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# Southwestern Rare and Endangered Plants:

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# From the Greenhouse to the Field: Cultivation Requirements of Arizona Cliffrose (*Purshia subintegra*)

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**Abstract:** Central to the conservation of the federally endangered *Purshia subintegra* (Arizona cliffrose) is development of an understanding of its cultivation requirements. This knowledge will enable us to augment declining or threatened populations as well as preserve genotypes impacted by human activities. We studied seed and stem cutting propagation of *P. subintegra*. The highest success rate for rooted stem cuttings of *P. subintegra* that were collected wild was 34 percent. The stem cuttings of *P. subintegra* survived best when they were taken in the fall, rooted in perlite with Hormex 8, and watered once daily. Stem cuttings can take from 1 to 11 months to root depending on treatment and time of cutting. Propagation from seed was much more successful (88%) when we used fresh seed sown in perlite with cold stratification for 4 weeks. Seeds did not require native soil to germinate, nor did seedlings require native soil to survive. This means that *P. subintegra* can propagate and survive under conditions far different than it experiences in the field. Because road construction is destroying *P. subintegra* and its habitat, transplanting propagules to unoccupied habitat may prove to be an important avenue for conserving genotypes. Following our successful propagation trials, we introduced *P. subintegra* into its natural habitat in the Verde Valley in February 2000. We tested whether irrigating plants for 5 months increased survival and found that supplemental water was important to the survival of transplanted plants. For rare plants, we need a much greater knowledge base on how to grow and transplant them. This process is time consuming, but will be necessary as more and more species that are impacted by human disturbance must be conserved ex situ.

Both in situ and ex situ conservation can be important components of rare plant conservation (Frankel et al. 1995). Although plant conservation in the wild is most important for maintaining a population, ex situ populations can serve many purposes. Knowledge gained from ex situ cultivation will enable us to augment declining or threatened populations, to restore or transplant individuals impacted by disturbance, to conduct experiments on ecological requirements of the species, and to germinate seeds from in-house or long-term storage.

Several propagation methods are available to grow plants in a greenhouse setting. Ex situ seed banks are standard practice to conserve the genetic diversity of a species and safeguard it against the demise of the wild population. Germination studies are needed to determine seed viability and proper propagation techniques to successfully germinate and establish seedlings so that ex situ seed banks can be used if necessary for species recovery. Rooting stem cuttings is another propagation technique that can be used to preserve genotypes.

The federally endangered shrub *Purshia subintegra* (Kearney) Henrickson is threatened by

rapid development of its habitat in the Verde Valley. Road expansion, new road construction, and land sales will eliminate about 600 individuals; therefore transplanting individuals may be an important mitigation solution. The recovery plan clearly states that restoration or transplantation should be considered a mitigation solution only if successful propagation techniques to grow and transplant *P. subintegra* are determined (U.S. Fish and Wildlife Service 1994). The ability to grow *P. subintegra* from seed or stem cuttings may provide an avenue to conserve genotypes as impacts and disturbances to its natural habitat continue to occur.

*Purshia subintegra* is a rare soil endemic. It is typically restricted to nutrient-poor, lacustrine limestone deposits (Anderson 1986, 1993). Four populations are known from central Arizona. We are interested in the largest population in the Verde Valley outside of Cottonwood. In the Verde Valley, the distribution of *P. subintegra* overlaps with the distribution of *P. stansburiana*. Plants that have characteristics of both parents have been observed, leading to some taxonomic questions about *P. subintegra* (see U.S. Fish and Wildlife Service 1994 for discussion). Using the descriptions

and definitions from the Arizona Cliffrose Recovery Plan, we refer to plants outside the definition of *P. subintegra* as the introgressed form.

Our two goals were to determine the best method to propagate *P. subintegra* from both seeds and stem cuttings, and to determine the feasibility of transplanting or relocating propagules. Maintaining plants *ex situ* in a greenhouse is not a feasible long-term solution with these large evergreen shrubs. Our intent was therefore to learn to grow *P. subintegra* for preservation and reintroduction of threatened genotypes.

## Past Propagation Trials

### Seeds

Previous seed germination studies reported little or no success with propagation of *P. subintegra*. We therefore examined germination studies conducted with the common cliffrose, *Purshia stansburiana* (Torr.) Henrickson. *Purshia stansburiana* requires cold-moist stratification to break dormancy, but germination success is related to length of stratification and temperature (Alexander et al. 1974, Young and Young 1986). A germination success rate of 55 percent occurred after 2 weeks of stratification at 5° C with incubation temperatures (night/day) at 10/20, 10/25, 10/30, and 15/25° C (Young and Young 1992). Even better germination success (88%) occurred when seeds were incubated at 21° C with light, and germination success was 76 percent with 3 months of cold stratification (Deno 1993). Seeds have remained viable for up to 7 years in dry storage (Young and Young 1986). These studies of *P. subintegra* and *P. stansburiana* suggested that we should examine seed for viability before germination trials and implement a cold-moist stratification treatment.

