

## **Germination of Redberry Juniper (*Juniperus pinchotii*) Seed in Western Texas**

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### **ABSTRACT**

We investigated germination/seedling emergence characteristics of redberry juniper (*Juniperus pinchotii* Sudw.) seeds harvested in December 1993 from 20 trees in the central Edwards Plateau and in December 1996 from 21 trees. Germination of seeds collected from raccoon (*Procyon lotor*) and bird (American robin [*Turdus migratorius*] and cedar waxwing [*Bombycilla cedrorum*]) feces in the northwestern Edwards Plateau in December 1996 were also tested. Seedling emergence from the 1993 seeds planted in soil in a greenhouse averaged 5.7% and emergence ceased after one year. Seedling emergence occurred primarily in winter (3.53%) and autumn (1.51%). Average laboratory germination at 68° F of the 1996 seeds was 8.8% at 3 and 52 mo after seed harvest. Scarification and stratifications treatments produced evidence of impermeable seed coats, embryo dormancy, and/or germination inhibitors, but these constraints seem of little biological or ecological significance. Redberry juniper seed germination is relatively low, but a single tree can produce thousands of seed in a favorable year and the seeds can potentially survive for one to several years. Long-range redberry juniper management plans should consider controlling trees before they reach reproductive maturity and using prescribed fire to control seedlings soon after years when climatic conditions favor juniper seed production, germination, and establishment.

**KEYWORDS:** after-ripening, birds, dormancy, ingestion, longevity, raccoons, scarification, stratification, sulfuric acid, temperature

## INTRODUCTION

Redberry juniper (*Juniperus pinchotii* Sudw.) is a dioecious or sometimes monoecious, basal-sprouting, evergreen conifer native to the southwestern United States and northern Mexico (Adams and Zanoni 1979). It occurs on about 11.7 million acres of Texas rangeland, primarily in the Edwards Plateau and Rolling Plains resource areas (Soil Conservation Service 1985). Redberry juniper was primarily restricted to rocky outcrops, canyons, and shallow range sites, but since the late 1800's, it has increased in density and spread into adjacent grasslands (Ellis and Schuster 1968). Its distribution increased about 60% during the period from 1948 to 1982 in a 65-county region in northwestern Texas by seedling recruitment (Ansley et al. 1995). Dense stands of redberry juniper suppress the growth of forage plants and threaten species biodiversity, rangeland watersheds, livestock production and wildlife habitat quality (Steuter and Wright 1983, McPherson and Wright 1990a, Dye et al. 1995). The conversion of grassland to shrubland has been attributed to the interactions of overgrazing, reduced fire frequency and intensity, drought, and perhaps climate change that favors woody plants (Smeins 1983, Archer et al. 1988).

Seeds of most *Juniperus* spp. are characterized as long-lived because germination can be delayed for several years by embryo dormancy, a pericarp covering the seed, an impermeable seed coat that interferes with imbibition, and/or the presence of germination inhibitor(s) (Johnsen and Alexander 1974). However, the germination requirements of redberry juniper seeds have not been thoroughly studied. Smith et al. (1975) reported greatest germination of redberry juniper cones at 64E F in distilled water. Warren (2001) reported only 0.07% seedling emergence from 1500 depulped redberry juniper seeds planted on tilled soil surfaces in 1997 at a site in the Rolling Plains of Texas.

Several monoterpenes are present in redberry juniper cones (Erica Campbell; Texas Agricultural Experiment Station, San Angelo, Texas, personal communication, December 2004). Volatile monoterpenes inhibit seed germination and seedling growth in some plants (Vaughn and Spencer 1993). Germination rates of several juniper species have been increased by dormancy-breaking treatments such as stratification, scarification, or an after-ripening period (Johnson and Alexander 1974, Fisher et al. 1987, Young et al. 1988, Chambers 1999). Removal of the pericarp increased germination of one-seed juniper (*J. monosperma* [Engelm.] Sarg.) seeds in northern Arizona (Johnsen 1962).

Knowledge of the population dynamics of redberry juniper is essential for developing ecologically and economically sound integrated management systems, aids in understanding historical patterns of invasion, and provides information to guide management decisions and predict their consequences. Whisenant (1991) described the potential of population modeling to direct research efforts and improve management strategies. Sensitivity analysis of an Ashe juniper (*J. ashei* Buchholz) population model identified seedling mortality as the most critical transition impacting Ashe juniper populations.

