germination testing; his is one of several standard methods available to the researcher. Thus it is important to carry out germination and growth studies as well as viability testing of seeds in storage.

Pollen Storage

Essentially the same procedures can be applied to pollen storage (Matthews and Kraus, 1981). Pollen storage can be useful when breeding regimes are interrupted or for highly depaupered populations, and used to enhance the genetic variability of captive or wild plants. An extreme example can be found with a species in the spectacular silversword genus, ***Argyroxiphium,*** in Hawaii. Fewer than 50 individuals remain of one species, but the opportunities for genetic management through controlled cross pollination are severely limited because each plant dies when it flowers, and only a few plants flower each year (R. Robichaux, personal communication with E. Guerrant). With stored pollen from plants that flowered and died in years past, the opportunity exists to maximize retention of what little genetic diversity remains.

culture laboratory at Harvard's Arnold Arboretum, is culturing species to aid with propagation and distribution as well as to preserve disease-free stock. Embryo and cell culture are emerging activities, whose applicability will be tested in time. Bramwell (1990) refers to the possibilities of cell and protoplast culture as well, kept in liquid media. Each of these activities is highly intensive and carries its own risks, but may be important for extremely rare species where conventional methods have failed (Bramwell, 1990). A recent report (Perez and Fernandez, 1990), for example, shows some success in embryo culture of walnuts, an important crop not amenable to seed storage by conventional means.

Simulated Natural Environments

Susan Wallace (1990), who for many years was Curator of Endangered Plants at Bok Tower Gardens, advocates an experimental approach in Central Florida whereby plants could be introduced to degraded natural habitats and allowed to reproduce naturally. She states "habitat destruction has been widespread in Florida . . . Two-thirds of the ancient scrubs in central Florida have been destroyed." Many of the plants she works with have recalcitrant seeds and cannot be maintained in seed banks. Her efforts at field genebanks are costly and labor intensive, leading her to pose this new strategy. The concept of simulated natural environments has not received wide review, but is perhaps worth discussing when entire ecosystems are in danger and other more standard conservation approaches have failed.

Relevance of Ex Situ Conservation: Why Have Off-site Collections at All?

Off-site conservation must be considered in context with other conservation activities, including outplantings to enhance existing wild populations, reintroduction to a historical site, introduction of species within an existing range to enhance species survival, and experiments with introduction or reintroduction. In our view, reintroductions and other similar activities are far from an exact science. In some ways, are all experimental, since the experience of the conservation community with these strategies is relatively recent, and long-term monitoring studies are just beginning at best. Few if any examples of these activities have been ongoing for over 20 years.

Although we do not present here a complete discussion of any of these activities, we do believe they are worth mentioning in the context of *ex situ* activities.

Mitigation activities often specify certain types of *ex situ* conservation, such as seed storage or transplantations of individual plants to new sites. Experience with these shows that transplantations of existing plants in the wild to new sites are rarely successful, even in the short run (Fahselt, 1988; Fiedler, 1991; Hall, 1987). Indeed, translocation is a controversial technique, possibly with limited conservation value. The above mentioned references note that most failed because of lack of site preparation and post-establishment care. Many sites of transplantation require continued watering, mulching, shade protection, grazing protections, insecticides, pest and weed control. The more successful projects tended to be those with more planning and care invested in the project. Gordon (1994) presents a 'decision tree', in the form of a dichotomous key, that aims to inform land managers when and where translocation might be considered appropriate.

Revegetation or restoration seems to be somewhat more successful, particularly when plants are propagated specifically for this purpose. Evans and Bohn (1987) report success in cultivating many California species, particularly woody plants, for restoration projects. For example, cuttings and seeds of wild populations of *Malacothamnus clementinus* (a rare San Clemente Island endemic) and seeds and salvaged plants of *Monardella linoides* ssp. *viminea* (San Diego Willowy Mint, endemic to SW San Diego County), and seeds of *Carpenteria californica* (a rare California endemic) produced hundreds of plants from wild material for re-establishment under contracts for mitigation or Fish and Wildlife Service recovery plans.