The germination of *P. subintegra* has critical implications for its survival in the Verde Valley. First, we need to understand the methods of seed propagation for *P. subintegra* in the event that plants must be grown from seed that had been stored *ex situ* as a method to conserve genotypes. In addition, knowledge about its germination requirements will help us understand the biology of *P. subintegra*. In the Verde Valley, *P. subintegra* occurs in a restricted habitat that can also be occupied by the introgressed form. The germination requirements of *P. subintegra*, *P. stansburiana*, and the introgressed form may help explain the distribution of each taxa as well as competition in the natural habitat.

### Stem Cuttings

Previous studies on the cultivation of *P. subintegra* from stem cuttings had variable and low success (Asplund 1989). Results from a trial to root stem cuttings from 1986 to 1989 found that cuttings collected in summer (June-July) had a higher rooting rate than cuttings collected during other seasons. Hormex 3 and Hormex 8 treatments had a higher rate of rooting than Dip 'N Grow or Wood's Rooting Compound. Intermittent mist also increased the success rate. After the cuttings rooted, they were transplanted to pots using standard potting mix. None survived transplanting (Asplund 1989).

The Arboretum at Flagstaff tried to root *P. subintegra* cuttings in 1990 and 1991 with varying success. In 1990, 51 percent of the stem cuttings rooted that were collected in October. All of the rooted cuttings were planted in potting mix, but none survived more than 4 months (Maschinski 1990). In 1991, only 3 percent of the stem cuttings collected in December rooted. However, the plants were potted in native soil and two large plants still survive to date.

In June through September 1996, the Arboretum at Flagstaff collected stem cuttings of *P. subintegra* and the introgressed form. Rooting trials in germination mix (8 perlite: 1 vermiculite: 1 peat) with Hormex 3 had 35 and 53 percent rooting success for *P. subintegra* and the introgressed form. In comparison, perlite and Hormex 8 had a success rate of 18 percent with *P. subintegra*, whereas the introgressed form success was only 8 percent.

In general, time of year, site of cutting, nutrient status, rooting hormone, amount of light, and juvenility have been shown to affect rooting success (Macdonald 1986, Dirr and Heuser 1987). We examined the influence of woodiness of stem, time of collection, propagation technique, and rooting hormone on stem cuttings of *P. subintegra*. In addition, we determined how to keep seedlings and rooted cuttings alive after they were transplanted to pots.

## Materials and Methods

### Seeds

Using the cold stratification period recommended for *P. stansburiana*, we investigated the effects of the propagation media on germination and compared the rate of germination of *P. subintegra*, *P. stansburiana*, and the introgressed form. We used three propagation media: perlite, germination mix (8 perlite: 1 vermiculite: 1 peat), and

native soil mix (2 soil: 1 perlite: 1 sand). We collected native soil in the Verde Valley in an area where *P. subintegra* grows and passed the soil through a sieve to remove large rocks. We planted each taxon in each of the three propagation media.

We sorted seeds visually for viability with about 40 percent of the collected seeds appearing viable. In January 1998, we planted five seeds in each individual pot. Only five seeds could be used due to the limitations of working with an endangered species. We planted 30 pots of each species divided equally among the three propagation media. The seeds then received a cold-moist treatment at 5° C for 4 weeks in dark plastic bags. We placed the pots in the passive solar greenhouse at the Arboretum at Flagstaff and watered by mist. Due to differences in the propagation media, we misted seeds in perlite and germination mix for 16 seconds every 64 minutes during the day, and we misted seeds in the native soil mix once daily. The greenhouse had daily fluctuations from 10 to 35° C during this period. We monitored seedlings every 3 to 4 days to measure germination and survival. Germination began 4 days after the pots were placed in the greenhouse and continued for 24 days. We counted a seed as germinated when the hypocotyl elongated.

We analyzed the percentage of seed germination (arcsin transformed) with a two-factor analysis of variance with all assumptions met (SYSTAT 6.0 – GLM). We calculated the percentage of seeds germinating as the total number of seeds that germinated in a pot over the 24-day period divided by the total number of seeds per pot. The two factors were species and propagation media. Not all seedlings survived the 24 days and we recorded mortality for the seeds that germinated.

