Title: Propagation of the Blue Diamond Cholla (*Cylindropuntia multigeniculata*), a rare cactus of the northeast Mojave Desert, USA

Authors: Sara Scoles-Sciulla, Alex Stosich, and Lesley DeFalco

Affiliation: US Geological Survey, Western Ecological Research Center, 160 N. Stephanie St., Henderson, NV 89074 USA

Abstract

Cylindropuntia multigeniculata (*Cactaceae*) is a rare cholla of the Mojave Desert, and we explored whether nursery cultivation can support vulnerable populations and inform management of the species. Joints were collected from adult plants at the type locality and grown in a shade house to promote root growth that might benefit out-planting survival: we tested two soil mixes that varied in the ratio of inorganic and organic components (50:50 and 85:15) and two watering frequencies (250 mL delivered every 5 d, and 500 mL every 10 d). Joint cuttings grown in the 50:50 soil mix produced plants with greater shoot and root biomass, more joint production, and higher survivorship over the 4-mo study. Neither soil nor watering treatments shifted biomass allocation to greater root-to-shoot ratios as intended, but frequent watering (250 mL delivered every 5 d) produced longer roots that may benefit reintroduced plants by establishing deep roots where soil moisture is more reliable in the desert environment. Despite their vigor during collection, freshly-cut joints declined in condition during callus development resulting in more than 50% mortality during the first month of establishment, which was not explained by the identity, condition, or size of the maternal plant. Other factors such as prior-year weather patterns and collection procedures may influence quality and durability of joint cuttings and requires further study. While vigorous plants from joint cuttings were produced

from the 50:50 soil mix, and rooting depth was promoted by frequent watering, reintroduction of nursery stock will verify whether such treatments influence establishment in the dry, stressful environments where this species occurs.

Introduction

Artificial propagation and reintroduction of rare or endangered plants can be an important aspect of mitigation measures and a valuable method for supporting wild populations (Maunder 1992). Propagation and reintroduction studies that evaluate requirements for survival and growth are lacking for rare *Cactaceae*, and no known propagation studies have focused on *Cylindropuntia multigeniculata* specifically. Cactus species can be propagated by seed (Rojas-Aréchiga and Vázquez-Yanes 2000), but erratic precipitation patterns in desert ecosystems can alter seed set and quality and make seed production unreliable (Montiel and Montaña 2003). *Cylindropuntia* species and similar cacti have been successfully propagated through stem cuttings (Fiedler 1991, Kelly 2009, Buhanan and Briggs 2011). Cuttings mimic *Cylindropuntia* species' ability to vegetatively reproduce when stem segments (joints) disperse from the parent plant and produce roots while lying in contact with the soil surface (Bobich and Nobel 2001, Allen et al. 1991, Rebman and Pinkava 2001). For some cactus species, joint dispersal may be the primary mode of reproduction (Allen et al. 1991, Bobich and Nobel 2001). A comparison of *ex situ* establishment of cuttings among 10 *Cylindropuntia* species and 15 *Opuntia* species from the southwestern U.S. showed that 32% of *Cylindropuntia* joints produced adventitious roots compared with 71% of *Opuntia* pads that rooted successfully (Evans et al. 2004). This rooting difference may reflect the larger pads of *Opuntia* species that store more water and carbohydrates and thus survive longer before establishment (Flores-Torres and Montaña 2012). Joint establishment is also correlated with the number of joints per stem, and species with fewer joint or pad segments per stem have a higher probability of rooting from terminal segments (Evans et al. 2004). The number of stem segments for *C. multigeniculata* (mature plants with 30 – 40 joints per stem; AS, pers. obs.) is comparable to *Cylindropuntia* species that have low rates of establishment from joints (*sensu* Evans et al. 2004). Species with a low number of segments per stem and high joint dispersal success may invest in producing large numbers of offspring, rather than permanence through individual longevity (Evans et al. 2004). Propagation success from cuttings may thus be dependent upon the species' growth form and life history.

In arid environments, the development of a strong and efficient root system is crucial for out-planting success (Rundel and Noble 1991), and propagation techniques can precondition plants, thereby increasing survival after reintroduction (Franco et al. 2010). High root-to-shoot ratios promote water conservation by reducing transpiration rates and allocating solutes to the root system to maintain the water gradient necessary for absorbing water from the soil (Lynch 1995, Canadell and Zedler 1995, Banon et al. 2006). *Cylindropuntia* species, and cactus species in general, have fibrous root systems with shallow lateral roots that rapidly develop short-lived root hairs in response to precipitation, allowing the plant to take advantage of seasonal rains while also tolerating periods of drought (Balandran-Quintana et al. 2018, Rebman and Pinkava 2001, Lynch 1995, Gdaniec and Grace 2019). Even large arborescent cactus species have a majority of roots within 30 cm of the soil surface, with an average root depth of 10 cm for many species, allowing the plant to benefit from small rain events (Gibson and Nobel 1986, p.147). Basic root structure varies, and some *Cylindropuntia* species are able to grow deeper and less-differentiated root systems with changes in watering, soil type, and soil volume (Cannon 1913).

Root development in cacti can be encouraged with good drainage, such as soil mixes composed primarily of pumice or perlite and small amounts of organic matter to retain moisture and provide nutrients (Kelly 2009). In desert ecosystems, nitrogen, phosphorus, and potassium are localized and recycled within "islands of fertility" around perennial shrubs, while other elements such as calcium, sodium, and magnesium are more abundant and evenly distributed in soils (Schlesinger et al. 1996, Titus et al. 2002). Plants can respond to low soil nutrients with enhanced root growth in order to maintain overall nutrient uptake (Franco et al. 2010). Conversely, organically rich greenhouse soils with high levels of nitrogen and phosphorus can decrease root growth and favor shoot growth, yielding low root-to-shoot ratios (Nobel et al. 1989, Bainbridge et al. 1995). Some cactus species can respond quickly to pulses of nitrogen fertilizer with increased shoot and root growth and increased root nitrogen concentrations, allowing them to survive long periods of stress while increasing responsiveness to transient resources (Buhanan and Briggs 2010). Pre-conditioning treatments during propagation are meant to reduce mortality after plants are transplanted under drought and heat conditions (Franco et al. 2011), although increased plant biomass overall may improve outplant survival to some degree.