The berrylike cones of most juniper species are well adapted for dispersal by vertebrates. Frugivorous mammals and birds, and heteromyid rodents have likely accelerated the rate of juniper expansion (Chavez-Ramirez and Slack 1993, 1994, Chambers et al. 1999). Large numbers of redberry juniper seeds have been observed in the feces of frugivorous birds and mammals, however, little information is available

regarding their post-digestive effect on seed germination and seedling recruitment

The objectives of this study were to investigate the germination ecology of redberry juniper, specifically the variability in seed germination among trees within a population, the effects of stratification, scarification, after-ripening, ingestion of seed by birds and small mammals, seasonality of germination, and seed embryo longevity. Understanding germination requirements provides information about the environmental conditions required for seedling recruitment and the conversion of grasslands to juniper woodlands.

## MATERIALS AND METHODS

### Greenhouse Seedling Emergence Experiment

One experiment was conducted at the Texas Agricultural Experiment Station 28 miles southeast of Sonora (Edwards County), TX (lat 30E17'N; long 100E32'W) in the central Edwards Plateau to evaluate seasonality of seed germination/seedling emergence and seed embryo longevity under greenhouse conditions. Elevation at the seed collection site is about 2075 ft and mean annual precipitation is 22.6 in. More than 10,000 cones were hand harvested from 20 randomly selected, mature, redberry juniper trees with heavy cone production growing during December 1993 to January 1994 on Tarrant silty clay and Tarrant stony clay soils (clayey-skeletal, montmorillonitic, thermic, Lithic Haplustalls) (Wiedenfeld and McAndrew 1968). Cones were harvested from different parts of the trees to randomize any positional effects. Cones collected from the 20 trees were composited, air dried, and pericarp was removed from the seeds by rolling a 4-in. polyvinyl chloride (PVC) pipe over the cones. Seeds were stored in a refrigerator at 37E F until planting. Two hundred 1-qt (4.5- ×5.0-in.) pots were filled with soil from the seed harvest site and placed in a greenhouse. Fifty fully developed seeds were planted about 0.5 in. deep in each pot on 8 March 1994. Pots were hand-watered as needed to keep the soil moist for the duration of the trial. The greenhouse was heated with a natural gas heater and cooled with an exhaust fan and evaporative cooler as needed during temperature extremes. Seedling emergence was recorded biweekly and emerged seedlings were marked with 24-gauge colored telephone wire to prevent multiple counts of the same seedling. Data were composited seasonally (spring = March through April; summer = May through September; fall = October through November; and winter = December through February).

### Laboratory Germination Study

About 1000 cones were hand harvested from each of 21 mature redberry juniper trees in December 1996 on an undulating Kimbrough soil (loamy, mixed, thermic, shallow Petrocalcic Calciustolls) (lat 31E38'N; long 100E32'W) (Wiedenfeld and Flores 1976) about 10 miles northwest of San Angelo in Tom Green County, Texas. Elevation of the site is about 2165 ft and mean annual rainfall is 21 in. with peak periods generally in late spring and early fall. The trees were arbitrarily selected from plants 12- to 18-ft tall with a heavy cone crop. Cones were collected from different parts of the trees to randomize any positional effects and the 21 trees were permanently marked to facilitate resampling in subsequent years. Cones from each tree were bagged separately and dried in a forced-air oven at 100E F. Pericarps were removed by rubbing the cones between two wooden boards. Fresh raccoon (*Procyon lotor*) and bird (probably American robin

[*Turdus migratorius*] and cedar waxwing [*Bombycilla cedrorum*]) feces containing redberry juniper cones were collected at the same time and site. Bird feces were generally concentrated around watering areas and under perch sites, whereas raccoon feces were collected in interspaces between juniper trees, along trails and roads, and near watering areas. The feces were air dried at 68E to 75E F (drying the seed in a forced-air oven at 100° was not necessary because the animals' digestive processes had removed most of the pericarp). Pericarp not removed by passage through the digestive track of the animal was removed as described above. Seeds were stored in the laboratory at room temperature in cloth bags.

