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SEEDLING RECRUITMENT AND SURVIVAL OF AN ENDANGERED LIMESTONE ENDEMIC IN ITS NATURAL HABITAT AND EXPERIMENTAL REINTRODUCTION SITES[1]

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The largest and most fecund population of the endangered *Purshia subintegra* is restricted to limestone mesas in Verde Valley, Arizona, USA, where habitat destruction is imminent. To examine factors limiting its distribution and potential for expansion, we compared recruitment and survival of seedlings growing in soils from occupied and unoccupied habitat in caged field experiments and compared survival of caged and wild seedling cohorts from 1998 to 2003. In field tests, seeds germinated in soils from occupied and unoccupied habitats. Seedling survival, however, was greatest in currently occupied habitat and dropped to zero in some unoccupied habitats with the onset of severe drought. Among 16 factors measured, soil moisture significantly explained between 62% and 71% of the variation in recruitment in both wild and caged plots. Shrubs conferred protection to wild seedlings, but decreased caged seedling survival. For 5 yr following germination, caged seedlings had greater survival than natural seedling cohorts indicating that reintroduction was comparatively more successful than natural recruitment. Expansion of *P. subintegra* into novel habitats is limited by soil moisture capacity, and this condition varied during the experimental time frame. Reintroductions to limestone mesas are possible and most promising if cages and supplemental watering are used.

Key words: edaphic endemic; endangered species; germination; limestone endemic; rare species distribution; recruitment; reintroduction; survivorship.

Many of the world's rare plant species are edaphic endemics whose unique soils, habitats, and restricted distributions make them especially vulnerable to human activities (Hopper et al., 1990; Shultz, 1993; Briggs and Leigh, 1996; Kelso et al., 1996; Sivinski and Knight, 1996; IUCN, 2002). Because habitat loss and degradation are believed to threaten 91% of the rare plants in the world (IUCN, 2002), many conservationists around the world are seeking to expand conservation and research efforts on the biology of rare species (ANPC, 1997; Hopper, 1997). Understanding the factors that restrict the establishment and growth of edaphic endemics can aid efforts to conserve rare species in their habitats, can help us identify suitable and occupied habitats for protection, and can expand options for species recovery, including restoration, translocation, and ex situ conservation (Schemske et al., 1994; ANPC, 1997; Maschinski and Holter, 2001). In many cases, human assistance will be vital for endangered species recovery.

Human-assisted expansion of geographic range has become a critical issue for conservation of the federally endangered shrub, *Purshia subintegra* (Kearney) Henrickson (Arizona cliffrose), whose distribution patterns suggest a high fidelity to one soil stratum of limestone, known as the Verde Formation, in the Upper Sonoran Desert (U.S. Fish and Wildlife Service, 1994). In the next year, approximately 600 individuals (10% of the Verde Valley population) and 5.5 acres (2.23 hectares) of potential and occupied *Purshia subintegra* habitat will be developed for a highway to serve the rapidly growing community of Cottonwood, Arizona. The highway will fragment the largest, most fecund population, alter physical conditions of the plant's habitat, and most likely affect water relations of the entire valley. Land managers have proposed to use reintroduction and restoration of former habitat to conserve the species, yet little was known about the efficacy of this strategy. In particular, the edaphic limitations of *P. subintegra* and its microsite requirements for recruitment are unknown; these would be key factors for planning a reintroduction effort.

Edaphic limitations and microsite requirements of *P. subintegra* are influenced by the unusual soils on which it grows and the aridity of the Upper Sonoran Desert. We conducted experiments to test several key hypotheses related to factors likely to influence the distribution *P. subintegra* and the efficacy of translocation. Adult distribution patterns of *P. subintegra* suggested that seed germination and recruitment would be greatest on the Verde Formation soils, yet little was known about the reasons for this distributional pattern. There is conflicting evidence about whether calicole endemics require limestone soils to germinate. Rorison (1960) found that calicole endemics failed to germinate and establish on acid soils, whereas other researchers have found that limestone endemics have no preferential soil requirements for germination (Mustart and Cowling, 1993). Under greenhouse conditions, *Purshia subintegra* germinated in limestone, sterile media, and other soils (Baggs, 1998), but it was unknown whether seeds could germinate in novel soils under field conditions.

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Survival of edaphic endemics in unoccupied or novel soils may be affected by complex interactions of abiotic and biotic factors, which can ultimately constrain the species' distribution. Reciprocal transplant tests of South African fynbos Proteaceae (two limestone endemics and two colluvial sands endemics) revealed that all species germinated in limestone and colluvial sands soils, but all species had maximum germination in limestone soil (Mustart and Cowling, 1993). Survival was not necessarily optimal for each species on its native soil during the 17-mo trial. Both limestone and colluvial sand endemics survived best on limestone soils, where soil moisture was higher and maximum soil temperature was lower than of colluvial soils. It was unknown whether *P. subintegra* could survive on novel soils, which would effectively expand its range. Given findings of Mustart and Cowling (1993), we hypothesized that the highest germination and survival of *P. subintegra* would occur on limestone soils.

It is possible that seed dispersal is so limited that seeds never reach adjacent novel habitats. Granivores in North American deserts consume most of the each year's seed crop—estimates ranging from 69% (Price and Joyner, 1997) to 95% (Soholt, 1973). Such extreme depletion of a seed crop limits the propagules available for dispersal and can limit establishment (e.g., Quintana-Ascencio et al., 1998). We hypothesized that human-assisted dispersal and sowing could expand the range of *P. subintegra* to novel habitats.

Because water is the most limiting resource in desert ecosystems (Noy-Meir, 1973), it significantly influences seedling emergence and survival. Several studies have shown that species' distributions are limited primarily by moisture (Sacchi and Price, 1992; Dunne and Parker, 1999; Escudero et al., 1999; Germaine and McPherson, 1999). In northern Arizona, lack of soil surface moisture was the most significant mortality factor in *Salix lasiolepis*, accounting for 70% of observed seedling mortality in the first two years of life (Sacchi and Price, 1992). Similarly, in semi-arid Spain, drought within the first two years following establishment caused 96% and 97% mortality of *Helianthemum squamatum* (Escudero et al., 1999). We hypothesized that *P. subintegra* seed germination and seedling survival would be significantly related to soil moisture.