Intermittent watering (i.e., more water delivered infrequently compared with less water delivered more frequently) can produce desired pre-conditioning effects in much the same way. Deep, infrequent irrigation can produce morphological and physiological benefits such as deeper rooting, decreased shoot growth, and improved drought tolerance (Qian and Fry 1996). Cactus species alternate between growth when water is available and vegetative stasis under dry conditions and are attuned to fluctuating soil water availability (Kelly 2009, Gdaniec and Grace 2019). However, cacti have shallow root systems that can respond quickly to small rainfall events through the formation of "rain roots" (Rebman and Pinkava 2001) which may result in increased root biomass with small, frequent waterings. Reduced watering amount, as opposed to intermittent watering, is a more-studied type of pre-conditioning treatment, which can create more robust and efficient root systems, lower shoot growth with an incidental increase in root to shoot ratio, and improved drought tolerance (Lu et al. 2014, Bañon et al. 2006, Fernández et al. 2006, Snyman 2004, Franco et al. 2010). These are important adaptations to consider when propagating plants for eventual out-planting into an environment where they will experience water stress.

We evaluated the use of *C. multigeniculata* joint cuttings as a viable propagation technique in a shade house study. We tested two soil mixes and two watering frequencies to determine a suitable combination to produce plants with high root-to-shoot ratio, increasing the chance of out-planting success. We hypothesized that plants grown in the high inorganic soil mix would have lower root and shoot biomass; however, treatments with both high inorganic soil mixes and infrequent watering regimens would produce plants with greater root-to-shoot ratios.

Methods

Cylindropuntia multigeniculata is a rare cholla cactus that grows in the northeastern Mojave Desert in southern Nevada and northwestern Arizona (Baker 2016). *C. multigeniculata* occurs in Joshua tree (*Yucca brevifolia ssp. jaegeriana*) woodland and Mojave Desert scrubland (*Larrea tridentata – Yucca* spp., *Larrea tridentata – Ambrosia dumosa*, *Coleogyne ramossisima – Yucca* spp. associations) on rocky limestone, basalt, granite, and rhyolite soils between 1035 m and 1400 m in elevation (Baker 2005, 2016). *C. multigeniculata* was federally listed as a candidate species under the Endangered Species Act in 1999 but removed from the candidate list in 2001 based on active management of lands and the execution of the multispecies conservation plan agreement (*C. whipplei* var. *multigeniculata*; USFWS 1999, 2001). However, the type locality at Blue Diamond Hill has been threatened by mining, residential development, and the proposed building of a hydroelectric plant over the past twenty years (Baker 2005). *C.*

multigeniculata is a Bureau of Land Management Special Status Species, fully protected in the State of Nevada and covered under Clark County's Multiple Species Habitat Conservation Plan (Nevada Natural Heritage Program 2001, RECON Environmental Inc. 2000).

Plant material

Joints were collected from *C. multigeniculata* plants on Blue Diamond Hill (36° 03' 13" N lat., 115° 24' 3" W long.) directly north of Blue Diamond, Nevada on June 11, 2020. The collection site has an average (± SD) annual precipitation of 239.4 ± 118.8 mm, average annual minimum temperature of 9.5 ± 0.7° C, and average annual maximum temperature of 23.4 ± 0.7° C (Oregon State University PRISM Climate Group). Plants were located on steep slopes and rocky ledges composed of a mixture of limestone, dolostone, gypsiferous red shale and claystone (Page et al. 2005).

Plants were verified as *C. multigeniculata* (Baker 2016), and four terminal joints were collected from each of 20 plants for a total of 80 joints. We selected only plants with 50 or more terminal joints to minimize damage to the individual (Fig. 1A). We measured the size of each maternal plant (greatest canopy diameter, perpendicular canopy diameter, and height to calculate volume of inverted cone), estimated the number of terminal joints, and rated plant condition on an ordinal scale (1 = no dead stems, 2 = some stems dead at base, 3 = whole stems dead). Terminal joints were removed at the nodes with a serrated knife, sanitized with 70% isopropyl alcohol between cuts, and placed in plastic containers at ambient temperature (30° C) for transport to a shade house (two-hour transit time). The base of each terminal joint was dusted with sulfur, and cuttings air dried in a well-ventilated shaded area for 25 d to ensure cuts formed a callus (Fig. 1B). Although only green joints were harvested, the condition of the joints changed while the callus formed; thus, each joint was rated on an ordinal scale 1 wk after collection (1 = entirely green, 2 = some browning at the callus, 3 = browning throughout the surface of the joint).

Experimental design

Prior to potting on July 6, joints were measured (joint height and greatest girth, excluding spines), and each of the four joints from a maternal plant was randomly assigned to one of four levels in a 2 (soil mix) × 2 (watering frequency) factorial experiment (20 replicate joints per treatment × 4 treatment levels = 80 joints). Random assignment ensured that estimated size of joints at the onset of the experiment was not different among treatments (one-way ANOVA on log-transformed volume, $F_{3,76} = 0.52$, $p = 0.07$). The two soil mixes differed in ratio of inorganic (⅛" pumice; General Pumice Products, Carlsbad, CA) and organic components (Organic Growers Potting Mix, Vermont Organics Reclamation, St. Albans, VT) and included 85:15 and 50:50 ratios of inorganic:organic. Each joint was situated in a 1-gal pot, buried to approximately ⅓ of its height, and supported with three wooden bamboo skewers (Fig. 1C). All pots were watered with 500 mL tap water administered 1 – 2 times per week during the first month after potting (4,500 mL total for each pot during first month). All pots experienced the same light and temperature at the USGS shade house in Boulder City, NV (Table 1).