Three replications of 50 seeds from each tree were placed on double layers of #2 blotter paper in 3.9-in. diameter petri dishes on 13 March 1997 (3 mo postharvest) to determine early post-harvest germinability and to quantify variability of seed germination among trees. Captan (cis-N-[trichloromethyl]thio-4-cyclohexene-1,2-dicarboximide) fungicide was applied to the seeds and 0.27 oz of distilled water were applied to each dish. Seeds were placed in an environmental chamber at alternating temperatures of 81E F; 12 h light/54E F; 12 h dark. These temperatures approximate average daily maximums and minimums, respectively, for spring and fall in the study area. Three 50-seed replications from each tree were placed in another environmental chamber at a constant 68E F (intermediate between the alternating temperature regime) and 12 h light/12 h dark at the same time. Distilled water was added as necessary to keep the blotter paper saturated. Seeds were examined at 7-d intervals for 56 d and were considered germinated when radical length was  $\geq$  seed length. Six 50-seed replications from each tree were used in a germination trial at constant 68E F (12 h light/12 h dark) initiated on 29 March 2001 (52 mo postharvest) to test seed embryo longevity.

Equal numbers of seed from each of the 21 trees were composited, thoroughly mixed, and separated into three equal groups (weight basis) on 13 March 1997 for stratification treatments. Seeds collected from the raccoon and bird feces were treated similarly. Seeds were spread between two layers of cheesecloth within dampened vermiculite at 39E F for 30 d (cool-moist stratification), placed in dry cloth bags at 39E F for 30 d (cool/dry stratification), or stored in cloth bags at room temperature (no stratification). Scarification treatments were superimposed on these three composited, hand-harvested seed lots after stratification and immediately before the germination trials. Seeds not used in germination trials conducted 4 mo after harvest were stored in cloth bags at room temperature until additional germination trials were conducted 10 mo postharvest. Seed scarification treatments applied to hand-harvested seeds included: 1) 45 min in concentrated sulfuric acid; 2) boiling water for 10 s; 3) rubbing between coarse-grit emery paper attached to boards; and 4) no treatment. No scarification treatments were applied to seeds ingested by raccoons or birds. Six 50-seed replications from each treatment combination were subjected to 56-d germination trials at 68E F (12 h light/12 h dark) in mid April 1997 (4 mo postharvest) and late October 1997 (10 mo postharvest) by the same methods described above.

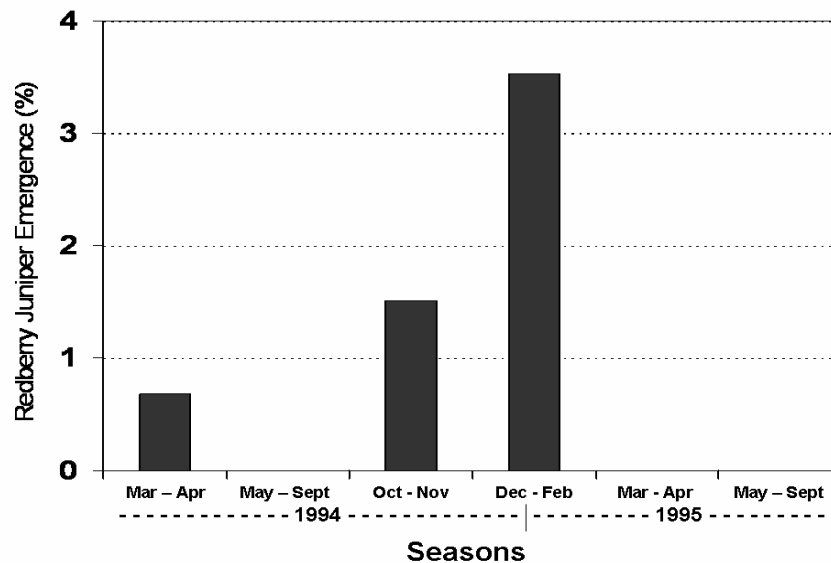
Analysis of variance (ANOVA) was conducted on final percent germination data for the laboratory study. Data were square root or arc sine transformed to meet assumptions of normality and homogeneity of variances. The observed means are presented for ease of interpretation. Analyses for the seed stratification/scarification study were conducted using a split-split-plot design with postharvest interval (4 mo vs. 10 mo) as the whole plot, stratification as the subplot, and seed scarification as the sub-subplot effect. ANOVAs were performed similarly but separately on data from raccoon-

and bird-ingested seeds because these free-ranging animals did not likely ingest cones from the same trees or those from which cones were hand harvested. Means were separated by Fisher's Protected LSD tests at the 5% level where appropriate, and 95% confidence intervals (CI) were calculated for each treatment mean (Gomez and Gomez 1984).

