Executive Summary

Dehesa nolina (*Nolina interrata*) and variegated dudleya (*Dudleya variegata*) are narrow endemic species that are priorities for management under the San Diego Management and Monitoring Program's Management Strategic Plan for western San Diego County. Both species declined precipitously on the South Crest Preserve after fire and subsequent invasion by nonnative plants. To stabilize and increase populations, CBI conducted a 3-year program that included seed collection, nursery propagation, and outplanting of nursery-grown plants into restored habitat. The Dehesa nolina population is one of the three largest in the U.S. and thus critical for species persistence. Propagation and outplanting of this species had not been demonstrated previously in a natural setting.

Dehesa nolina mass-flowers following fire but flowers only sporadically in the absence of fire. We collected 93 seeds in 2013 and 14 seeds in 2015 for propagation. No seeds were produced in 2014. We obtained an additional 150 seeds from Rancho Santa Ana Botanic Garden (RSABG) that had been collected near the site and held in long-term storage.

A majority of the field-collected seeds were malformed and inviable. Only 6 germinated and 5 were grown successfully for 2 years before outplanting. Of the 150 RSABG seed, 135 germinated and 66 were grown successfully for 1 year before outplanting. Plant losses in the nursery were due to aphids and root rot.

Low levels of seed production and inviable seed were likely due to (1) high rates of insect herbivory, particularly early in the season, and (2) low number of flowering plants. Dehesa nolina is insect-pollinated and presumably requires a sufficient number of male and female plants in proximity, although there may be other factors governing production of viable seed.

Recon Environmental, Inc. outplanted 71 Dehesa nolina plants onto South Crest in November 2015. We provided supplemental water, weeded planting basins through August 2016, and monitored plant survivorship through November 2016. Over this period, 51 (72%) plants survived, including 5 (100%) 2-year old plants and 46 (70%) 1-year old plants. Nearly a third of all plant mortality (30%) occurred within a few weeks of planting, and may have been due to transplant shock and/or immature root systems. Another 25% of plant mortality occurred prior to the onset of supplemental watering, and the remaining plants were lost during the driest, hottest part of the year (June-August).

Based on low levels of seed production and viable seed in the absence of fire or other disturbance, and relatively high outplanting success, we recommend the following:

1. Develop a permanent Dehesa nolina seed source (seed bank) that consists of both conservation and propagation collections.

2. Collect seed during mass-flowering events (i.e., post-fire).
3. Conduct additional studies during mass-flowering events to determine (a) breeding system, (b) effective pollinators, (c) herbivory threat, and (d) population ratios, and use this information to assist with long-term Dehesa nolina management and conservation.
4. Grow Dehesa nolina plants in a nursery setting for a minimum of 2 years before outplanting to maximize survivorship.
5. Protect outplanted individuals with wire cages and/or treepees for at least 2 years after planting to minimize herbivory and maximize survivorship.
6. Provide supplemental water, and weed planting basins of outplanted Dehesa nolina individuals for at least 2 years after planting.

In contrast, seed viability for variegated dudleya was relatively high, and nursery production of plants was successful with no major plant mortalities. Recon Native Plants, Inc. has developed Best Management Practices for outplanting variegated dudleya, which led to high plant establishment and survivorship. Recommendations to ensure continued success of outplanted individuals in the field include:

1. Maintain caging around variegated dudleya plants to protect from herbivory, but modify the cages to accommodate plant growth and maintenance activities.
2. Continue hand-weeding nonnative grasses and other emergent invasive species within and around planting cages, beginning in late fall 2016.
3. Provide supplemental irrigation during drought or low rainfall years only for plants that emerge naturally to allow them to survive.

In addition, we recommend enhancing the small, naturally-occurring variegated dudleya population on the western slope of Skeleton Flats with nursery-grown plants as resources and funding are available.

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Introduction

The Conservation Biology Institute (CBI), in collaboration with the Endangered Habitats Conservancy (EHC), Earth Discovery Institute (EDI), Recon Native Plant Nursery (RNP), and Recon Environmental, Inc. (Recon), conducted a 3-year program to enhance populations of two narrow endemic species, Dehesa nolina (*Nolina inter*rata) and variegated dudleya (*Dudleya variegata*), on the South Crest Preserve (South Crest) in San Diego County, California. Both species are covered under the San Diego Multiple Species Conservation Plan (MSCP) and are priority species for management under the San Diego Management and Monitoring Program's (SDMMP) Management Strategic Plan (MSP) for western San Diego County (SDMMP 2013).