In arid environments, nurse plants commonly provide safe sites for seed germination and for many species are the only means of avoiding harsh conditions (McAuliffe, 1984; Fowler, 1988; Valiente-Banuet and Ezcurra, 1991; Suzan et al., 1994; Tewksbury et al., 1999; Ibanez and Schupp, 2001). The trade-off between the facilitative and competitive effects of shrubs on seedlings varies in magnitude depending upon climatic conditions (Leirana and Parra, 1999; Ibanez and Schupp, 2001). Seedlings have improved growth and survival under nurse shade especially in drier sites and drier years (Holmgren et al., 1997). Often shrubs have positive affects on early stages, but then competition becomes stronger as recruits grow in size (Keyes et al., 2001), and their continued survival under trees or shrubs will depend upon soil water availability (e.g., Chambers, 2001). We tested the hypothesis that seedling establishment of *P. subintegra* would be enhanced by nurse plants, but long-term survival would not.

Selecting suitable sites for reintroductions or translocation of rare species must consider physical, biological, logistical, and historical critieria; however, it is "far from self-evident" what suitable sites will be (Fiedler and Laven, 1996). Because we rarely know enough before doing a reintroduction effort, conducting reintroductions in an experimental framework is essential for understanding the underlying ecology of the rare species' distribution that may influence reintroduction success. These studies represent such and address the following questions: (1) Can *Purshia subintegra* seeds germinate and seedlings become established in novel soils where adult plants do not currently grow? (2) How does seedling recruitment and survival in the wild compare to that of the experimental reintroduction? (3) Do shrubs facilitate or compete with young seedlings? (4) What factors influence seed germination and seedling survival in natural and experimental habitats? (5) Which factors will influence the success of future reintroductions?

MATERIALS AND METHODS

Species and study site—The federally endangered shrub *Purshia subintegra* is known from four disjunct populations in central Arizona, where it usually occurs on lacustrine outcrops with a distinct chalky white appearance (U.S. Fish and Wildlife Service, 1994). The largest population occurs in the Verde Valley, Arizona, where a Pliocene limestone deposit called the Verde Formation forms finger-like protrusions into the basin (Phillips et al., 1996).

Verde Valley is located approximately 10 km north of Cottonwood, Arizona (34°44.381′ N, 111°59.026′ W). Mean annual precipitation is 30 cm and mean high and low temperatures are 26.8°C and 8.6°C, but temperature extremes in the last seven years have been 47° and −8.3°C. Severe drought conditions have been occurring in the last three years with higher than normal temperatures and lower than normal precipitation (NOAA, 2003). Tuzigoot National Monument, located approximately 6 km from the study sites, has recorded weather information since 1977 (National Oceanic and Atmospheric Administration/National Weather Service Climatological Data). Vegetation is the Upper Sonoran desert scrub community, part of the *Larrea tridentata–Canothia holacantha* association (Brown, 1982). Within the Verde Valley along a 100-m elevation gradient from the top of the valley to the bottom, there are distinct changes in plant communities (Baggs, 1998). The upper strata is dominated by *Larrea tridentata–Canotia holocantha* woodlands; intermediate mesa strata support *P. subintegra*, where carbonate and clastic sediments mix; the slopes below support a *Canotia holocantha–Ceanothus greggii* community; and the alluvial bottoms are predominantly *Ceratoides lanata–Stipa neomexicana*. Within the context of these studies, we refer to the latter three habitats as mesa, slope, and alluvial, respectively (Fig. 1).

Usually less than 2 m tall, *P. subintegra* (Arizona cliffrose) is a xeric rosaceous evergreen shrub with pale yellow flowers and entire leaves that lack glands. It is closely related to *P. stansburiana* (Stansbury cliffrose), a widespread western U.S. species that has lobed leaves with glands and stalked glands on the hypanthium and young stems. In the Verde Valley, adult *P. subintegra* plants bloom from late March through early June (Fitts et al., 1993; Baggs and Maschinski, 2000). Seeds disperse from late June to early July, and seed germination occurs the following spring from March through June depending upon temperature and rainfall. In dry years, plants may not produce any viable seed and there may be no recruitment (Baggs and Maschinski, 2000).

Caged field study—To determine whether *P. subintegra* seeds could expand its range by germinating and surviving in soils and sites where adult plants do not grow, we conducted field experiments. In the spring of 1997 we collected *P. subintegra* seeds from plants growing close to the field site in the Verde Valley. Until we initiated germination experiments, we stored dry seeds at 5°C in sealed plastic bags. The field experiment was conducted near Cottonwood, Arizona, Yavapai County, on land managed by the Arizona State Land Department, where the previously described "mesa," "slope," and "alluvial" habitats are in close proximity (Fig. 1). *Purshia subintegra* is generally restricted to the mesa habitat and is rarely, if ever, found on slope or alluvial bottoms. On 25 November 1997, we randomly assigned and sowed 20 seeds of mixed parentage into each of 80 hardware cloth (1.2 cm mesh) cages in