Like it or not, strategies to enhance wild populations through outplantings are increasingly becoming a part of conservation strategy. To do any of these activities properly, good procedures for *ex situ* storage and growing out seeds to plants is necessary. Bowland (1978) reports on a project to revegetate the Guadalupe Dunes in California to restore a pipeline right-of-way. Reid and Walsh (1978) report extensive efforts to reclaim habitats for both rare plants and the host plants of rare butterflies in California's San Bruno Mountain. Ferreira and Smith (1978) report methods of increasing native populations of *Erysimum menziesii*, in which seeds from a remnant population produced 3,500 seedlings for a restoration project. These are but a few examples of hundreds of projects underway with varying degrees of planning, attention to genetic considerations, and follow-up care and monitoring.

Genetic Considerations

Although engineers designing new projects probably know little about genetics, biologists certainly do, and they have been vocal about following guidelines to protect and enhance genetic integrity. Many biologists refer to genetic considerations in the published literature when undertaking any conservation activity. Indeed, it seems to be a uniting theme--as if it is not said enough, no one will hear. In the context of mitigation, this loud and repetitive voice is certainly appropriate. In the context of species recovery, *ex situ* conservation, restoration, and management, it is equally important. Ferreira and Hillyard (1978) report an open forum discussion of this topic at a conference on rare plants in California, in which much concern, both general and specific, came to light. Included were needs to define "local" population, the need to be very careful with the genetics of plant species, the need to know genotype and location of all material used in any vegetation enhancement, and the need to tighten up contracts to account for genetic credibility.

For seed banks, protocols are becoming firmly established. Brown and Briggs (1991) advocate collecting a high amount of genetic material for seed banks--material kept separate for each maternal parent plant, collected at different times and from different plants, and from varying numbers of populations. Advice from this and other sources have been compiled into guidelines for the Center for Plant Conservation (CPC, 1991), which is included in full as an appendix to this report.

Fenster and Dudash (1994) advocate the need to understand genetic consideration for any restoration project. They cite factors such as inbreeding and outbreeding depression, genetic diversity of stock material, for example. In the same book on restoration, Pavlik (1994) cites the need to monitor projects adequately. He considers monitoring "crucial" to the success, and points out that monitoring is a highly developed science involving statistical trends analysis--census data alone are not enough.

Although we will not go into detail about these considerations in this paper, we agree that genetic considerations are extremely important in any *ex situ* effort--or *in situ* effort for that matter.

Polarity or Seamless Whole?

Occasionally, negative reactions to off-site conservation activities have emerged, whether in private conversations, at conferences, and in the literature. We can summarize some commonly encountered arguments, and equally as common responses, as follows:

1. Off-site conservation efforts dilute the already small amount of money and effort available to conserve rare and endangered plants. The limited resources available ought to be devoted to habitat acquisition and management.

Hamilton (1994) offers a what at first glance appears to be a devastating condemnation of *ex situ* conservation using seed banks. His basic genetic argument is that the sampling designs advocated by the Center for Plant Conservation is fundamentally flawed because it is based on genetic information obtained solely from electrophoretic analyses of proteins. Hamilton says we need lots of other kinds of genetic information to be able to sample species intelligently. The kinds of studies he says are *necessary* (quantitative genetic studies that look into the interaction of genotype x environment interactions) have not even been done of very many species at all, and are incredibly time consuming and expensive. In response, we would counter that these data, while sometimes perhaps useful, are certainly not necessary to gather genetically representative samples.

The question of limited resources is always with us. Certainly, we must make priorities whenever possible and make sure that any overall conservation plan for a species or habitat
is appropriate for that site or species. In some ways, off-site conservation brings new resources by enlisting the aid of seed storage laboratories, botanic gardens, and the research community. For example, the money spent on our new seed vault, which came primarily from foundations and individuals, would not have otherwise gone to habitat conservation, but to another program at The Berry Botanic Garden.

Although it would be nice to have the sort of information advocated by Hamilton (1994), it is probably not necessary in most cases, and in any case, can't be obtained for rare species because of high sample sizes necessary. His argument loses most of its force because of some implicit assumptions he has about the role of *ex situ* conservation as it relates to *in situ*. He sees *ex situ* as being offered as an alternative to *in situ*, and that the two are competing for the same resources: any resources that go to *ex situ* are resources that would, otherwise, have gone to *in situ*.