After one month of establishment in the two soil treatments (August 13), 39 of the original 80 *C. multigeniculata* joints remained alive. We reassigned several pots to ensure equal representation of joint size and condition across the watering treatments (i.e., two pots reassigned from frequent to infrequent watering for both 50:50 and 85:15 soil treatments, and two pots reassigned from infrequent to frequent watering for the 85:15 soil treatment), resulting in 9 – 12 joints per treatment combination. Based on measurements on August 13, the remaining live joints had equal estimated size between the two watering treatments (two-factor ANOVA on log-transformed joint volume, $F_{1,35} = 0.08$, $p = 0.77$). Soil effects on joint volume resulted in 50:50 joints having 3.4 times the volume of those in the 85:15 soil mix at the start of the watering treatment (log-transformed, $F_{1,35} = 28.06$, $p < 0.01$). We initiated watering at two different frequencies while holding the total volume constant: 500 mL of water administered to each pot, as half the volume every 5 d (“frequent watering”) or the entire volume every 10 d (“infrequent watering”) (5,000 mL total for each pot during first month). Three pots from each soil × watering treatment combination were weighed before and after each watering to document water addition and water loss from pots for each treatment through time.

Joint size was measured shortly after collection (June 19), at the onset of watering treatments (August 13), and at the end of the study (November 20). Joint height (ht, in cm) and greatest width (wid, in cm) were used to calculate volume approximating a cylinder: volume = $\pi * (wid/2)^2 * ht$. At the onset of the watering treatment, joints that were still green but rotting at the base were recut, re-dusted with sulfur, and allowed to callus before being repotted. Joints that were completely tan, desiccated, and hardened before the onset of the watering treatments or midway through the study (October 2) were discarded. We estimated the water-holding capacity (WHC) of the soil treatments (*i.e.*, the amount of water retained and stored in soil after watering and subsequent drainage and is important to plant growth) by comparing oven-dried soils at the onset of the experiment with the experimental pots that had 150 mL and 350 mL added on July 6 and July 8, respectively, and weighing pots on July 15 before joints were actively growing: WHC = (weight of soil + pot – empty pot) / (weight of oven dried soil) * 100%.

Instead of destructively harvesting plants to determine biomass and root-to-shoot ratio, we used a gravimetric water displacement approach at the end of the study (November 20) to measure root and shoot volume as an indicator of plant biomass (Harrington et al. 1994, Pang et al. 2011, Burdett 1979). Whole plants were removed from pots by carefully loosening soil from roots to preserve root structure, rinsed with water, and patted dry with paper towels (Fig. 1F). Roots and shoots were separately inverted and immersed in a 2 L beaker of water set on a 5 kg balance (reading to 0.1 g). Weight of the displaced water approximates plant mass (1 g water = 1 cm^3). We measured maximum length of the roots, total number of joint segments, and the height and width of each joint. Joints that were yellow, browning, and not rooted were not measured. Joints were subsequently repotted into original soil mixes after weighing and watered to pot capacity. Saturated pots dried down for 17 d before we started watering at reduced frequency due to colder temperatures during the onset of winter: 250 mL every 10 d for "frequent" and 500 mL every 20 d for "infrequent." We are maintaining these plants at their assigned soil × watering levels for potential use in future out-planting or other research.

Statistical analyses

We used an information-theoretic approach (Burnham and Anderson 2002) to determine plausible explanations for joint mortality during establishment prior to implementing the watering treatments (July 13 – August 13) and during watering treatments (August 13 – November 20). We first compared several logistic models using the LOGISTIC procedure (SAS, version 9.4, Cary, NC) to determine which factors influenced survival during initial establishment. Models included: intercept only, separate models for initial size (joint volume), joint condition at planting (ordinal scale 1, 2, or 3), the soil and watering treatments and their interaction, and joint condition or volume as covariates with soil and watering treatments and their interaction. Treatment levels for the most significant model based on Wald's χ^2 were compared using odds ratios.

We compared survival models after watering treatments began by incorporating the same independent variables as described for establishment and computing an AICc value for each model using the LIFEREG procedure in SAS. Prior to model development, we selected the most appropriate distribution type (i.e., lognormal) for the failure model by comparing AICc values of intercept-only models. Multicollinearity did not occur among variables used within the same model based on Pearson's $|r| < 0.75$ and variation inflation factors < 10 (Neter et al. 1996). The importance of each variable (a value ranging from 0 to 1 for least to most important) was derived by summing the Akaike weights (w_is) across all candidate models where the variable occurred (Burnham and Anderson 2002).

We used two-factor ANOVA (soil mix × watering frequency) to analyze soil moisture dynamics and joint growth. We analyzed water addition and water loss totaled across the initial establishment and post-watering treatment phases. Effects of soil mix and watering frequency were tested on plant responses including relative growth rate of whole plant (RGR, d^{-1}), root and shoot biomasses (g), root-to-shoot ratio (R:S, $g\ g^{-1}$), maximum root length (cm), and number of joints (R, version 3.6.1, Vienna, Austria or SAS, version 9.4, Cary, NC). RGR was calculated based on Blackman (1919) where initial biomass was derived from water displacement extrapolated to dimensions measured on June 19, and final biomass was the actual water displacement on November 20. Assumptions of normality and equal variance were verified prior to analysis using D'Agostino-Pearson Normality test-and Levene's test, respectively. Root length and root-to-shoot ratio were log-transformed prior to analysis to meet the assumptions of equal variance. Joint count data were analyzed by comparing model fits for Poisson and negative binomial models of soil and water effects and selecting the best model based on deviance statistics (GENMOD procedure in SAS).

Three joints remained green but did not root by the end of the experiment (November 20) and were excluded from statistical analyses of joint growth and post-establishment survival. New growth on one joint was eaten by a woodrat (*Neotoma lepida*) at the beginning of the experiment, but bird netting over the joints and regular live-trapping resulted in no further incidents. Furthermore, exclusion of this plant did not change the statistical outcome for survival ΔAICc values or for any growth response, thus, the plant was included in all analyses.

Results

Shade house temperatures were 2 – 3° C warmer than the summer and fall temperatures at Blue Diamond Hill where *C. multigeniculata* was collected, reflecting the lower elevation of Boulder City (Table 1). Maximum daily temperatures (± SD) at the shade house were highest in July (40.3 ± 0.6° C) and August (41.1 ± 1.7° C) and minimum daily temperatures were lowest toward the end of the experiment (October, 17.1 ± 1.4° C; November, 7.2 ± 0.9° C). Precipitation at Blue Diamond Hill during the nine months preceding joint collection was 42 mm lower than average and highly variable based on comparison to 30-year normals for the same location (Oregon State University PRISM Climate Group). Monthly precipitation was greater than the upper 95% CI during November 2019 and March 2020 and was less than the lower 95% CI for October 2019 and January, February, May and June 2020 (Fig. 2).