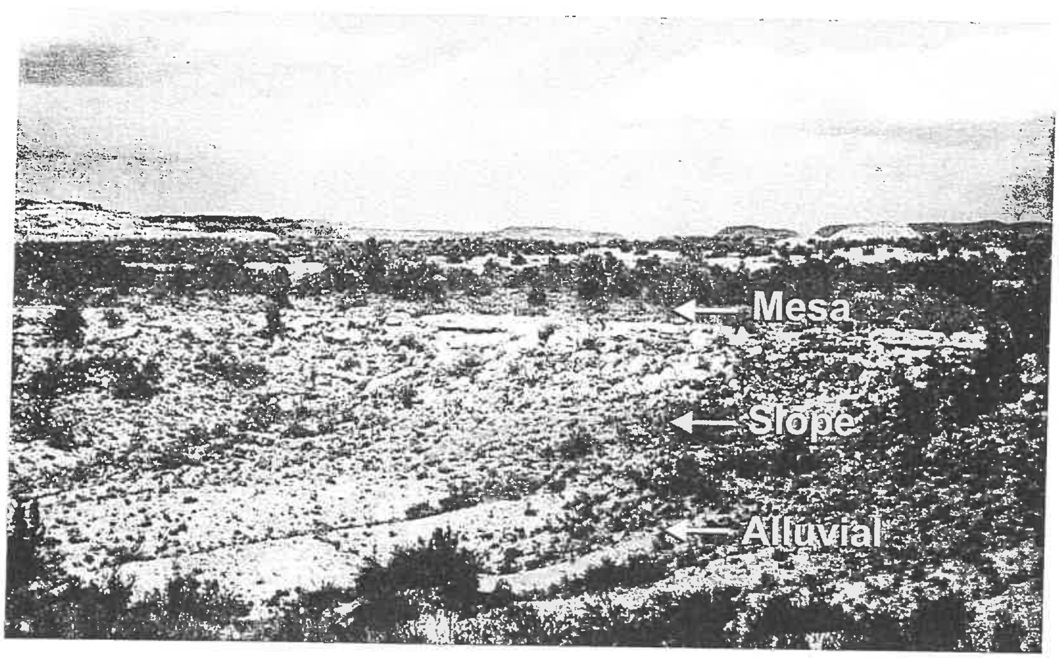

Fig. 1. Three habitats along an elevation and Upper Sonoran plant community gradient in the Verde Valley, Arizona, USA. *Purshia subintegra* is generally restricted to the mesa habitat and is rarely, if ever, found on slope or alluvial bottoms.

each of the three habitats (mesa, slope, or alluvial). We placed cages at one of two distances from shrubs interspersed within the habitats (40 cages within 0.2 m of the north side of a shrub or 40 cages >0.5 m from a shrub). These treatments were designated as shrub and open, respectively. Shrub species included *P. subintegra*, *Ceanothus greggii*, *Canotia holocantha*, *Ephedra viridis*, *Quercus undulata*, *Juniperus osteosperma*, *Rhus trilobata*, *Larrea tridentata*, *Gutierrezia sarothrae*, *Atriplex canescens*, *Berberis haematocarpa*, and *Ceratoides lanata*. Because we anticipated that mammalian herbivory could significantly undermine our ability to detect effects of experimental treatments (e.g., Soholt, 1973; Price and Joyner, 1997), we placed seeds into closed cylindrical hardware cloth cages measuring 15 cm in diameter. Cages excluded small and large mammalian herbivores from reaching the seeds from above or below ground, but did not exclude insects. Installing cages meant that the soils were disturbed, which may have created mixed, aerated conditions for the seeds.

To account for recruitment and survival, we recorded seedling recruitment of *P. subintegra* and other species from March 1998 through May 2003. In the first eight months, we recorded recruitment and survival weekly; from 1999–2000, we made monthly observations spring through summer and in 2002 and 2003 we made observations in May only. Seedlings had distinct cotyledons (2 × 3 mm) that remained green for approximately 2 mo; cotyledons usually dropped after more than four true leaves emerged. It was relatively easy to detect signs of herbivory or desiccation in seedlings. Stems persisted for months or sometimes years following mortality. Because cotyledons and leaves are large, any damage caused by insects was easily detectable. In one case, all seedlings within the cage had cotyledons and meristems removed, leaving only the stem present. We sometimes observed grasshoppers within cages, but this was relatively uncommon. Seedlings that had been established over eight months could persist even after dropping all of their leaves. We determined seedlings to be dead only if stems were completely brittle and no leaves or buds were present.

We examined recruitment vs. survival in two separate analyses to avoid confounding the two phenomena. We analyzed maximum recruitment in the three habitats and two shrub proximities using an analysis of variance with habitat and shrub proximity as the two fixed effects (SYSTAT 10.0, SPSS, 2000). We calculated percentage survival of the 1998 spring seedling cohort by dividing the number of seedlings that survived until 1998, 1999, 2000, 2001, 2002, and 2003 by the maximum number of recruits in the cage. We examined percentage of seedling survival (arcsine square-root transformed) in only those cages with seedling emergence ($n = 234$) using a repeated measures analysis of variance with habitat and shrub proximity as the two fixed effects (SYSTAT 10.0, SPSS, 2000). Because some cages were lost to vandalism, the final analysis had unequal sample sizes across habitats and proximity to shrubs. To account for uneven sample sizes, we used a weighted means model (Yates, 1934; Speed et al., 1978).

We measured soil composition, hardness, and moisture at the study site. To determine the soil composition of soils from the three habitats, we collected 10 15 × 5 × 5 cm soil samples. With a mechanical soil shaker, we separated soil fractions and weighed each particle size fraction using USDA classifications: clay and silt <0.05 mm, very fine sand >0.05–0.1 mm, fine sand >0.1–0.25 mm, medium sand >0.25–0.5 mm, coarse sand >0.5–1.0 mm, very coarse sand >1.0–2.0 mm, and gravel >2.0 mm (Brady, 1990). Using discriminant function analysis (SYSTAT 10.0, SPSS, 2000), we determined whether the proportions of soil particle constituents were distinct across the three habitat types.

We measured soil hardness taking eight measurements within and adjacent to 20 cages in each of the three habitats using a pocket penetrometer (Soiltest, Inc.). We analyzed differences in soil hardness (in kilograms per square centimeter) among the three habitats within and outside of cages using a nested analysis of variance with habitat as the fixed effect and measurement location as the nested factor (SYSTAT 10.0, SPSS, 2000).

For the first eight months following germination, we quantified change in gravimetric soil moisture within the site by collecting 10 core soil samples from each habitat on each seedling survey date. We used standard gravimetric methods to quantify the amount of water present in the soil samples passed through a 2-mm sieve after heating the soil to a constant mass at 100°C (Day, 1965). Using analysis of variance with date and habitat as the main fixed effects, we analyzed the percentage of water across habitats (SYSTAT 10.0, SPSS, 2000). Results are reported as % ODM (percentage oven dry mass) determined from (g H_2O lost in drying/g oven dry soil) × 100. Regression analysis determined what percentage of variation in soil moisture contributed to pooled seedling recruitment in each habitat (SYSTAT 10.0, SPSS, 2000).

Recruitment and survival in natural populations—We also assessed recruitment and survival of naturally occurring seedling cohorts in the wild population. As part of a larger demographic analysis, we established a total

TABLE 1. Maximum recruitment (as a proportion) of seedlings of *Purshia subintegra* established within experimental cages in three habitats and two proximities to shrubs in Verde Valley, Arizona, USA. Values are means ± 1 SE. Means with the same letter are significantly different at the $P \leq 0.05$ level.