## RESULTS AND DISCUSSION

### Greenhouse Seedling Emergence Experiment

Ambient internal greenhouse temperatures varied from 32° to 100° F during the greenhouse experiment and were generally similar to external ambient temperatures. No seedlings emerged after the 1994-95 winter season. Seedling emergence by season averaged 0.68, 0.00, 1.51, and 3.53% for the spring, summer, fall, and winter seasons after the seeds ripened, respectively (Figure 1). Total redberry juniper seedling emergence was 5.7% from the 10,000 seeds planted. These results suggest the presence of a dormancy mechanism, an after-ripening requirement, the presence of a germination inhibitor, and/or that high summer temperatures in the Edwards Plateau inhibit germination. Owens and Schliesing (1995) reported 0% germination of Ashe juniper seed collected from a soil seed bank and 5% for seeds collected from trees in the southeastern Edwards Plateau. Smeins and Fuhlendorf (1997) suggested that bacteria and other degrading factors destroyed Ashe juniper seeds buried 0.5 in. below the soil surface under field conditions after 18 mo Smith et al. (1975) reported greatest germination of redberry juniper seed collected in the Texas High Plains (Garza County) at 64E F (range 3.3 to 15% in 3 experiments with one seed lot ) and no or only limited germination at 50 and 81E F, respectively.



**Figure 1.** Seasonal percent (%) emergence of redberry juniper seedlings from 10,000 seeds harvested from 20 trees in December 1993 in Edwards County, TX and planted in pots in a greenhouse, 8 March 1994.

### **Laboratory Germination Study**

Seeds were abundant on the female redberry juniper trees sampled at the site near San Angelo in December 1996, presumably because total rainfall during 1995 and 1996 was 10 and 8% above the long-term average, respectively, and seasonal distribution was favorable (data not shown). Cones were rare or not present on trees at the site during the drought of 1997 through 2001. All mature junipers at the study site were killed by mechanical grubbing during 2002, thus preventing any quantification of year-to-year variation in germination of seeds from the marked trees. Because this experiment was not repeated temporally or spatially, the inferences presented herein apply specifically to the study site, the population of redberry juniper trees sampled, and the environmental conditions that existed during this study.

Mean germination at 3 mo postharvest was  $8.8 \pm 4.8\%$  (95% CI;  $n = 21$ ) at a constant 68E F, but only  $2.9 \pm 1.6\%$  ( $n = 21$ ) at an alternating 81/54E F (data not shown). No seeds germinated for 23 and 36% of the trees at the constant and alternating temperatures, respectively. Mean germination at 68E F 52 mo after harvest ( $8.8 \pm 3.8\%$ ) (range 0-24%;  $n = 21$ ) was not different than that at the same temperature 3 mo (range 0-36%) postharvest. The variability in seed germination among juniper trees suggests that large numbers of trees should be sampled to obtain reliable estimates of germination for a tree population. Seed germination at 68E F varied from 0 to 36% among trees even though rainfall was above average during development and maturation of the fruits. Warren (2001) concluded from visual examination of x-ray film of seeds from four tree populations that redberry juniper, as a taxon, produces fully developed, empty seeds. Seed fill was only 17.0, 9.5, 18.1, and 9.9% for seed harvested from tree populations at Palo Duro Canyon State Park, Justiceburg, San Angelo, and Salt Flat, Texas, respectively in September 1999 during severe drought conditions (National Oceanic and Atmospheric Administration [NOAA 2004]). Smeins and Fuhlendorf (1997) reported significant tree to tree variation in germination (0 to 55%) of Ashe juniper seeds harvested in the central Edwards Plateau. Our finding that germination was greater at a constant 68E F than at an alternating 81/54E F temperature regime suggests that germination of redberry juniper seeds may be enhanced during warm-moist periods when diurnal temperature fluctuations in the surface soil are minimal. Low germination within the temperature regimen that included 81E F in our study was consistent with findings of Smith et al. (1975) and Owens and Schlieshing (1995).