2. Off-site conservation efforts lead to a false security, both for conservationists and developers. Developers may sometimes claim that a species or group of species are "saved" once in off-site storage, so their activities can go forward as planned. In this sense, off-site activities are not really conservation at all. Even conservationists can be lured into complacence if genetic material is stored off-site. Ultimately, the conservation value of off-site collections will be determined by how they have contributed to the perpetuation of the species in the wild.

Indeed, off-site conservation can lead to false security if it is not part of an integrated conservation plan. By itself, it does little more than conserve genetic material at best, an activity that has little significance without the context of its habitat. At worst, it can lure us into thinking that we have actually done something for conservation, only to later learn that we cannot germinate seeds in storage or that all suitable habitat has disappeared. The conservation strategy involving *ex situ* methods must be part of an overall plan and not act in a vacuum.

3. Maintenance of off-site collections are poor at best, and the genetic erosion such that they are not at all effective methods (see for example Elias, Thomas S., 1987).

Living collections and other off-site storage methods have their risks. As Elias (1978) points out, living collections are far from secure in cultivation. Natural mortality and difficulty in maintaining propagated stock make the maintenance of living collections highly challenging if not impossible in the long run. For shorter durations, they may be useful if the context is right. For example, The Berry Botanic Garden entered into an agreement to maintain clones of wild collected ***Penstemon barrettiae*** for eventual new plantings at Bonneville Dam. Even under these ideal circumstance and less than a 10 year time frame, some of the material was lost because of different survivorship in the garden and differing success in clonal propagation between different clones.

Even more secure methods such as seed storage and tissue culture have their risks, including mutations, mechanical failure, and natural disasters. All methods need to be approached with sound science and humility.

4. Off-site activities remove germplasm from the environment where it might lead to increased survival in the wild.

Removing plant material from the wild for off-site conservation activities does, at least minimally, diminish plant reproduction in the wild. Since most seeds never grow to reach reproductive maturity, seed collection is probably the least intrusive of off-site methods. Demographic modelling by Eric Menges (1992) has shown that activities like seed collecting probably have minimal impact on survival. "The threat posed to population survival by environmental variation appeared almost entirely due to variation in mortality, growth, and reproduction status and not to variation in reproductive output. In fact, the addition of reproductive variation to stochasticity in mortality and growth did not consistently increase or decrease extinction probability. Variation in reproductive output by itself was not sufficient to generate nonzero EP [extinction probability], except for extremely high levels of variation."

Seed collection for storage is a form of "environmental stochasticity" in reproductive output. Whether it is cold weather nipping buds, elk eating fruits, or folks collecting seeds for conservation, it is all environmentally induced variation in reproductive output. This is not to say that we should subject the same population to repeated collection.

The Role of Botanic Gardens

Botanic Gardens are relatively new partners for certain aspects of plants conservation. For many years, botanic gardens, particularly the larger gardens with research staff, have participated in research on plant taxonomy and distribution. Some botanic gardens may have worked on propagation of certain rare species when creating educational plant displays.

In the 1980's, botanic gardens in general began to become more interested in plant conservation, both in the United States and abroad. Before that time, the major role of botanic gardens was considered to be public educations through productions of educational materials and building outdoor plant displays.

By 1978, David Bramwell (Bramwell, 1978) was able to provide a report of five years of progress on how the Jardin Botanico in Gran Canara of the Canary Islands was carrying out a conservation program. This fine garden, which has continued its activities up to the present and has become a model for many other national programs, was already looking at establishing living collections of the island's endemic plants, studying pollination mechanisms, displaying plants for educational purposes, working with local educators, and various reintroduction projects. Since that time (personal communication), they have established a seed bank as well, and are participating in island-wide efforts at habitat restoration.

In 1985, the Center for Plant Conservation (CPC), a non-profit organization dedicated to using botanic garden resources to further plant conservation, formed to work out of Harvard University's Arnold Arboretum (Falk, 1987). In the program, botanic gardens were considered to have many roles, from scientific and taxonomic expertise, knowledge of germination and growth requirements, facilities for long-term maintenance, integration into academic and research institutions, and the high visibility important for public education. The Berry Botanic Garden became a charter participating institution of the Center for Plant Conservation in 1985, largely based upon the garden's Seed Bank for Rare and Endangered Plants of the Pacific Northwest, established in 1983 with a grant from the Meyer Memorial Trust. In 1990, the Center for Plant Conservation moved to the Missouri Botanical Garden in St. Louis.