During initial establishment when the same volume of water was delivered to all pots, the two soil mixes had the same amount of total water added ($F_{1,5} = 2.38$, $p = 0.20$) and water lost between waterings ($F_{1,5} = 0.58$, $p = 0.49$) – as determined through weighing pots before and after water addition (Table 2). The total water over the first month of establishment was 16% to 19% lower than the 4,500 mL added per pot, reflecting the small drainage of water accumulated over waterings. Although we also delivered the same total volume of water for each pot once we started the watering treatments (5,000 mL), 7% – 15% of water drainage occurred over 3 mo. The frequent watering treatment had more water delivered than infrequent watering, likely due to accumulated measurement error for the smaller volume of water ($F_{1,11} = 32.94$, $p < 0.01$); frequent watering also lost more water between waterings as plants grew ($F_{1,11} = 54.66$, $p < 0.01$) (Table 2). Less water was retained in the 85:15 mix when watered ($F_{1,11} = 8.03$, $p = 0.02$) due to drainage through the greater inorganic component, and greater water loss than the 50:50 soil mix ($F_{1,11} = 13.82$, $p < 0.01$). The difference in water holding capacity of soils was not statistically different between soil mixes ($F_{1,5} = 2.70$, $p = 0.13$).

Plant survival prior to and after watering treatments

Initial survival of joints measured before watering treatments were implemented (39 of 80 joints = 49%) was principally influenced by joint condition (condition index was best model with all other models with ΔAICc >>2; Table 3). Joints that were initially rated as green were 19.929 times as likely to survive as those rated with some browning of the callus and 147.250 times as likely to survive as those rated as fully brown (Wald's $\chi^2 = 25.30$, $p < 0.01$). Other models tested did not significantly influence initial survival (Table 3).

Survival of the remaining joints after 3 mo of watering treatments was 56% (20 of 36 joints), and soil mix was the most influential factor (w_is = 0.9909, summed over all candidate models). Joint survival was 2.1 times higher for plants in the 50:50 soil mix compared with the 85:15 mix (Fig. 3). Several models containing both soil mix and watering frequency had some support, but these models all included soil mix (summed w_is = 0.9909; Wald's $\chi^2 = 11.337$, $p < 0.01$), while watering frequency had far less support (summed w_is = 0.4828; Wald's $\chi^2 = 1.004$, $p = 0.32$). Initial joint condition (summed w_is = 0.0873) and initial joint volume (summed w_is = 0.1012) were not well-supported as explanations for survival in the most plausible models (Table 3).

Growth and biomass allocation

Joints grown in the 50:50 soil mix had greater shoot biomass ($F_{1,16} = 15.98$, $p < 0.01$) and root biomass ($F_{1,16} = 6.53$, $p = 0.02$) after 4 mo compared with joints grown in the 85:15 mix regardless of watering frequency (Fig. 4). However, root-to-shoot ratio was not significantly different among soil and watering treatments (log-transformed R:S; soil, $F_{1,16} = 0.30$, $p = 0.59$; water, $F_{1,16} = 3.83$, $p = 0.07$; soil × water, $F_{1,16} = 0.19$, $p = 0.67$). Joints with frequent watering had significantly increased root length (log-transformed root length, $F_{1,16} = 6.00$, $p = 0.03$) compared to joints grown with infrequent watering, regardless of soil mix (Fig. 4C). Whole-plant relative growth rate was more complex, with joints having similar growth rates across soil mixes with frequent watering, but with infrequent watering, joints in the 85:15 soil mix had significantly lower relative growth rates than joints in the 50:50 soil mix (soil mix × watering frequency, $F_{1,16} = 6.42$, $p = 0.02$; Fig. 5). The number of joints at the end of the experiment was significantly higher for the 50:50 soil mix (soil effect best fit model, Deviance = 0.9512, Pearson's $\chi^2 = 1.0324$): the number of joints for plants in 50:50 soil (3.5 joints) was 2.311 ± 0.83 times the number of joints for plants in 85:15 soil (1.5 joints) (Wald's $\chi^2 = 5.40$, $p = 0.02$).

Discussion

Cylindropuntia multigeniculata can be propagated from joint cuttings, but the condition of the collected joints is key to initial establishment while the soil medium played an important role in post-establishment survival and growth. Even though we collected green terminal joints during summer when plants were active, the surface color of some joints changed from green to brown during development of the callus. Our index of joint condition demonstrates that during one month of initial establishment, declining green tissue explained the lower survival better than joint size or soil mix. In addition, joint condition was not correlated to the identity, size or condition of the maternal plant nor was joint condition of maternal plants clustered spatially within the population (data not shown). Over-collecting terminal joints to overcome the more than 50% initial mortality and increase the probability of vigorous joints is not desirable given the sensitive status of this rare *Cylindropuntia*. Further study is needed to understand whether certain seasonal and interannual precipitation and temperature patterns impair joint condition so that timing of collections can optimize joint quality and improve nursery propagation. Improved collection and pre-planting practices may enhance joint vigor, such as whole-joint disinfection before callusing to reduce fungal growth (Sims et al. 2016) and transporting cuttings in a cooler to reduce cutting stress (St. John et al. 2009). We partially buried joint cuttings to offer them some support, but resting the cuttings on the soil surface or supporting them upright with the callus in contact with the soil surface has been used in successful joint propagation for other *Cylindropuntia* species (Holthe and Szarek 1985) and may reduce rot by limiting the surface area of the unrooted joint in direct contact with moist soil. With low overall rooting of joints after 4 mo in our study (25%), application of rooting hormones may also improve establishment of *C. multigeniculata* joints by encouraging rooting before cuttings desiccate (Alabaster 1996).