#### Stem Cuttings

Because previous propagation trials with cuttings had low success, we examined whether the condition of plants, the age of stem cuttings (woodiness), different propagation techniques, or time of year influenced rooting success. Although we experimented with many propagation techniques, we used some standard procedures in all the experiments. The factors that vary from the standard procedure are propagation media, rooting hormone, watering treatment, and bottom heat (Table 1). They are described in detail in the following sections. Stem cuttings were approximately 10 cm long and were stripped of the lower half of their leaves. We were careful not to damage the stem when removing leaves. Before inserting the

ends of stem cuttings, we soaked them in 10 percent bleach solution for 10 minutes and then applied a small amount of rooting hormone to the cut end. We maintained the cutting flats in the passive solar greenhouse at the Arboretum at Flagstaff. We also needed to determine how much time a cutting would need to root; we therefore monitored the stem cuttings for up to 1 year as long as the cuttings maintained green leaves. After cuttings had developed roots, we marked the date and transplanted the cuttings to pots to be used in later greenhouse experiments.

#### Origin of Cuttings

To determine whether the origin of cuttings influenced rooting success, we collected stem cuttings from both wild plants and greenhouse-grown plants (Table 1). We took wild stem cuttings from both the rare *P. subintegra* and the introgressed form from September 1996 to November 1996. During the same period, we took cuttings from two large *P. subintegra* plants that survived 1996 propagation trials and that are maintained in the greenhouse at the Arboretum at Flagstaff. These plants are well watered, healthy, and robust. We treated all the stem cuttings with our standard procedures as described. We applied Hormex 8 to the cuttings before placing them in perlite.

#### Woodiness

We used greenhouse-grown plants as material for cuttings in order to classify cuttings based on amount of lignification or woodiness. New tissue, less than a month old, is pliable, whereas older tissue quickly becomes woody. We took cuttings ( $n = 126$ ) from greenhouse-grown *P. subintegra* and scored them for woodiness in September of 1996. We classified cuttings in four categories: new, where terminal growth was green and pliable; early, where stems had begun to lignify but were still pliable; 1 year, where the stem was completely woody and 1 year old; and old, where stems had woody growth greater than 1 year in age. We applied Hormex 8 to the cuttings before placing them in perlite (Table 1). We analyzed the proportion of rooted cuttings for each category using Pearson chi-square analysis.

#### Propagation Techniques

Previous trials of propagating stem cuttings suggested that propagation techniques could affect the success of rooting cuttings. The main difficulty was getting cuttings to root before they rotted. We performed three trials that manipulated

Table 1. Details of each propagation trial with stem cuttings.

Trial	Date	Taxa	Origin	Propagation medium	Rooting hormone	Watering treatment	Bottom heat	Other
Origin of cutting	Sept.-Oct. 1996	<i>P. subintegra</i>	wild and greenhouse	perlite	Hormex 8	once daily	no	
Woodiness	Sept. 1996	<i>P. subintegra</i>	greenhouse	perlite	Hormex 8	once daily	no	age of stem
Propagation techniques	1.	Introgressed	wild form	germination	Hormex 8 mix	once daily	no	
				perlite	Hormex 8	once daily	no	
				perlite	Hormex 8	once daily	yes	
				perlite	Hormex 8	mist every 32 min	no	
	2.	Introgressed form	wild	perlite	Rootone	once daily	yes	
				perlite	Hormex 3	once daily	yes	
				perlite	Hormex 8	once daily	yes	
				germination mix	Rootone	once daily	yes	
	3.	<i>P. subintegra</i>	wild	germination mix	Hormex 3	once daily	yes	
				perlite	Hormex 3	once daily	no	
				perlite	Hormex 8	once daily	no	
Time of collection	variable	<i>P. subintegra</i>	wild	perlite	Hormex 8	once daily	as needed	
		Introgressed form	wild	perlite	Hormex 8	once daily	as needed	

propagation medium, rooting hormone, and watering treatment (Table 1). We used three rooting hormones: Rootone (Black Leaf Products, Louisville, KY 40222), Hormex 3, and Hormex 8 (Brooker Chemical Corporation, P.O. Box 9335, N. Hollywood, CA 91609). We also used two propagation media to root cuttings: perlite and germination mix (8 perlite: 1 vermiculite: 1 peat).

In the first trial we examined the differences of propagation media and watering treatment. In October of 1996 we collected cuttings of the wild introgressed form of *Purshia* ( $n = 191$ ). We compared four treatments: germination mix watered once daily, perlite watered once daily, perlite watered once daily with bottom heat of 20° C, and perlite misted at 32 minute intervals for 16 seconds for 3 months. Because of low temperatures in the greenhouse, we placed all of the cutting flats on heating mats during week 13. We analyzed the proportion of rooted cuttings after 1 year for each treatment using Pearson chi-square analysis.