Habitat	Microhabitat	Spring 1998
Mesa	Open	0.33 ± 0.26 A
	Shrub	0.29 ± 0.26 A
Slope	Open	0.33 ± 0.24 A
	Shrub	0.33 ± 0.24 A
Alluvial	Open	0.13 ± 0.13 B
	Shrub	0.19 ± 0.17 B

TABLE 2. Repeated measures analysis of variance for proportion of *Purshia subintegra* seedlings surviving (arcsine square-root transformed) in Verde Valley, Arizona, in three habitats and two distances from shrubs in each year from 1998 to 2003.

Source	df	MS	*F*	*P*
Habitat	2	9.85	12.6	0.0001
Shrub	1	8.77	11.2	0.001
Habitat × Shrub	2	0.50	0.64	0.53
Error	189	0.78		
Year	5	5.05	83.41	0.0001
Year × Habitat	10	0.10	1.72	0.07
Year × Shrub	5	0.19	3.14	0.008
Year × Habitat × Shrub	10	0.10	1.63	0.09
Error	945	0.06		

of 30 plots (4 × 10 m) in four mesa habitats that span the 5 × 2.5 km range of *Purshia subintegra* in the Verde Valley, Arizona. We randomly selected 24 plots and systematically selected six plots where we observed high densities of relatively young *P. subintegra*. We monitored all plots during the period of maximum seedling recruitment and seed production between April and June from 1998 through 2003. For each seedling within a plot, we recorded location in plot, whether it was established beneath a shrub canopy or in the open, height, maximum width and its perpendicular width, basal diameter, number of true leaves, and if obvious, the cause of mortality. We determined canopy volume of individuals from height and average width using the formula for half of a spheroid, canopy volume = $4/3\pi h^2 W$ (Ludwig et al., 1975). Seedlings had cotyledons present, basal diameters ≤0.2 cm, and canopy volumes $\leq 9 \times 10^{-6}$ cm^3.

To examine the relationship of wild seedling recruitment to abiotic and biotic ecological factors, we measured elevation, slope, aspect, gravimetric soil moisture (in April 1997 and April 1999), total nitrogen, total phosphorus, total potassium, soil particle size distribution, soil hardness (in kilograms per square centimeter), bulk density, species diversity, and percentage cover in all demographic plots. We collected three 0–10 cm soil cores from each plot and combined them for analysis of soil chemistry, moisture, and composition. Using the same methods described for the caged field experiment, we quantified gravimetric soil moisture, hardness, and particle size composition. Concentrations of N, P, and K were measured using a Kjeldahl digestion of subsamples of the soils at the Bilby Research Center at Northern Arizona University. We measured community attributes within the demographic plots using circular plots (3 m radius), counting the abundance and number of each species and visually estimating the percentage vegetative cover and bare area. We analyzed the relationship between the number of seedlings recruited in plots (log transformed) and the 16 ecological factors using stepwise regression (SPSS 10.1, SPSS, 2001).

To assess differences in seedling survival between experimental and natural conditions, we performed a survival analysis using the Kaplan–Meier method (SYSTAT 10, SPSS, 2000). We compared survival curves and mean survival time of caged seedlings growing in the three habitats and two proximities to shrubs with naturally recruiting seedlings emerging beneath shrubs or in the open in all demographic plots. We tested the differences in shapes of survival curves of seedlings emerging from 1998–2003 by the log-rank test (Pyke and Thompson, 1986). In addition, we examined the relationship between seedling survival and size (basal diameter at last census, height, and volume) using regression analysis (SYSTAT 10.0, SPSS, 2000).

RESULTS

Caged field study—*Purshia subintegra* seedlings recruited in all three habitats. In March 1998, we detected the first emergence of seedlings and over the next 2 mo the majority of seedlings emerged. Approximately equal recruitment occurred in mesa and slope habitats, while the lowest occurred in the alluvial habitat ($F_{2,234}$ = 10.81, P = 0.001, Table 1). There was no significant difference in the number of seeds that recruited near or distant from shrubs ($F_{1,234}$ = 0.77, P = 0.38), and there was no significant interaction between habitat and proximity to shrubs ($F_{2,234}$ = 1.27, P = 0.28).

Seedling survival, however, was significantly influenced by habitat and proximity to shrubs. Seedlings in mesa habitats had significantly higher survival than did seedlings from slope or alluvial habitats (Table 2, Fig. 2). In all habitats, there was significantly lower seedling survival near shrubs than in the open until 2002, when there was a reversal in alluvial habitat. Hence, there was a significant interaction between time and proximity to shrub (Table 2, Fig. 2).

From 1998 to 2003, seedling survival decreased in all habitats, but remained highest in the mesa open group (Table 2, Fig. 2). By 2003, no seedlings survived in the alluvial habitat, regardless of proximity to shrub, 7% survived in mesa open, 4% survived in mesa shrub, 1.5% survived in slope open, and 0.8% survived in slope shrub habitat. Overall mean survival of seedlings was 24.2 mo in the mesa habitat, 13.86 mo in the slope habitat, and 8.6 mo in the alluvial habitat (Mantel log-rank test = 539.17, 2 df, P < 0.0001, Fig. 2). Mean survival time for seedlings emerging near shrubs in all habitats combined was 12.86 mo and in the open was 21.1 mo (Tarone—Ware, X^2 = 20.77, 1 df, P < 0.0001, Fig. 2). Mean survival time for seedlings growing in the open vs. shrubs was 26.5 vs. 20.93 mo in mesa (Tarone—Ware, X^2 = 1, 1 df, P < 0.31), 18.64 vs. 9.89 mo in slope (Tarone—Ware, X^2 = 12.50, 1 df, P < 0.0001), and 11.11 vs. 6.85 mo in alluvial habitat (Tarone—Ware, X^2 = 12.70, 1 df, P < 0.0001).

Factors influencing seedling recruitment and survival in the caged field study—Corresponding to the pattern in seedling recruitment and survival in the caged field study were changes in soil physical characteristics and moisture in the three habitats. Soil physical characteristics significantly changed along the 100-m elevation gradient and were significantly discriminated (F = 2.45, df = 12, 44, P < 0.01). In general, the soils had a greater proportion of large particle sizes at the top of the mesa and became significantly finer at the bottom of the elevation gradient in the alluvial habitat (Fig. 3). The slope habitat soil was a sandy loam intermediate between the mesa and alluvial soils.