Currently, the Center for Plant Conservation has a network of over 25 botanical gardens, including several in Hawaii, and is working cooperatively with many other countries to enhance other conservation programs. The Center has cooperative agreements with the USDA National Seed Storage Laboratory, the U.S. Bureau of Land Management, and is working on agreements with the U.S. Forest Service and other federal agencies.

At a conference sponsored by the Center for Plant Conservation in 1989 (CPC, 1991), and the discussions that followed that conference, guidelines were formed for collecting both for seed banks and for living collections. Some of these guidelines in general form are also available in a botanic gardens publication (McMahan and Guerrant, 1991). Steps to carry out a conservation program are outlined in this article as follows: (1) Choose the entity you with to conserve, and evaluate whether it is a realistic goal; (2) Collect adequate samples to fully represent the germplasm you wish to conserve, without harming native populations in the process; (3) Document the plant material thoroughly; (4) Determine and use appropriate methods to conserve genetic diversity; and (5) Once you have a collection, use appropriate methods to retain the genetic diversity you have amassed.

The role of botanic gardens does not stop with off-site germplasm storage, however. There is active work in research, such as that carried out at The Arnold Arboretum to try to establish disease-free tissue cultures of *Torreya taxifolia*, and many projects aimed at re-establishing wild populations (McMahan, 1990). Specific projects include The Berry Botanic Garden's role is reintroduction of *Stephanomeria malheurensis* (Parenti and Guerrant, 1990), reintroduction of *Styrax texana* with stock plants grown at the San Antonio Botanical Garden (Cox, 1990), and transplantation of *Penstemon barrettiae* by The Berry Botanic Garden (Guerrant, 1990).

A Working Model-- The New England Plant Conservation Program (NEPCOP)

Several years ago, the New England Wild Flower Society, which runs a native plant botanical garden known as Garden the Woods in Framingham, Massachusetts, began a new collaboration which may provide a model for similar activities in other parts of the country (New England Wild Flower Society, 1992). They formed a voluntary association of 65 different organizations termed the "New England Plant Conservation Program," called NEPCOP for short. The purpose of the program is develop integrated conservation for the survival of species and ecosystems in all New England states. Through a series of meetings and discussions, funded by several national and regional foundations, these organizations worked out mutually agreeable protocols for conservation strategies. Groups included botanic gardens, state and federal conservation programs, offices of The Nature Conservancy, The Center for Plant Conservation, and many other similar groups.

As part of this program, the Seed Bank at Garden in the Woods became the repository for storing off-site material of species that were part of the program.

Concerted groups and efforts such as this dispel the hostility that may exist among advocates for different conservation strategies by providing a forum for airing views and discussion. The group moves forward by consensus, with all parties cooperating in the joint efforts. An effort on this scale in the Pacific Northwest or for any subregion could provide similar results and provide an avenue to work towards new kinds of cooperation among all interested parties.

Applicability for the Columbia River Basin Project

Basin-wide planning for conservation is admirable, and like the New England experience, could prove useful in other contexts. Viewing off-site and activities as part of the whole conservation picture allows the step-wise development of priorities. Although we are not aware of all activities relating to off-site activities on plants within the basin, and could not have discovered them within the time-frame of this contract, we have contacted the two other botanic gardens in the Center for Plant Conservation network working within the region to learn of their off-site collections for the taxa on the list. These are summarized in Table 1. The botanic gardens referred to are The Berry Botanic Garden in Portland, Oregon, Red Butte Gardens and Arboretum in Salt Lake City, and the Denver Botanical Garden in Denver Colorado. Of the 161 taxa in the list, 63 are kept in off-site seed storage at these institutions.

Table 1. Number of accessions of rare plant taxa from the Columbia River Basin for which seeds or living collections are maintained at botanic gardens.