In the second trial we compared propagation media and rooting hormone. In March of 1997 we

collected stem cuttings from the introgressed form ( $n = 171$ ). We used two rooting media: perlite and germination mix. In perlite, we compared the three rooting hormones, Rootone, Hormex 3, and Hormex 8. In germination mix, we compared rooting hormones Rootone and Hormex 3. We placed all cuttings on heating mats at 20° C and watered once daily. We did not perform a statistical analysis because most treatments had no success.

In the third trial we examined the effect of the rooting hormones Hormex 3 ( $n = 705$ ) and Hormex 8 ( $n = 699$ ) in perlite on stem cuttings of *P. subintegra* we collected in September of 1997 from the Verde Valley. We placed the stem cuttings in perlite and watered once daily. We analyzed the proportion of rooted cuttings for each rooting hormone using Pearson chi-square analysis.

#### Timing of Wild Collection

Previous results indicated that the season in which wild cuttings are taken is important; we took cuttings systematically over the year to determine the optimal time to take cuttings for the

greatest success. We collected stem cuttings of *P. subintegra* in fall 1996 (October and November), winter 1997 (March), spring 1997 (May), and fall 1997 (September) and the introgressed form in summer 1996 (June), fall 1996 (October and November), winter 1997 (February and March), and spring 1997 (May). We treated the stem cuttings using our standard procedures as described previously. We placed the cuttings in perlite after treating them with Hormex 8 (Table 1). We watered the cutting flats once daily and placed them on bottom heat as the low temperatures in the greenhouse required for 12 months. Using Pearson chi-square analysis, we compared rooting success in the four seasons for each taxon.

#### Greenhouse Plants

Little is known about the cultural requirements of soil endemics. Previous trials suggested that *P. subintegra* required native soil even in a greenhouse situation; however, native limestone soil is difficult to use in pots because it resembles concrete after a few weeks. We therefore tested the success of growing *P. subintegra* in different pot sizes and media. The container sizes tested were 3.25 inch square pots (volume 500 ml), 10 inch seedling tubes (volume 650 ml), and 1 gallon pots (volume 3.0 L). We also tested three types of potting media: standard potting mix (8 Supersoil, Rod McLellan Co., 914 S. Claremont St., San Mateo, CA 94402, 4 perlite: 1 sand), native soil mix (2 limestone soil: 1 sand: 1 perlite), steam-sterilized native soil mix (2 limestone soil: 1 sand: 1 perlite), and a mix of equal parts of the native soil mix and our standard potting mix. When stem cuttings had rooted or seedlings were large enough, we transplanted them to pots. In 1 gallon pots, we usually planted three stem cuttings per pot. We calculated survival after 6 months from the time of transplant. We maintained potted plants in the passive solar greenhouse and research greenhouse at the Arboretum at Flagstaff.

#### Reintroduction Trial

One of our main goals was to reintroduce plants to the wild as a conservation measure for the species, so we relocated plants generated from cuttings or seeds into the Verde Valley to determine the optimal method for transplanting success. Initially, we were interested in the amount of care necessary to ensure survival of the plants. We therefore chose to water half the plants for 5 months in this initial trial and the other half for 1 month.

We selected 98 plants propagated from seeds and cuttings of *P. subintegra* from the Verde Valley to use for the reintroduction. All of the plants had been established in the greenhouse for approximately a year in 1 gallon pots in standard potting mix. On February 25, 2000, we transplanted them into the Verde Valley in wash bottoms and the sides of washes where *P. subintegra* grows naturally. We thought the sides of the large washes would give the plants the best chance of survival due to higher soil moisture. We dug holes 25 cm deep x 25 cm wide and wetted the hole with approximately 2 gallons of water before planting. After transplanting, we gave the plants additional water until saturation. We installed a drip irrigation system to deliver approximately 1 gallon of water per week to 49 of the plants for 5 months. The remaining 49 plants received water for 3 weeks by hand and then received no additional water.

We monitored survivorship from February to August 2000. We will continue to monitor monthly for a year and then seasonally for the next year. We analyzed survivorship of each treatment with a Pearson chi-square test at 6 months.

## Results

#### Seeds

*Purshia subintegra*, *P. stansburiana*, and the introgressed form germinated after 4 days in each propagation medium. Germination varied between species and media treatment (Table 2). All taxa had their highest germination in perlite and lowest in the native soil mix (Figure 1). The introgressed form had the lowest germination rate in all of the propagation media, whereas *P. subintegra* and *P. stansburiana* had similar germination rates.

All three taxa had the highest mortality in native soil (Table 3). In perlite and germination mix, mortality was less than 10 percent, but in native

Table 2. Analysis of variance on the proportion of the total number of seed germinated (arcsin transformed) for species (*Purshia stansburiana*, *P. subintegra*, and introgressed form) and propagation media (perlite, germination mix, and native soil mix).