Soil compaction significantly varied across habitats ($F_{2,54}$ = 23.09, P < 0.0001, Table 3). Alluvial soils had the least compact soils of the habitat types; mesa and slope habitats had similar hardness. In general, soils were more compact outside the cages than inside the cages ($F_{1,54}$ = 7.68, P = 0.01). However, there was a significant interaction between compaction inside or outside cages and habitat ($F_{2,54}$ = 8.84, P = 0.0001);

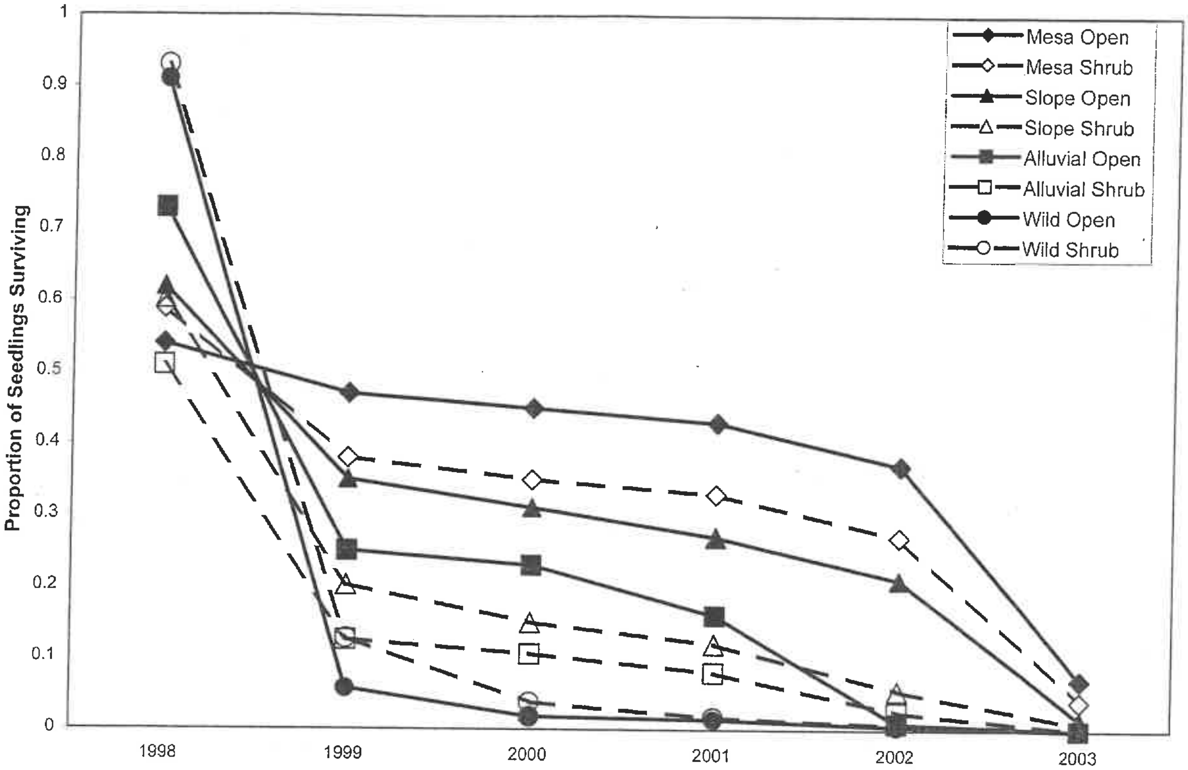

Fig. 2. Seedling survival curves for *Purshia subintegra* seedlings established cages in mesa, slope, and alluvial habitats and in the wild cohorts growing near and distant from shrubs in Verde Valley, Arizona.