We collected joints in June when root development for potted cacti is favored by nighttime temperatures above 15.6° C (Kelly 2009), such as the temperatures from May through September at the Boulder City shade house (30-yr normal, Oregon State University PRISM Climate Group). Cactus joint cuttings are treated as softwood cuttings and collected during spring or early summer when plants are actively growing (Luna 2009). New stem growth for *C. multigeniculata* occurs from late spring to fall (Baker 2005). Further study is needed to elucidate whether the timing of collection across the active growing season and collecting actively growing versus previous years' terminal joints improve joint condition. Our collection of joints in June 2020 followed a period of sporadic rainfall (Figure 2). Most *C. multigeniculata* fruits produced in summer 2020 at the Blue Diamond Hill population had low seed set, with many aborted seeds (AS, pers. obs.). The lack of rainfall in January and February 2020 may have also reduced vegetative growth for the species and reduced the mobilization of resources into stems needed to establish new plants from joints (Beatley 1974).

We intended to administer the same total volume of water across watering treatments and only vary frequency to disentangle the two effects that are sometimes confounded in irrigation experiments. Varying watering amount rather than frequency may have produced different results as reduced watering can also improve drought tolerance, produce more robust root systems, and increase root-to-shoot ratios (Franco et al. 2010, Franco et al. 2011, Lu et al. 2014, Fernández et al. 2006, Bañon et al. 2006). Holding the frequency constant while lowering the amount of irrigation may provide more insight into how *C. multigeniculata* allocates biomass during drought or stressed conditions.

Cylindropuntia multigeniculata cuttings survived better and had increased root and shoot biomass in soils with higher levels of organic matter (50:50) despite recommendations for propagating cacti in high inorganic soils (Neisen (no date), Alabaster 1996). *C. multigeniculata* joints that survived initial establishment were already an average of three times larger after 1 mo when grown in the high organic soil, and this impact carried through to increased survival and greater above-ground and below-ground biomass after 4 mo. Our watering and soil treatments, however, did not promote allocation of biomass to increase root-to-shoot ratios for the cuttings.

The greater survival and growth of *C. multigeniculata* cuttings in the 50:50 mix suggests that nurse plants, where soil organic matter and nutrients are concentrated (Schlesinger et al. 1996, Titus et al. 2002), may promote survival and growth of joint cuttings after out-planting. Seedlings of other *Cylindropuntia* species often establish under nurse plants (Cody 1993, Flores-Torres and Montaña 2012, Martínez-Berdeja and Valverde 2008) where they primarily benefit from greater water availability (Martínez-Berdeja and Valverde 2008). Other cactus species, including *Cylindropuntia* spp., studied in association with shrub and perennial grass species emphasize the various benefits of nurse plants including lower physiological stress (Badano et al. 2016), protection from extreme temperatures (Nobel and Bobich 2002), joint capture and shading (Cody 1993), enhanced soil fertility (Méndez et al. 2004), and protection from herbivores (Mandujano et. al.1998). Joint establishment in inter-canopy areas is variable (Flores-Torres and Montaña 2012), and factors influencing joint establishment may differ from those influencing seedling survival and establishment. Some *Cylindropuntia* joints, like those of *C. leptocaulis*, establish exclusively under nurse plants, while others like *C. imbricata* establish readily in inter-canopy areas (Flores-Torres and Montaña 2012). The life history of *C. multigeniculata* may also prioritize individual longevity and/or sexual reproduction over asexual reproduction, making propagation from seed a supplemental area of study for this rare species. The number of stem segments on *C. multigeniculata* (30 – 40 per stem) is similar to other *Cylindropuntia* species with low levels of joint establishment (Evans et al. 2004), consistent with our 25% establishment and survival of joints 5 mo after collection over all treatments. The mechanics of joint stress failure have been used to help describe the regeneration strategy of cacti (Evans et al. 2004), but this has not been studied for *C. multigeniculata* in particular.

Although our soil and watering pre-conditioning treatments did not increase biomass allocation to roots, frequent watering did produce longer roots. Thus, infrequent watering appears to produce shorter roots in greater density or diameter in contrast to frequent watering producing longer, potentially sparser or thinner, roots. The cuttings grown in organic soils (50:50) with frequent watering had greater root biomass and length, but these cuttings also had greater shoot biomass, which can reduce survival under water stress if above- and belowground demands are out of balance. Reintroduction studies will ultimately determine whether propagation pre-conditioning treatments translate into long-term survival in protected desert habitat. Cacti may be largely inflexible in the proportional allocation of root and shoot biomass with their wide environmental tolerance and ability to take advantage of transient resources allowing changes in relative growth rate with minimal change in root-to-shoot ratios (Martínez-Berdeja and Valverde 2008). Desert succulents can also survive arid conditions with relatively low root-to-shoot ratios compared with non-succulents due to the water storage capacity of their shoots and an ability to produce new roots quickly after rains (Jordan and Nobel 1984). In this study, we did note an increase in RGR for joints growing in the high organic soils (50:50) with infrequent watering compared to joints grown in low organic soil with infrequent watering, but this difference did not carry over to the frequent watering treatment and we found no significant change in root-to-shoot ratios for all soil and water treatments.

Propagation of *C. multigeniculata* from joint cuttings provides a viable option for conservation of the species with promise for reintroduction into protected habitats, especially when unpredictable seed production and viability typical of desert environments may limit propagation from seed. The challenges of joint condition still need to be understood when harvesting joints from *C. multigeniculata* populations. However, the use of a 50:50 mix of inorganic:organic components can enhance survival of nursery stock and promote growth of root and shoot tissues; frequent irrigation using small volumes of water is preferred over infrequent watering of greater volume to enhance rooting depth. An understanding of habitat requirements for the species in combination with the optimal season of reintroduction and the potential benefits of nurse plants will promote conservation efforts of the species before it becomes critically vulnerable.

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Table 1. Mean (± SD) monthly temperatures for collection site at Blue Diamond (30-yr normal, Oregon State University PRISM Climate Group) and USGS shade house in Boulder City, Nevada during study period (2020).