Source of variation	DF	MS	F-value	p-value
Species	2	0.89	3.70	0.0291
Propagation media	2	2.81	11.72	<0.0001
Species * propagation media	4	0.21	0.89	0.4759
Error	81	0.24		

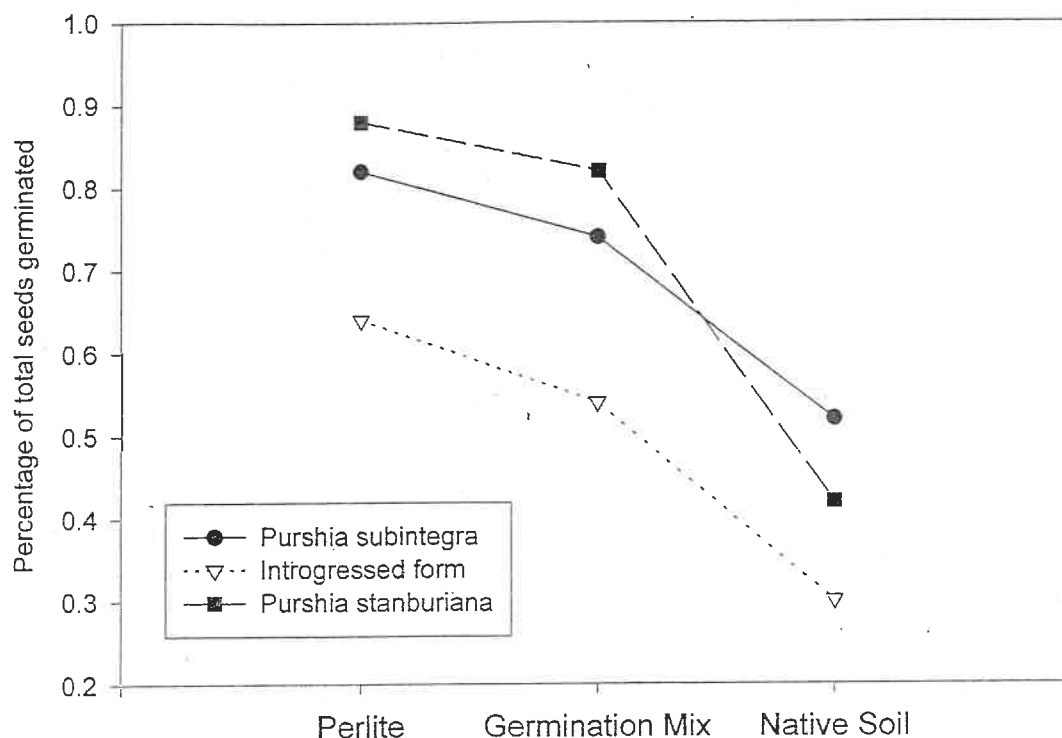


Figure 1. Percentage of total seeds germinating in each propagation medium for the species *Purshia subintegra*, *P. stansburiana*, and the introgressed form.

soil mortality ranged from 44 to 68 percent. Mortality was due to several causes. Many seedlings began to germinate but never developed enough to shed the seed case from the cotyledons. Other seedlings wilted or damped off at the surface layer.

#### Stem Cuttings

##### Origin of Cutting

The success rate varied as a function of the origin of the cutting (Table 4). Wild cuttings taken in September to November 1996 had an overall success of 34 percent for *P. subintegra* and 19 percent for the introgressed form. Stem cutting success from greenhouse-grown *P. subintegra* plants was much greater at 81 percent. Wild-collected cuttings took 8 to 31 weeks to root, whereas cuttings from greenhouse-grown plants took 8 to 25 weeks.

##### Woodiness

The woodiness of stems did not significantly influence rooting success (Table 4,  $\chi^2 = 3.347$ ,  $df = 3$ ,  $p = 0.34$ ). The oldest woody cuttings had lower

rooting success than the other classifications. The trend is similar to what is observed with wild-collected cuttings. Stem cuttings that rooted most successfully were from the early growth (where stems had begun to lignify but were still pliable). All cuttings rooted in 8 to 24 weeks with no difference between categories in the length of time needed to root.

##### Propagation Techniques

In the first trial, propagation techniques significantly affected the proportion of stem cuttings rooted (Table 5,  $\chi^2 = 58.6$ ,  $df = 3$ ,  $p < 0.0001$ ). Cuttings with bottom heat were most successful at 60 percent after 13 weeks, whereas cuttings in perlite

Table 3. Mortality of seedlings for each species and each propagation media.