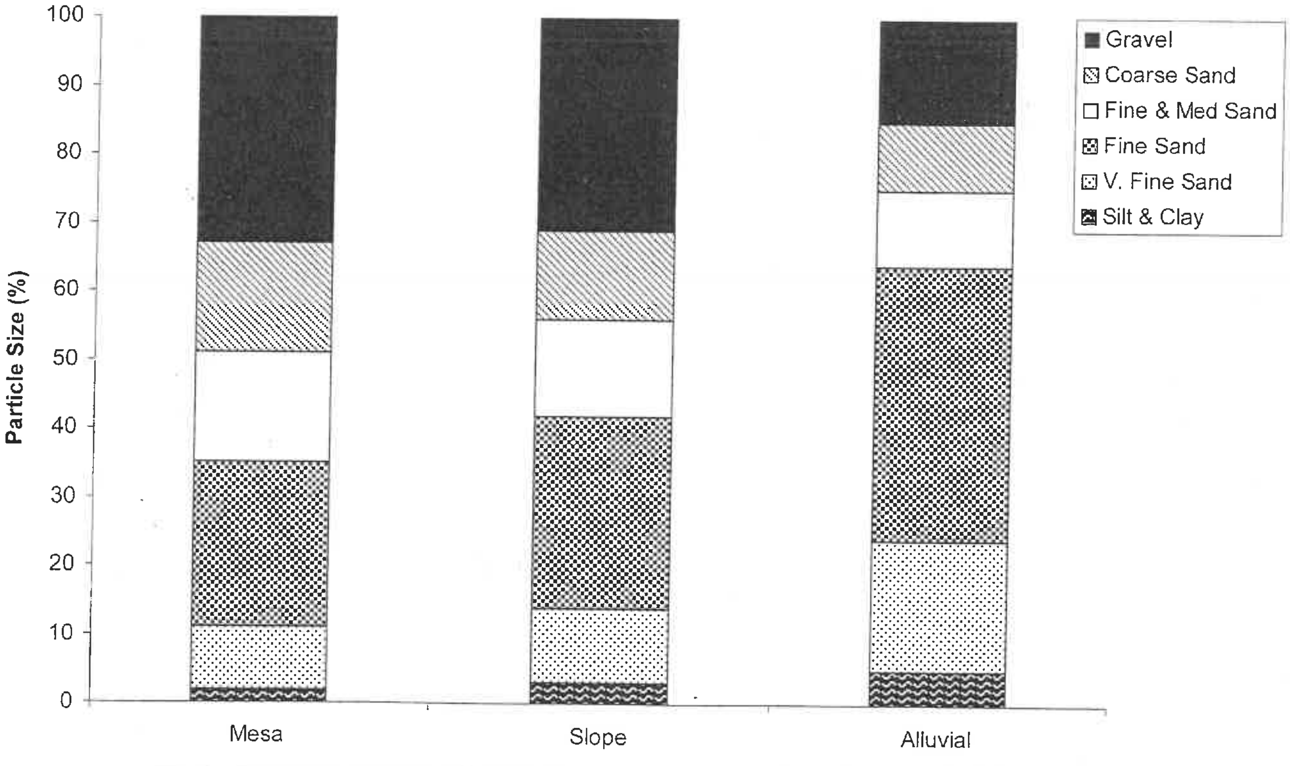

Fig. 3. Comparison of soil particle composition in mesa, slope, and alluvial habitats in Verde Valley, Arizona.

TABLE 3. Comparison of soil compaction inside and adjacent to seed cages in three habitats and two proximities to shrubs in Verde Valley, Arizona. Values are means ± 1 SE.

Location	Proximity to shrub	Inside cage (kg/cm²)	Outside cage (kg/cm²)
Mesa	Open	0.12 ± 0.02	0.14 ± 0.01
	Shrub	0.10 ± 0.01	0.13 ± 0.02
Slope	Open	0.12 ± 0.01	0.14 ± 0.01
	Shrub	0.11 ± 0.01	0.14 ± 0.01
Alluvial	Open	0.06 ± 0.01	0.04 ± 0.02
	Shrub	0.06 ± 0.01	0.05 ± 0.01

digging soil to install cages significantly loosened soil in the mesa and slope habitats, but not in the alluvial habitat, which was comparatively more loose and sandy (Table 3).

Soil moisture significantly influenced seedling recruitment and survival in the caged field studies. Soil moisture explained a significant proportion of the variation in total seedlings recruiting within each of the mesa, slope, and alluvial sites ($R^2 = 0.69, 0.71, 0.65$; $F_{1,16} = 35.74, 38.36, 30.23$; $P < 0.0001$, respectively). As was true with soil composition, soil moisture also followed the elevation gradient. On all but five of the sample dates, the mesa habitat had significantly greater soil moisture than the other two habitats (Fig. 4, $F_{2,537} = 95.84$, $P < 0.001$). The first seedling emerged when soil moisture across the three sites was greater than 20% in early March (Fig. 5). By mid-April, seedlings began to die and soil moisture dropped significantly (Figs. 4, 5). By the beginning of June, soil moisture had dropped to approximately 7% in all sites. Despite summer monsoons in July, seedling survival continued to decline (Figs. 4, 5). These data suggest that winter or early spring precipitation is critical for seed germination and that the water-holding capacity of the soil is critical for seedling survival.

Recruitment and survival in the natural population—Of 728 seedlings established in the demographic plots in 1998, 419 (58%) established in the open (≥0.5 m from a shrub) and 309 (42%) established under shrub canopies. Survival curves were significantly different for seedlings established between these microsites (Mantel log-rank test = 15.75, $P < 0.0001$, Fig. 2), especially in the first 3 yr. During this period, seedlings growing in the open had significantly lower survival than those established under shrub canopies. Mean survival time of seedlings growing in the open was 7.6 mo, whereas it was 9 mo for seedlings growing with shrubs. By 2003, only a single seedling survived, and it was under a shrub.

Survival of seedlings was significantly and positively correlated with size; basal diameter ($R^2 = 0.27$, $F_{1,723} = 255$, $P = 0.0001$), height ($R^2 = 0.009$, $F_{1,726} = 6.44$, $P = 0.01$), and volume ($R^2 = 0.016$, $F_{1,726} = 10.31$, $P = 0.001$). However, there was no clear demarcation of threshold seedling size to guarantee survival.

Seedling recruitment in 1998 was significantly positively correlated with soil moisture in 1997. Stepwise regression indicated that soil moisture in 1997 explained 62% of the vari-