	Blue Diamond (1,067 m elev)	Boulder City (770 m elev)
	36°03’20.6”N 115°24’12.7”W	35°58’44.3”N 114°50’28.9”W
	° C	
June	25.5 ± 0.14	28.3 ± 0.78
July	30.0 ± 0.49	32.0 ± 0.35
August	30.8 ± 1.77	33.2 ± 0.99
September	26.0 ± 1.27	28.9 ± 0.71
October	20.1 ± 1.91	23.9 ± 1.91
November	11.3 ± 0.71	13.8 ± 0.07

Table 2. Water dynamics of soil treatments prior to watering treatment implemented (Pre-watering period = July 6 – August 13) and after implementation (Post-watering period = August 13 – November 16). Water dynamics were not significantly different between soil mixes before watering was implemented. Differences after watering was implemented are denoted with different lowercase letters (watering frequency main effect) and uppercase letters (soil mix main effect) (see text for statistical results). No interactions were significant. Weight of water (g) = mL.

	Water added (mL)	Water lost (mL)	WHC (%)
Pre-watering treatment			
15:85 soil mix	3647 ± 51	-3090 ± 12	16.83 ± 1.90
50:50 soil mix	3764 ± 56	-3039 ± 66	24.24 ± 4.09
Post-watering treatment			
85:15 Infrequent	4228 ± 66 Aa	-3936 ± 61 Aa	n/a
85:15 Frequent	4570 ± 40 Ab	-4259 ± 38 Ab	n/a
50:50 Infrequent	4431 ± 53 Ba	-4051 ± 59 Ba	n/a
50:50 Frequent	4638 ± 20 Bb	-4563 ± 65 Bb	n/a

Table 3. Comparison of ordinal logistic regression models for survival of *C. multigeniculata* joints prior to initiating watering treatment (July 6 – Aug 13) and comparison of failure-time models for survival of joints during watering treatment (Aug 13 – Nov 20). Models are ranked by ΔAICc. Only those models with some level of support (ΔAICc < 7) and the intercept model for comparison are presented. Akaike weights (w_i) are included for comparing the relative importance of the initial joint and treatment attributes. Joint and treatment attributes: Joint condition index (CI), joint volume (vol), maternal plant (mat), soil mix (soil), and watering treatment (water).

Survival Prior to Watering Treatment[a]

Model	AICc	ΔAIC	w_i
Intercept	112.9053	45.8534	0.0000
CI	67.0518	0.0000	0.8963
CI Soil|Water	71.3648	4.3130	0.1037

Survival During Watering Treatment[b]

Model	AICc	ΔAIC	w_i
Intercept	57.316	9.678	0.0040
Soil	47.638	0.000	0.5101
Soil Water	49.191	1.553	0.2347
Soil Water Vol	51.355	3.717	0.0795
Soil Water CI	51.571	3.933	0.0714
Soil|Water	51.894	4.256	0.0607

[a] Analysis prior to watering treatment (1 month) included all 80 *C. multigeniculata* joints collected.

[b] Analysis during watering treatment included the 39 *C. multigeniculata* joints remaining alive after 1 month, minus 3 joints that never rooted during the experiment even though the shoots remained green and succulent (N = 36).

Figure captions

Figure 1. Terminal joints of *C. multigeniculata* were collected from adult plants on Blue Diamond Hill, Nevada (1A), dusted with sulfur (1B), and dried in a shade house for 25 d before planting under two soil and two watering treatments (1C). Potted joints were arranged on a bench at the USGS Boulder City, Nevada shade house (1D), and growth was measured on actively growing plants for the duration of the experiment (1E) before being removed from pots to assess root and shoot growth (1F).

Figure 2. Precipitation prior to joint collection at Blue Diamond Hill, Nevada. The shaded area represents 95% CI around monthly 30-year normals, and the line represents monthly rainfall preceding collection in June 2020. Data from Oregon State University PRISM Climate Group.

Figure 3. Survival functions for the best explanatory variable, soil mix (highest weight in model with lowest AICc value), for *C. multigeniculata* joints at the greenhouse after watering treatments were initiated on 13 August 2020 (N = 36).

Figure 4. Measurements of *C. multigeniculata* joints (lsmean ± SE) after 4 mo in the USGS shadehouse: (A) shoot biomass and (B) root biomass by soil mix, and (C) root length by watering schedule. Root length lsmean and SE values are back-transformed for clarity. Statistical differences at the $p < 0.05$ level are denoted by different lowercase letters within each graph.

Figure 5. Relative growth rate (lsmean ± SE) over soil and watering treatments for *C. multigeniculata* joints at the USGS shadehouse in Boulder City, Nevada from 6 July through 20 November 2020. Statistical differences at the $p < 0.05$ level are denoted by different lowercase letters within the graph.