	Perlite	Germination mix	Native soil mix
<i>P. stansburiana</i>	2%	6%	44%
<i>P. subintegra</i>	5%	2%	61%
<i>Purshia</i> hybrid	10%	0%	68%

Table 4. Results of propagation trials with stem cuttings examining the origin and woodiness of cutting. Values are listed as percentage of stem cuttings that rooted.

	<i>Purshia subintegra</i>	Introgressed form
Origin of cutting (Sept.-Oct. 1996)		
Wild collected	34%	19%
Greenhouse grown	81%	
Woodiness (Sept. 1996)		
Terminal new growth	76%	
Branch still pliable	87%	
Woody about 1 year old	70%	
Woody > 1 year old	68%	

that were watered daily rooted between weeks 4 and 12, with only 14 percent success. In February (week 13), we placed all cuttings on heating mats due to low temperatures in the greenhouse. It was only after placement on the heating mats that the cuttings in the misting treatment began to root, increasing the success rate to 43 percent between weeks 17 and 39. Cuttings in germination mix also developed roots from week 16 to week 24, achieving a success rate of 14 percent after we placed cuttings on the heating mats.

The March 1997 (second) trial had very low success rates with no difference between treatments (Table 5), and cuttings of *P. subintegra* and

*P. stansburiana* during this period were unsuccessful. Cuttings with the germination mix and Rootone treatment were most successful at 12 percent, but they also took a longer time to root, from week 16 to week 41.

In the third trial, to examine the effect of the rooting hormone on *P. subintegra*, we compared rooting success with Hormex 3 and Hormex 8 (Table 5, September 1997). Cuttings treated with Hormex 8 had a success rate of 27 percent compared to 16 percent for cuttings treated with Hormex 3 ( $\chi^2 = 24.97$ ,  $df = 1$ ,  $p < 0.0001$ ). Most cuttings rooted within 11 to 31 weeks, with a few rooting as late as week 43.

#### Timing of Wild Collection

To determine whether the season the cuttings were taken in was important for their rooting success, we collected stem cuttings of *P. subintegra* and the introgressed form during different seasons (Table 6). Using Pearson chi-square analysis, we compared rooting success in the four seasons. Cuttings had significantly greater rooting success in the fall (*P. subintegra*:  $\chi^2 = 30.40$ ,  $df = 3$ ,  $p < 0.0001$ ; introgressed form:  $\chi^2 = 41.61$ ,  $df = 3$ ,  $p < 0.0001$ ). The success rate of cuttings for *P. subintegra* was similar in fall 1996 and fall 1997 even though the years were very different; 1996 was a drought year and 1997 had adequate rain in both winter and spring.

Table 5. Results of trials of different propagation techniques with stem cuttings. Values are listed as percentage of stem cuttings that rooted.

Propagation media	Rooting hormone	Watering treatment	Bottom heat	<i>Purshia subintegra</i>	Introgressed form
October 1996					
Perlite		Daily	no		14%
		Daily	yes		60%
		Misted	yes		0% (43%)
Germination mix	Hormex 8	Daily	yes		0% (14%)
March 1997					
Perlite	Rootone	Daily	yes		0%
	Hormex 3				4%
	Hormex 8				7%
Germination mix	Rootone				12%
	Hormex 3				0%
September 1997					
Perlite	Hormex 3	Daily		16%	
	Hormex 8			27%	



Table 6. Results of propagation trials examining the timing of wild collection of stem cuttings. Values are listed as percentage of stem cuttings that rooted.

Time of wild collection	<i>Purshia subintegra</i>	Introgressed form
Summer 1996		8%
Fall 1996	34%	39%
Winter 1997	23%	7%
Spring 1997	7%	18%
Fall 1997	27%	

### Greenhouse Plants

The survival of rooted cuttings and seedlings varied with pot size and media used after transplanting. Transplants were most successful in native soil mixes that included additional sand and perlite (Table 7). Rooted cuttings actually survived the best in sterilized native soil mixes but grew very slowly. The plants grew fastest in potting mix, but had higher mortality. Seedlings transplanted to 3.25 inch pots survived best in native soil mix compared to our standard potting mix.

When plants were large enough, we transplanted them into 1 gallon pots. Survival was similar to the previous results, with the greatest survival in native soil mixes (93%) and lowest survival (73%) in the standard potting mix.

### Reintroduction Trial

Irrigation was important to the survival of transplanted plants. After 1 month only 2 percent of the transplants died regardless of treatment (Figure 2). After 3 months the transplants that were not watered had a much higher rate of mortality until the fifth month. The watered group had significantly greater survival after 6 months ( $\chi^2 = 27.88$ ,  $df = 1$ ,  $p < 0.001$ ). After the irrigation was

Table 7. The mortality rate of transplanted rooted cuttings and seedlings of *Purshia subintegra* in different pot sizes and potting media.