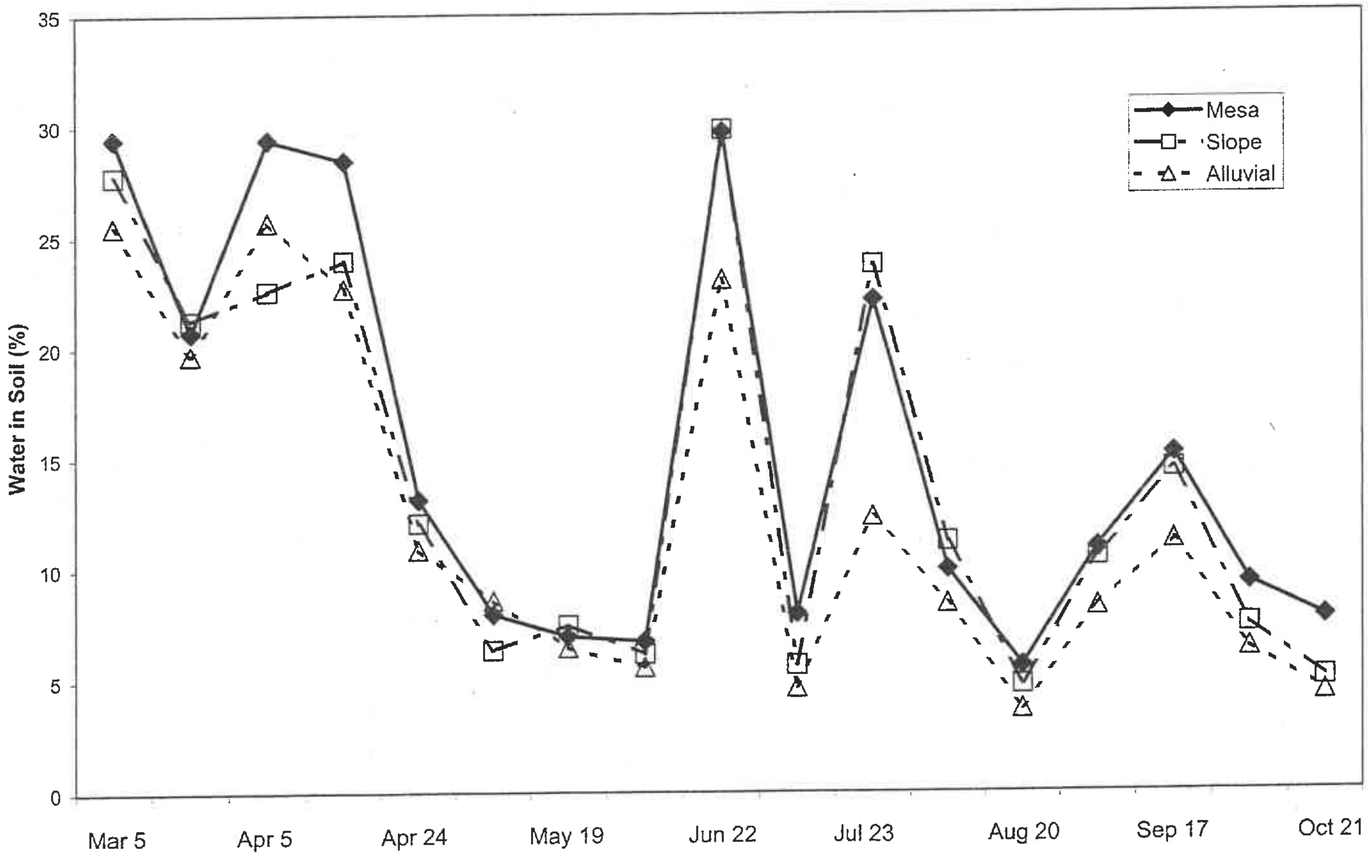

Fig. 4. Mean percentage soil moisture in mesa, slope, and alluvial habitats for 8 mo following germination of *Purshia subintegra* seeds in Verde Valley, Arizona.

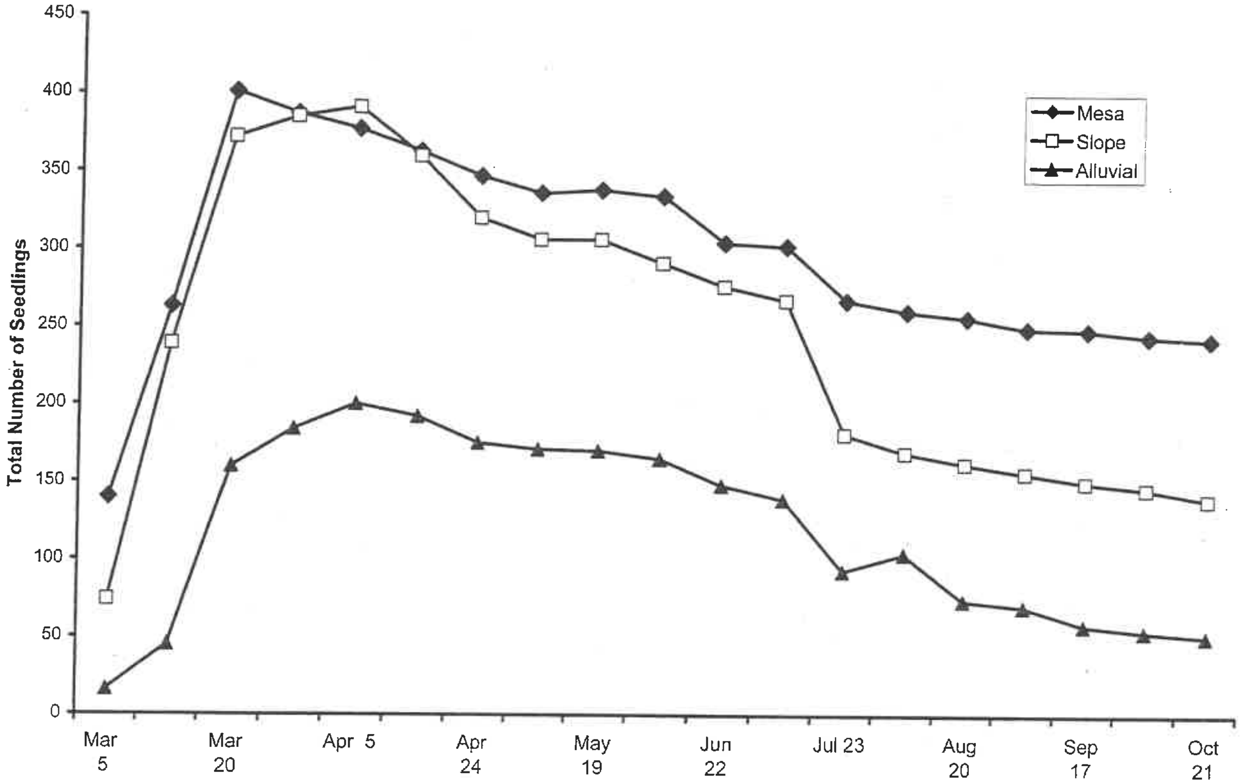

Fig. 5. Total *Purshia subintegra* seedlings present in mesa slope and alluvial habitats in Verde Valley, Arizona for the first 8 mo following germination.

ation in log seedling density (Table 4). No other variables made significant contributions to the model.

Seedling survival in natural population compared to the caged field study—Comparing the survival of seedlings in the caged field study vs. the natural population revealed that overall seedling survival was significantly greater in the cages than in the wild in some, but not all habitats. By 2003, the percentage of seedlings surviving in the wild was 0.3%, whereas depending upon habitat and proximity to shrubs, 0–7% of caged seedlings survived through the 2003 census; shapes of survival curves were significantly different (Fig. 2, Mantel log-rank test = 235, $P < 0.0001$). In habitat presently occupied with *P. subintegra*, caged seedlings had the highest probability of survival (mesa open 7%, mesa shrub 4%), whereas uncaged wild seedlings had only 0.3% survival. The two novel habitats tested indicated that alluvial habitat could not support *P. subintegra* for any significant amount of time; however, caged seedlings in slope habitat had somewhat higher seedling survival than the wild seedlings (slope open 1.5% and slope shrub 0.8%), though these low survival rates do not suggest long-term survival of seedlings in this novel habitat.

TABLE 4. Stepwise regression of log seedling recruitment of *Purshia subintegra* and 16 environmental and community variables in Verde Valley, Arizona.