	Botanic Garden/No. of Accessions		
Taxon	Berry*	Denver	Red Butte
Allium aaseae	1		
Amsinckia carinata	5		
Antennaria arcuata	1		
Arabis fecunda		1#	
Artemisia campestris var. wormskioldii	4		
Astragalus applegatei	4		
Astragalus diaphanus var. diurnis	1		
Astragalus mulfordiae	10		
Astragalus peckii	2		
Astragalus sinuatus	6		
Astragalus solitarius	3		
Astragalus sterilis	3		
Astragalus tegetarioides	3		
Astragalus tyghensis	3		
Calochortus longebarbatus var. longebarbatus	3		
Castilleja chlorotica	3		
Castilleja christii		1#	
Chaenactis cusickii	1		
Colloma mazama	2		

Cypripedium fasciculatum	1	
Delphinium viridescens	4	
Erigeron basalticus	1	
Eriogonum argophyllum		1
Eriogonum crosbyae	3	
Eriogonum cusickii	4	
Eriogonum prociduum	5	
Hackelia cronquistii	12	
Hackelia venusta	22	
Haplopappus radiatus	10	
Howellia aquatilis	living plants	
Ivesia rhypara var. rhypara	21	
Lepidium davisii	5	
Limnanthes floccosa ssp. bellingeriana	2	
Lomatium erythrocarpum	1	
Lomatium suksdorfii	5	
Luina serpentina	4	
Lupinus biddlei	8	
Mentzelia mollis	5	
Menzelia packardiae	8	
Mimulus hymenophyllus	1	
Mimulus jungermannioides	3	
Mimulus pygmaeus	1	
Mirabilis macfarlanei	39	
Penstemon barrettiae	33	
Penstemon peckii	201	
Perideridia erythrorhiza	7	
Phacelia lenta	3	
Pleuropogon oregonus	4	
Polemonium pectinatum	3	
Primula nevadensis	1	
Ranunculus reconditus	4	
Rorippa columbiae	1	
Senecio ertterae	9	
Sidalcea oregana var. calva	3	
Silene seelyi	4	
Silene spaldingii	23	1
Stephanomeria malheurensis	127	
Tauschia hooveri	1	
Thelypodium eucosmum	2	

Thelypodium howellii ssp. spectabilis	2
Trifolium leibergii	2
Trifolium owyheense	5
Trifolium thompsonii	2

* The number of accessions can indicate many things. In earlier years, some accessions from different plants in a population were accessioned together. Later, each plant from a population received a separate accession number.

Accession is split between the garden and the National Seed Storage Laboratory. May be more than one accession.

This summary information provides a rough estimate at best of off-site activities. Accessions may or not be adequate to represent the genetics of the wild populations. Germination testing has most likely been completed for only a few of the taxa. Although the number of taxa in off-site seed storage is surprisingly high, the "quality" of these collections has not been assessed for most and should not provide a sense of security that these taxa are well-represented off-site.

It would be interesting and desirable to provide a more in-depth assessment of these collections. Appropriate areas of inquiry would be how the off-site collections compare to natural population and their genetic representation, which additional species might be added to off-site storage and in which priority, which species provide challenges for germination and growth, what outstanding taxonomic questions might lead to better conservation action, and what kinds of research are being carried out in general for life history parameters or restoration/enhancement. We know for example that re-establishment projects are underway for ***Penstemon barrettiae*** (Guerrant, 1990) and ***Stephanomeria malheurensis*** (Parenti and Guerrant, 1990) because we are involved directly, however, others may exist that we could learn of with time to inquire. Research on germination and growth of ***Hackelia venusta*** is being undertaken by the Cincinnati Zoo and Botanical Garden with seeds to be provided by the Berry Garden. Likewise, the Berry Garden is undertaking a long-term soil seed bank study of ***Penstemon peckii***.

General Recommendations for Off-Site Germplasm Collections

1. Off-site methods should be considered as part of the overall conservation strategy for rare species or habitats. Careful analysis and discussion will determine if any of these activities are appropriate, helpful, superfluous, or possibly counterproductive. At the very least, plans should incorporate long-term seed storage, assuming the seeds are amenable to storage. Funding such activities in advance is like buying pork belly futures. A small investment now could save your bacon later.