This work described in this document was part of a larger effort to develop regional management goals and objectives for Dehesa nolina in accordance with the regional MSP, and supports preserve-level management actions for both species. The project was funded by a *Transnet* Environmental Mitigation Program (EMP) grant from the San Diego Association of Governments (SANDAG).

Background

Setting

South Crest is located in Management Unit 3 (MU 3) of the Management Strategic Plan Area (MSPA) (SDMMP 2013; Figure 1). The preserve is owned and managed by EHC, and includes a portion of Dehesa Mountain and lands to the west. Together with other EHC acquisitions in the vicinity, the preserve is part of the Greater Crestridge Ecological Reserve (GCER) Complex.

Species augmentation was focused in that portion of South Crest known locally as Skeleton Flats (Figure 2). This area is relatively level to gently sloping, supports clay and gabbro-derived (gabbroic) soils, and has a history of disturbance, including dryland farming, off-road vehicle activity, and repeated fires. At the project onset, vegetation on Skeleton Flats was dominated largely by the invasive grass, purple falsebrome (*Brachypodium distachyon*), and by scrub associations with a high percentage of *Brachypodium* in the understory (CBI 2014).

In 2011, CBI conducted baseline biological surveys on South Crest that included mapping and identifying threats and stressors to sensitive plant species (CBI 2012). We compared results to mapping conducted prior to the 2003 Cedar and Dehesa fires, which cumulatively burned the majority of the preserve (REC Consultants, Inc. 2004). Results indicated that approximately 15-20 percent (%) of the Dehesa nolina population onsite was eliminated by fire, primarily in and around Skeleton Flats. Variegated dudleya numbers also declined after the fires, although this is likely related to post-fire habitat invasion by *Brachypodium*. Prior to the fire, an estimated 350

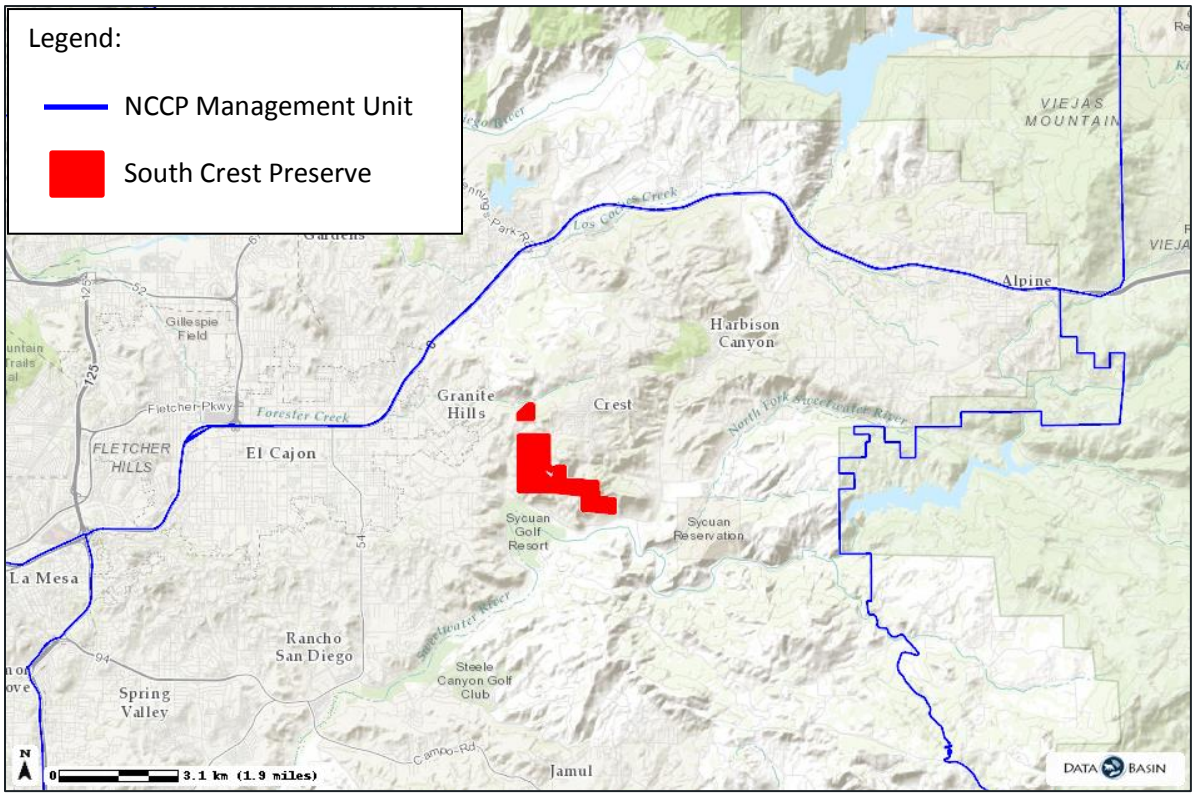

Figure 1. Project Location.

variegated dudleya occurred on Skeleton Flats and were part of a larger, offsite population[1] (ca. 3,550 individuals pre-fire, REC Consultants, Inc. 2004).