Average germination of seeds in our laboratory study (8.8%) was somewhat greater than that reported from other studies with cones (Smith et al. 1975) or depulped seeds (Warren 2001). Similar germination (8.8%) at 3 and 52 mo postharvest suggests that the embryos were mature when cones were harvested in December, and that dormancy mechanisms or germination inhibitors (if present) are stable over time at room temperature, and that seeds kept in dry storage may remain viable for several years. Warren (2001) reported 44% seed fill and 100% viability of embryos (determined by tetrazolium tests) in redberry juniper seeds harvested in early September, but only 14.8% seed fill and 72% embryo viability after these seeds were left on the soil surface for 22 mo. Smeins and Fuhlendorf (1997) reported no germination of Ashe juniper seeds planted 0.5 cm below the soil surface after 18 mo, suggesting that seed longevity is limited under field conditions. Data from our greenhouse seedling emergence trial indicate that redberry seeds in the soil seedbank may lose their germinability after about one year.

A significant postharvest interval  $\times$  scarification  $\times$  stratification interaction for

hand-harvested seeds ( $F = 3.0$ ;  $df = 6,120$ ;  $P = 0.010$ ) precluded comparing the main effects, so analyses were conducted separately for each postharvest interval. The stratification  $\times$  scarification interaction was significant ( $F = 4.2$ ;  $df = 6,60$   $P = 0.001$ ) at 4 mo postharvest, thus scarification effects were analyzed within each stratification treatment. The stratification  $\times$  scarification interaction was not significant at 10 mo postharvest, thus the main effects were compared. The 45-min sulfuric acid treatment apparently damaged many of the embryos (Table 1). This finding was inconsistent with Johnson and Alexander (1974), who reported that seeds of redberry juniper soaked for 45 min in concentrated sulfuric acid had slightly higher germination than seeds subjected to warm and cold stratification, but these authors did not indicate if the pericarp was removed prior to the acid treatment. Mechanical scarification of seeds following a cool/moist stratification enhanced seed germination at 4 mo postharvest, but did not affect germination of unstratified seeds. No scarification treatment enhanced germination of unstratified seeds at 4 mo postharvest or that of seeds in all stratification treatments at 10 mo postharvest. Germination at 10 mo postharvest averaged about 4% greater for seeds subjected to cool/moist stratification compared to that for seeds that received a cool/dry or no stratification treatment (Table 1). Cool/moist stratification enhanced seedling emergence of western juniper (*Juniperus occidentalis* subsp. *occidentalis* Hook.) and Utah juniper [*J. osteosperma* (Torr.) Little] (Young et al. 1988). This suggests that cool-moist stratification might overcome physiological dormancy mechanisms and/or dilute or eliminate germination inhibitors in the seed coat. Substantial new seedling recruitment might be expected during wet spring seasons that follow a cold, wet winter. There was little evidence that the seed coats of redberry juniper seeds must be scarified to facilitate imbibition and germination, especially after the seeds are about 1 y old.

The postharvest interval  $\times$  seed stratification interaction was not significant for germination of raccoon-ingested seeds, so the 4- and 10-mo postharvest data were pooled for analysis (Table 2). Mean seed germination was greater for cool/dry-stratified seeds than for unstratified seeds, with germination of cool/moist-stratified seeds being intermediate between these two treatments. Stratification treatments did not affect germination of bird-ingested seeds at 4 mo postharvest, but germination at 10 mo postharvest was greater for the cool/moist treatment compared to that for unstratified seeds (Table 2). Germination of bird-ingested seeds subjected to the cool/dry treatment was intermediate between that of unstratified and cool/moist stratified seeds. Germination of redberry juniper seeds ingested by raccoons and birds generally tended to be greater than that of untreated, hand-harvested seeds but similar to that of hand-harvested seeds that were cool/moist stratified and mechanically scarified (Tables 1,2). This indicates that ingestion by raccoons and birds may have removed germination inhibitors and provided a scarification treatment similar to our mechanical scarification. However, data from seeds harvested from trees were not compared statistically with that for ingested seeds for the reasons discussed above. Smith et al. (1975) reported that ingestion of redberry juniper seeds by small mammals did not affect germination, but they also collected seeds from the feces of free-ranging animals. Fuentes and Schupp (1998) reported that the seed-eating bird, plain titmouse (*Parus inornatus* Gambel) was more likely to prey on Utah juniper trees that had higher proportion of filled seeds. This may explain why redberry juniper seeds ingested by birds or raccoons in our study appeared more germinable than seeds we harvested from trees.