Pot type	Native soil mix	Potting mix	Sterile native soil mix	Half and half*
Rooted Cuttings				
3.25 inch pots	85%	72%	90%	
Seedling tubes	56%	78%		
Gallon pots	78%	60%		70%
Seedlings				
3.25 inch pots	94%	67%		

\*Half native soil: half potting mix.

discontinued from the watered group, five plants died in both groups between the fifth and sixth months. In this experiment we saw mortality due only to transplanting and environmental stress and not herbivore or pest damage.

## Discussion

### Seeds

Germination of *P. subintegra* seeds requires the use of fresh good seed in perlite with a cold stratification treatment for 4 weeks. Inviabile seed of *Purshia* is flat in appearance and easily detectable; viable seed appears plump and is larger than inviable seed. We performed the experiments with seed that had been stored for 6 months under cool and dry conditions. Tests will need to be performed to determine the length of time that *P. subintegra* seed can be stored. Cold moist stratification of seed is a common requirement for many native plants. We suggest using perlite as the rooting medium because seedling growth was usually faster in perlite than in germination mix.

Neither *P. subintegra*, *P. stansburiana*, nor the introgressed form required native soil to germinate in the greenhouse. In fact, seedlings sown in native soil had the highest mortality in comparison to seeds grown in perlite or germination mix. This contrasts with the fact that the best survival for rooted cuttings was in native soil. The difference can be attributed to the difficulty encountered in using native soil to germinate seeds. Regulating water to keep the soil moist without rotting the seedlings was difficult.

Several factors may explain the increased mortality of seedlings in native soil. Conditions differ for plants grown in pots and greenhouses than in the field. The greenhouse for this experiment had a high humidity level, usually greater 70 percent, and can experience large daily fluctuations in temperature (up to 40° F). We initially over-watered the seedlings in native soil and many seedlings died. When we reduced the amount of water, the survival of seedlings improved. Because of the pot effect, seedlings in native soil experienced more extremes with the drying and wetting of the soil.

### Stem Cuttings

Successful propagation of *P. subintegra*, *P. stansburiana*, and the introgressed form requires proper timing, media, hormones, heat, and time. The main challenge was to encourage stem cuttings to root before they died or rotted. The wild-collected stem cuttings of both *P. subintegra* and