Figure 1

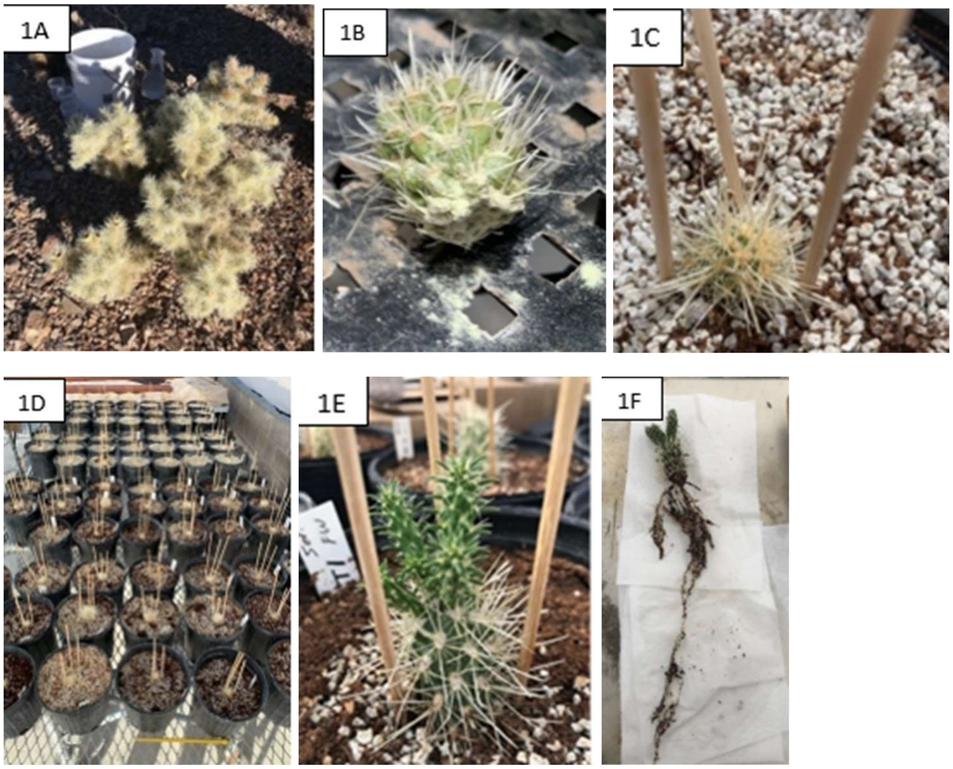


Figure 2

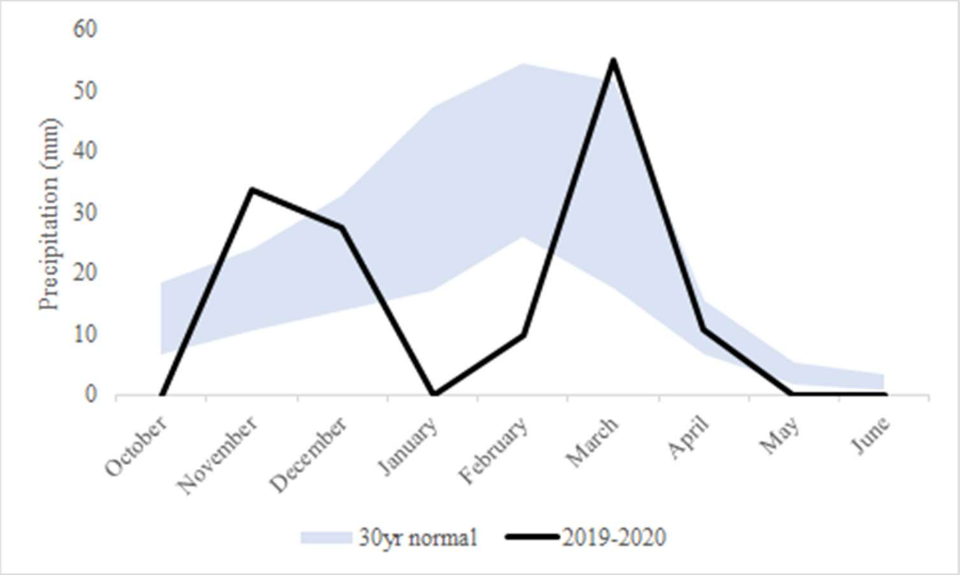

60
50
40
30
20
10
0
Precipitation (mm)
October
November
December
January
February
March
April
May
June
30yr normal
2019-2020

Figure 3

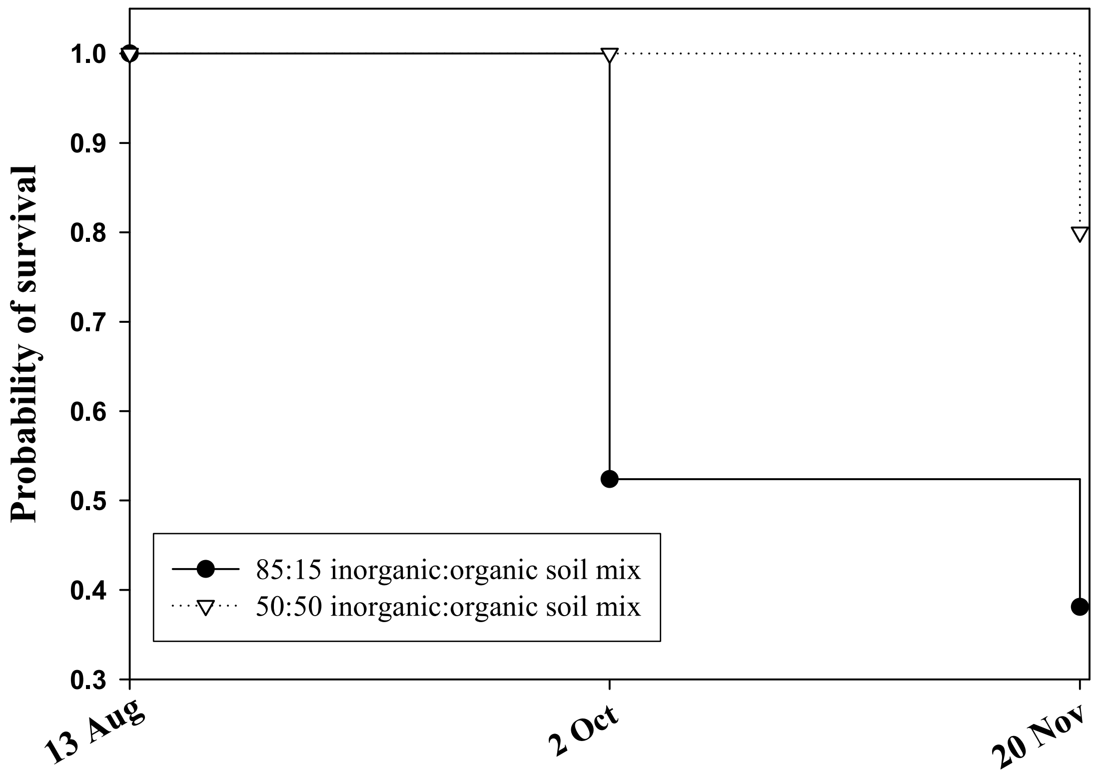

Probability of survival
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
13 Aug
2 Oct
20 Nov
85:15 inorganic:organic soil mix
50:50 inorganic:organic soil mix