**Table 1.** Mean percent (%) germination ( $\nabla$  95% confidence interval) (68E F constant temperature) of redberry juniper seeds subjected to stratification and scarification treatments in 56-d trials at 4 and 10 mo postharvest in December 1996 from 21 trees near San Angelo, Texas.<sup>1</sup>

4 mo postharvest			
Treatments	Seed stratification		
	Cool/dry	Cool/moist	None
Scarification ( <i>n</i> = 6)			
	%		
45 min in sulfuric	3.3 c $\pm$ 3.4	0.7 c $\pm$ 1.1	2.7 $\pm$ 1.1
10 s in 212° F water	5.3 bc $\pm$ 3.9	0.7 c $\pm$ 1.7	5.3 $\pm$ 3.4
Mechanical	10.3 a $\pm$ 4.1	13.3 a $\pm$ 5.4	5.7 $\pm$ 3.1
None	9.0 ab $\pm$ 3.2	6.7 b $\pm$ 4.3	6.7 $\pm$ 2.9
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10 mo postharvest			
Stratification ( <i>n</i> = 24)	Mean		
	%		
Cool/dry	7.4 b $\pm$ 1.4		
Cool/moist	11.2 a $\pm$ 2.3		
None	7.4 b $\pm$ 2.0		
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Scarification ( <i>n</i> = 18)			
45 min in sulfuric	5.3 b $\pm$ 2.0		
10 s in 212° F water	9.0 a $\pm$ 2.6		
Mechanical	10.7 a $\pm$ 2.5		
None	9.7 a $\pm$ 1.6		

<sup>1</sup>Means within a column and treatment category followed by a similar lower case letter are not different according to LSD<sub>0.05</sub>.



**Table 2.** Mean percent (%) germination ( $\forall$  95% confidence interval) of stratified and unstratified redberry juniper seeds at 68E F constant temperature after collecting from raccoon (averaged over trials at 4 and 10 mo postharvest) and bird feces (at 4 and 10 mo postharvest) collected in December 1996 near San Angelo, Texas.<sup>1</sup>

Seed stratification	Raccoon ( <i>n</i> = 12)	Bird ( <i>n</i> = 6)	
		mo postharvest	
		4	10
		%	
Cool/dry	17.5 a $\pm$ 2.9	12.0 $\pm$ 5.8	9.7 ab $\pm$ 2.5
Cool/moist	15.2 ab $\pm$ 3.4	9.7 $\pm$ 4.7	14.5 a $\pm$ 4.4
None	12.4 b $\pm$ 2.6	14.0 $\pm$ 5.0	6.6 b $\pm$ 5.9

<sup>1</sup>Means within a column followed by a similar lower case letter are not different according to LSD<sub>0.05</sub>.

Warren (2001) found that four populations of redberry juniper in western Texas differed in DNA polymorphisms. Because it is not known how or if these polymorphisms might affect life history, metabolism, or reproductive efforts, inferences from this study should not be extended to other redberry juniper populations or to environmental conditions that differ from those encountered during this study.

### MANAGEMENT IMPLICATIONS

Our data and those of others are conclusive that average seed germination is relatively low for redberry juniper populations. However, a single tree can produce thousands of seed in a year of favorable growing conditions, so the deposition of viable seeds to the soil seedbank during these years would be significant. Data from our study did not reveal any major constraints to redberry seed germination after they ripen on the trees, but indicated that seeds in the soil may lose their ability to germinate in about one year. Seed dispersal to safe microsites during years of abundant cone production, combined with favorable climatic conditions (cool, wet spring or fall or mild, wet winter), provides the potential for invasion into new sites or re-establishment in areas where juniper populations have been controlled. McPherson and Wright (1990b) reported that redberry juniper seedlings established during the second year of a wet (above-average rainfall) 2-year period at two Rolling Plains sites in western Texas. Furthermore, above-average cool-season rainfall in successive years was not rare—occurring 10 times during the period 1950-1979 at the two study sites. The ability of certain species to respond to an episodic event, such as precipitation, or to treatments that remove conditions that inhibit germination/establishment is not uncommon and must be considered in long-term management strategies (Owens and Schliesing, 1995). Land managers should control redberry juniper plants before they reach reproductive maturity to minimize the deposition of viable seeds in the soil seed bank.

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