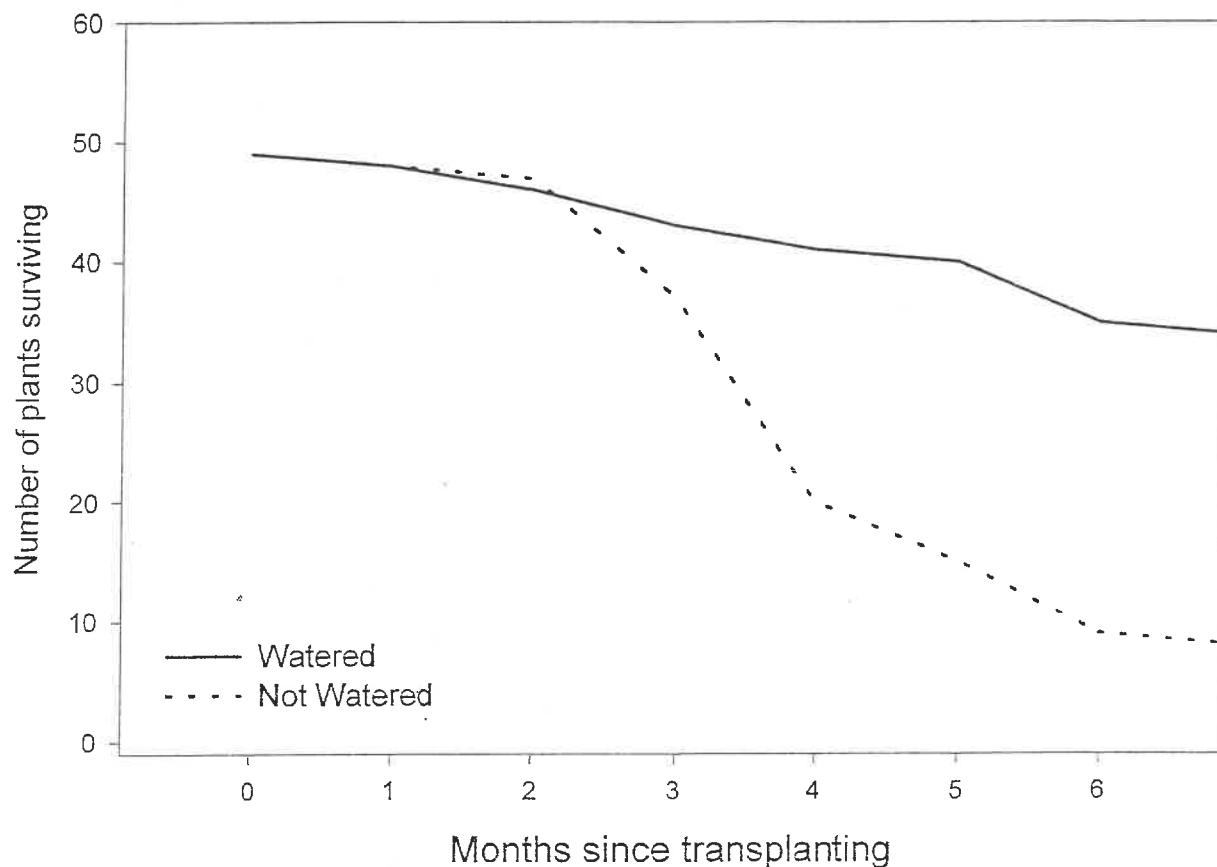


Figure 2. Number of plants that have survived since transplanting on February 25, 2000. All plants were watered for 3 weeks; 49 were watered for a total of 5 months.

the introgressed form survived best when they were taken in the fall, rooted in perlite with Hormex 8, and placed on heating mats. These techniques also produced roots in the least amount of time. The results from trials using the age of the cutting and the season suggest that cuttings taken from the current year's growth at the end of the growing season have the highest rate of success. The differing success rate through the year may be a result of either the time that cuttings are taken from the wild or the conditions in the greenhouse. In the fall, the plants may have a better physiological condition in response to the monsoons or a hardening off of growth with a buildup of storage products (Macdonald 1986, Dirr and Heuser 1987). Greenhouse-grown plants had the highest success rate. Plants from greenhouses have better nutrient and water status and often have high rates of rooting success (Dirr and Heuser 1987). An ex situ population of rare plant stock would enable experiments on its biology and would provide genetic material for experimental reintroduction without compromising the wild population.

#### Greenhouse Plants

To establish an ex situ population, you must also be able to grow plants in a greenhouse situation. We found that *P. subintegra* will grow in a variety of media, but each has its drawbacks. In potting mix the plants grew faster but were far more finicky in their water needs. The soil dried easily and would stress the plants. For soil mixes with native soil, the plants survived at a higher rate but grew slower than in other potting media. Obtaining native limestone soil can also prove problematic. We are still examining whether the success of plants transplanted in the wild will vary with size or potting mix. Plants potted in the native soil are already inoculated with the native soil flora, including arbuscular mycorrhizae.

We experimented with both seed germination and rooting stem cuttings to determine the cultivation requirements for *P. subintegra*. Although we had some information about congeners, we still had to experiment with this species. At the Arboretum, our results with rooting stem cuttings of *P. subintegra* are actually much better than with *P.*

*stansburiana*. Initial results suggested that *P. subintegra* required its native soil to survive even in the greenhouse. Further work showed that this was not the case. Using knowledge from congeners helped, but there is still a vast need for horticultural and cultivation information on rare species.

#### Reintroduction Trial

We thought that our transplanting procedures were adequate with almost no mortality in the first month and only 18 percent mortality in the group that received 5 months of water after transplanting. The drip irrigation was very successful even though the time involved to install and to maintain the system was great. A four-person crew could set up the system in 8 hours, but the system had to be checked weekly. Irrigation drip emitters would clog with dirt or would need to be adjusted based on their location on the landscape and the irrigation line. Surprisingly, we saw only minimal damage to the irrigation lines by animals. We will continue to monitor these plants for long-term survival and reproduction for another year. Other transplant trials are underway to examine the timing of transplanting, effect of propagation techniques, and microsites.

These results have implications for the success of future *P. subintegra* reintroduction trials and trials of other desert species. We recommend using plants generated from cuttings or seeds for transplantation or reintroduction. Because *P. subintegra* is a deep tap-rooted shrub, manipulating cultivated plants instead of digging up plants is more feasible. Digging up individual plants would be destructive to the old and new habitat and would be difficult with large plants. Growing plants from seeds or cuttings would also allow more flexibility in determining the number of plants and genotypes needed.

Because precipitation can be variable and the timing and amount can affect the outcome of transplant trials, it is necessary to supplement transplants with a constant, dependable source of water during establishment. We recommend watering for 5 months or more. It is important to note that the time involved for the care of transplants is significant and may mean that a reintroduction requires multiple years.

Finally, despite the additional expenditure in time and money, monitoring should be considered an important part of any transplant experiment (Sutter 1996). Monitoring is necessary to increase the knowledge base about reintroductions, and

monitoring also provides managers with reasonable expectations of the cost and time involved for a successful reintroduction.

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