2. Preferences for off-site storage should be in the following order:

a. Seed storage using CPC collecting guidelines and germination and growth studies to ensure that plants can be regenerated from stored seeds.

b. Seed storage without the full complement above.

c. Living collections maintained asexually, or living collections maintained as tissue cultures or grafts.

d. Living collections in artificial sites close to natural habitat (e.g. degraded but appropriate site; experimental plots).

e. Other off-site storage (if no other alternative exists and all other strategies will virtually eliminate the species)

We would like to reiterate here the value of looking at the species itself to determine the *ex situ* strategy, which will sometimes affect the order of the above activities. *Stephanomeria malheurensis*, for example, appears to be extremely uniform genetically. The germination and growth regime is well worked out. In this case, seeds of plants grown in cultivation are probably just as "good" as wild-collected seed, and their "captive" production and storage is a good conservation alternative. *Fritillaria gentneri* produces sterile seeds, so seed storage is not possible. Here, we might consider trying to maintain living collections or tissue culture. *Corydalis aquaegelidae* and *Howellia aquatilis* are both aquatic species and cannot be stored using conventional methods. For these, other habitat based strategies are preferable, although captive "populations" of *Howellia* are a possibility, as well as new techniques in embryo storage.

3. Set *ex situ* activities with particular goals in mind. Examples are as follows:

a. Seeds of species "a," for which germination and growth requirements are already known, are collected under CPC guidelines and stored against future loss of a species in specific habitats. This is the "insurance policy" approach.

b. Seeds of species "b" are obtained for storage under CPC guidelines, with the addition of research to establishment of germination and growth requirements. This is basically the insurance policy as well.

c. Research to determine some biological characteristics of species "c," such as amount of genetic variability among populations and individuals within a population, followed by collecting and storage of seeds following the CPC guidelines. This is sort of an "enhanced" insurance policy, making sure the collecting reflects the best known science.

d. Research to establish the genetic identity (e.g. species status) of species "d," followed by seed, plant, or tissue storage as appropriate. The research is completed first, unless species is immediately threatened, in case the species "d" turns out to not be a species at all. Such research could be genetic, as was carried out at The Berry Botanic Garden to determine the probable species status of *Fritillaria gentneri*, using enzyme electrophoresis. One "common garden experiment," where plants from difference habitats are grown together in one place, in this instance the New England Wild Flower Society, showed that two reported subspecies were in fact environmentally induced variations and not genetic (Bill Brumback, personal communication). Research at the Desert Botanical Garden on *Agave arizonica* showed this endangered species to be a probable hybrid, or at the very least of recent hybrid origin (Wendy Clark, personal communication). Knowing this information leads to better resource use by not "wasting" conservation funds and effort on plants that prove not to have individual species, subspecies,or varietal status.

e. In the event of the imminent destruction of a particular population, strategies might include collecting all seeds, transplanting plants to maintain as living collections, cross pollinating as appropriate to obtain seeds. The Desert Botanical Garden has become the repository for wild plants of several cactus species. However, not all plants can successfully be transplanted. Carol Dawson at the Denver Botanical Garden is currently working on *Astragalus osterhoutii*. It has a long tap root, which, given the rocky 'soil' it inhabits, cannot easily be extracted. Nevertheless, because the species is federally listed and many plants will soon be lost to a new reservoir, she was told to transplant the ones that will be inundated. Perhaps the only information they will gather is that successful transplantation of established plants cannot be accomplished for this species.

4. Contracts for seed storage should specify conditions, e.g. those specified by CPC guidelines. Seeds from each plant should be maintained separately, and adequate records maintained. The contracts should also specify other conditions, such as collection of certain populations on federal land, and include the amount of time the seeds are to be stored. If other activities are required, such as photographing sites or providing research

on germination, including standard germination trials, this should be included specifically and in detail.

5. Alternative approaches to seed storage should be considered for some aquatic species (e.g. *Corydalis aquagelidae*), plants that do not produce fertile seeds (e.g. *Calochortus longebarbatus* var. *peckii* and *Fritillaria gentneri*), or plants with seeds difficult to germinate (e.g. *Aster vialis*). In some cases, efforts at off-site storage of these species should be abandoned altogether, or if desirable in any case, altered to provide the best possible results. Using this approach for species difficult to maintain off-site will maximize the efficient use of monetary resources and effort.

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