Figure 4

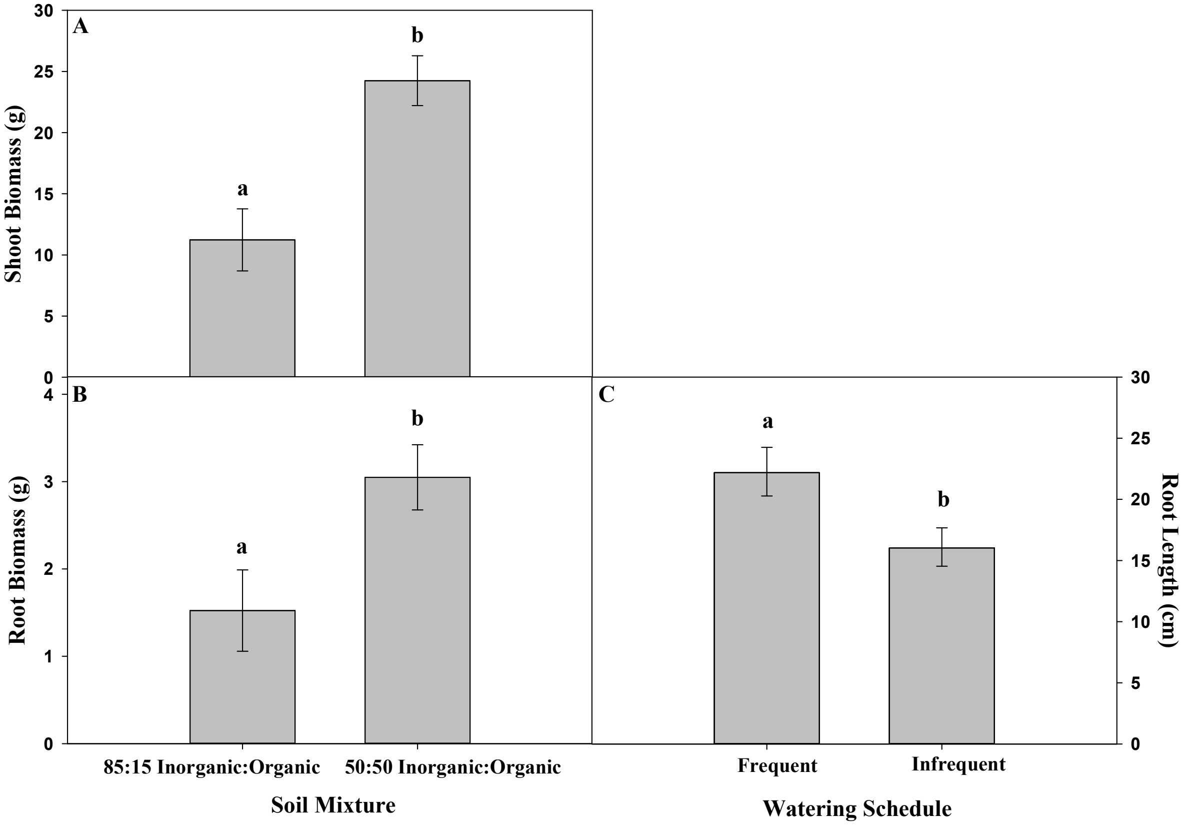
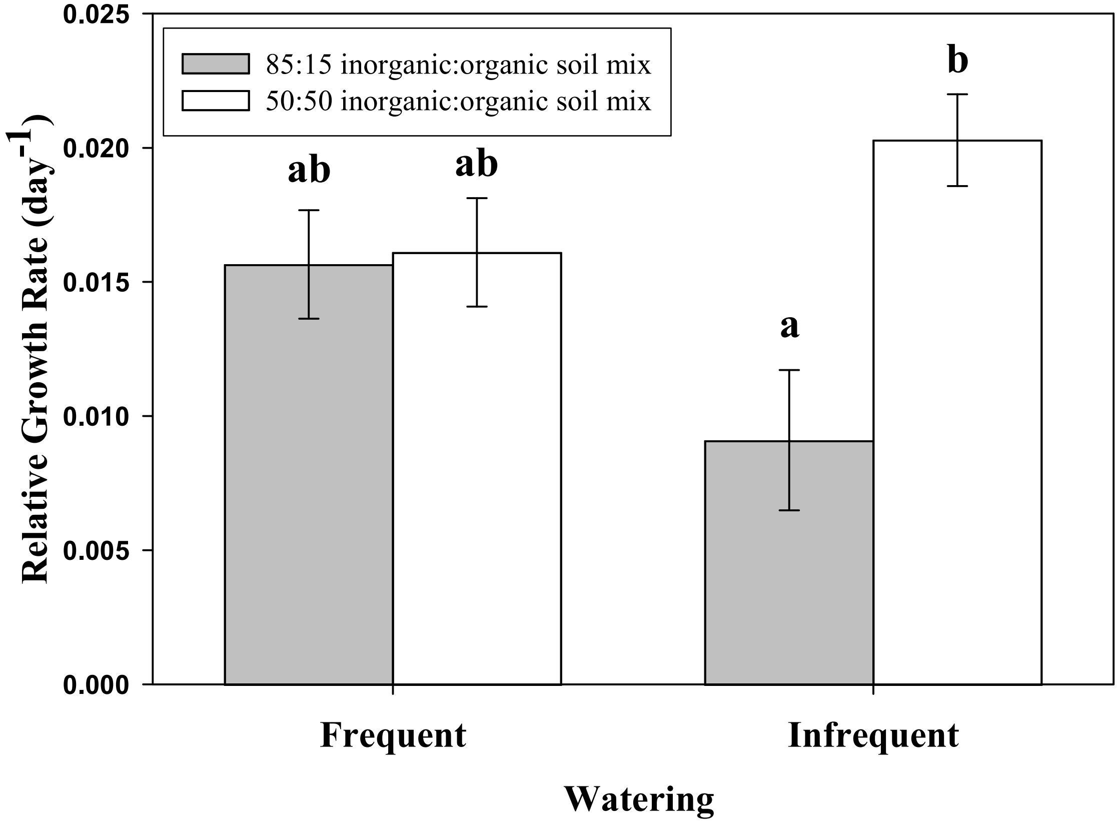

A
Shoot Biomass (g)
30
25
20
15
10
5
0
a
b
B
Root Biomass (g)
4
3
2
1
0
a
b
85:15 Inorganic:Organic
50:50 Inorganic:Organic
Soil Mixture
C
Root Length (cm)
30
25
20
15
10
5
0
a
b
Frequent
Infrequent
Watering Schedule

Figure 5


0.025
0.020
0.015
0.010
0.005
0.000
Relative Growth Rate (day^{-1})
85:15 inorganic:organic soil mix
50:50 inorganic:organic soil mix
ab
ab
a
b
Frequent
Infrequent
Watering