ANOVA	SS	df	MS	F	P
Regression	48.68	1	48.68	46.07	0.0001
Residual	29.59	28	1.058		
Total	78.26	29			

Model: Soil moisture 1997 (predictor): $R = 0.789$; $R^2 = 0.622$; adjusted $R^2 = 0.608$; standard error estimate = 1.028; df = 1; $P = 0.0001$.

DISCUSSION

Our results suggest that novel habitats are doubtful candidates for reintroductions. Although *Purshia subintegra* seeds germinated equally well in mesa and slope habitat, seedling survival was limited in the novel slope habitat. Alluvial habitat had poor germination and survival. Reintroduction success and the species' distribution were influenced by a combination of factors including habitat, soil moisture, caging, and proximity to shrubs. Survival was greatest in the habitat currently occupied by *P. subintegra* and generally declined along an elevation gradient in the slope and alluvial habitats.

Associated with the elevation gradient were decreased soil moisture and increased proportion of fine and very fine sand. Soil moisture was significantly related to seedling establishment in both the caged field study and wild plots, explaining between 62 and 71% of the variation in recruitment. Limestone has been shown to retain up to 20% of its mass in water, thereby acting as a water reservoir (Mustart and Cowling, 1993). The winter of 1997–1998, following establishment of experimental plots, was a wet El Niño period, and there was a sustained period where soil moisture remained above 14% (Fig. 4). Therefore, germination success and early seedling survival were probably higher than would be expected in a year with average precipitation. The onset of drought in 2000 significantly affected seedling survival in both wild and caged plots and completely eliminated seedlings from the alluvial habitat (Fig. 2). Baggs and Maschinski (2000) showed that *P. subintegra* flower production, seed production, and seedling recruitment are linked to precipitation and suggested that more than one year of adequate precipitation may be required for seedling recruitment.

The presence of shrubs differentially influenced seedling survival in the wild plots vs. caged field study. In the wild, shrubs enhanced seedling survival for the first three years, suggesting that under natural conditions shrubs confer some protection to seedlings as has been seen in other arid systems (e.g., Ibanez and Schupp, 2001; Keyes et al., 2001). Microenvironmental characteristics under shrubs, such as nutrients, cation exchange capacity, organic matter, and soil temperatures, may be more favorable for seedling establishment than open sites (Chambers, 2001; Ibanez and Schupp, 2001). By the third year after germination, there was no significant difference between seedlings growing in the open or under shrubs because total survival was generally low. By 2003, the only seedling surviving in the wild 1998 cohort was growing under a shrub.

Conversely, in the caged field study, shrubs negatively affected seedling survival. If we eliminate the effect of habitat and compare only the mesa habitat with the wild seedlings, we find that caged seedlings growing near shrubs had lower survival than those in the open, but higher survival than uncaged seedlings in the wild. In other words, caging had more of an influence on survival than did the presence or absence of shrubs when habitat was equal. The discrepancy between the two experiments suggests that cages offered greater advantages to seedlings than did shrubs (Fig. 2). Cages may offer protection from herbivores (Chambers, 2001; but see Juenger and Bergelsen, 2000), wind, and sun without the cost of competition that shrubs impose (Parikh and Gale, 1998). Another consequence of caging was disturbance. When the cages were installed, soil was loosened and mixed. In the mesa and slope habitats, soils were significantly less hard inside the cages than outside the cages (Table 3). It is likely that this disturbance promoted rapid root growth of seedlings, which in turn may have conferred greater drought tolerance. Cages offered seedlings a significant survival advantage, suggesting that protection provided by non-organic sources, such as rocks or cages, may be important for reintroduction of *P. subintegra*.

Because seedling establishment varies dramatically with abiotic and biotic factors, it is often difficult to assess the success of a reintroduction trial. Comparing seedling cohorts in the wild vs. the caged field study during the same time period allowed us to examine whether our manipulations enhanced seedling survival of this endangered species. Clearly, alluvial habitat was not appropriate for reintroduction of *P. subintegra*, however seedlings introduced to slope habitat away from shrubs had survival rates of 21% after 3 years, which is similar to the survival of caged seedlings in the mesa habitat and 19% greater success than seedling survival (2%) in wild demographic plots. The onset of drought dramatically decreased seedling survival such that after 5 years caged seedlings in only three microsites (mesa open, mesa shrub, and slope open) had higher seedling survival than in the wild (Fig. 2). The decline in survival of seedling cohorts in both the wild and caged field study illustrates that it is critical to monitor reintroductions over a long term to determine their success. Our conclusions about the appropriateness of using novel habitat for *P. subintegra* reintroductions drawn after 1, 3, and 5 yr were markedly different.

With road development imminent in the habitat of this endangered species, effectively conserving genotypes that will be lost and increasing the population of *P. subintegra* will require establishing plants into protected habitat. Our studies indicate that at least in the short term, seedlings can establish on limestone mesas, which is encouraging for reintroduction efforts. Although natural establishment on unoccupied limestone mesas has probably been limited by poor dispersal (Harper, 1977; Howe and Smallwood, 1982; Cain et al., 1998; Quintana-Ascencio et al., 1998), inhospitable soil moisture and surface characteristics (Meyer, 1986; Sacchi and Price, 1992; Escudero et al., 2000), predation (Cabin et al., 2000), and/or competition with other species (McGraw and Levin, 1998; Russell and Schupp, 1998), reintroductions of *P. subintegra* seeds or plants may be successful under certain conditions. We propose that both seed and whole plant reintroduction include caging protection for the first few years, soil disturbance to loosen compact soils, and supplemental watering to maintain soil moisture at or above 14% until plants are well established. Our intentional protocol in these studies was to examine survival of seedlings under ambient precipitation; however, to maximize seedling survival in a reintroduction would require irrigation. Reintroduction trials of whole plants of *P. subintegra* were more successful with supplemental watering (Baggs and Maschinski, 2001). Supplemental watering is more attention than most reintroduced plants usually receive (Cully, 1996; Guerrant, 1996), but most reintroductions could benefit from more attention to design, site selection, planting technique, and follow-up monitoring in order to assure their success (Falk et al., 1996), and those trials that have utilized these techniques have had greater promise of long-term success (Dixon and Krauss, 2001).

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