The Dehesa Mountain–South Crest population of Dehesa nolina is one of the three largest Dehesa nolina populations in the U.S. (CBI 2015). The majority of plants in this population occur on conserved lands within South Crest; thus, it is referred to as the South Crest population in the SDMMP's Master Occurrence Matrix (MOM) database. South Crest also supports one of the easternmost occurrences of variegated dudleya. Because of the regional importance of these populations, we proposed species augmentation to increase and buffer populations from existing threats and stressors, as well as future catastrophic events or environmental stochasticity.

Primary threats and stressors within South Crest are repeated fire and habitat invasion by nonnative plants, particularly *Brachypodium* in coastal sage scrub and native grasslands on clay and gabbroic soils. Nonnative forbs are also a concern in selected areas. Other threats include altered hydrology and subsequent erosion that undercuts individual Dehesa nolina plants, roads and trails, and recreational activity. Feral pig activity has been observed onsite, but appears to have been transient. Herbivory may impact Dehesa nolina reproduction. Some of these threats,

[1] The offsite population was on private lands at the onset of this project; however, these lands have since been acquired and conserved by EHC and will become part of the GCER Complex.

Figure 2. Skeleton Flats, South Crest Preserve.

such as invasive plants and altered fire regime, are exacerbated by landscape-level stressors such as climate change and nitrogen deposition.

Management is ongoing on South Crest to control *Brachypodium* and other invasive plants. Erosion control and access control measures (including signage, fencing, and road and trail closures) have been implemented under this grant and a separate *Brachypodium* control grant (SANDAG EMP grant 5001965). Efforts to identify and control herbivory are discussed in this document.

Goals and Objectives

This project contributes to regional goals of increasing Dehesa nolina and variegated dudleya resilience and long-term persistence by augmenting these species on portions of South Crest where individuals were lost in the 2003 wildfires and/or eliminated or suppressed by subsequent nonnative plant invasions. Methods for increasing and stabilizing populations included seed collection, nursery propagation of plants, and outplanting of nursery-grown plants into restored habitat. Actions under this grant complement ongoing *Transnet*-funded invasive control/habitat

restoration efforts on South Crest; techniques developed/refined in this project will be applicable to other conserved populations.

Three questions were addressed in this study:

1. Does Dehesa nolina produce viable seed in the absence of fire or other disturbance?
2. Can sufficient amounts of Dehesa nolina seed be collected in the absence of fire or other disturbance to produce adequate amounts of seed for species augmentation?
3. Can Dehesa nolina be propagated in a nursery-setting and outplanted successfully?

Variegated dudleya propagation and outplanting methods had been pioneered previously and implemented successfully by Recon, so there was less uncertainty about augmentation methods.

Species augmentation under this grant completes portions of the following MSP objectives for Dehesa nolina:

- Best Management Practices (BMP, regional)
- Seed Bank (SB, regional)
- IEX (population expansion, regional and/or local) (CBI 2015).

Augmentation is also a MU3 management objective for variegated dudleya populations that are threatened by eventual extirpation due to small population size or other factors (SDMMP 2013).

Dehesa Nolina Augmentation

Methods

Seed Collection

Dehesa nolina seed collection was a multi-step process that included identifying, protecting, and monitoring flowering plants to promote seed formation and determine the optimal time for seed collection.

Plant Identification. During baseline biological surveys in 2011-2012, we observed several flowering Dehesa nolina plants on South Crest, despite the lack of a recent burn.[2] Based on these observations, we conducted weekly surveys in late spring through summer 2013, 2014, and 2015 to identify flowering individuals on South Crest. We identified 6 flowering individuals in 2013, 1 flowering individual in 2014, and 12 flowering individuals in 2015. Individuals detected

[2] Dehesa nolina mass flowers following fire, but flowers only sporadically in the absence of fire or other disturbance (Dice 1988, USFWS 1995, Rombouts 1996).

in 2015 were flagged, numbered, and mapped using a Geographic Positioning System (GPS) (Figure 3).

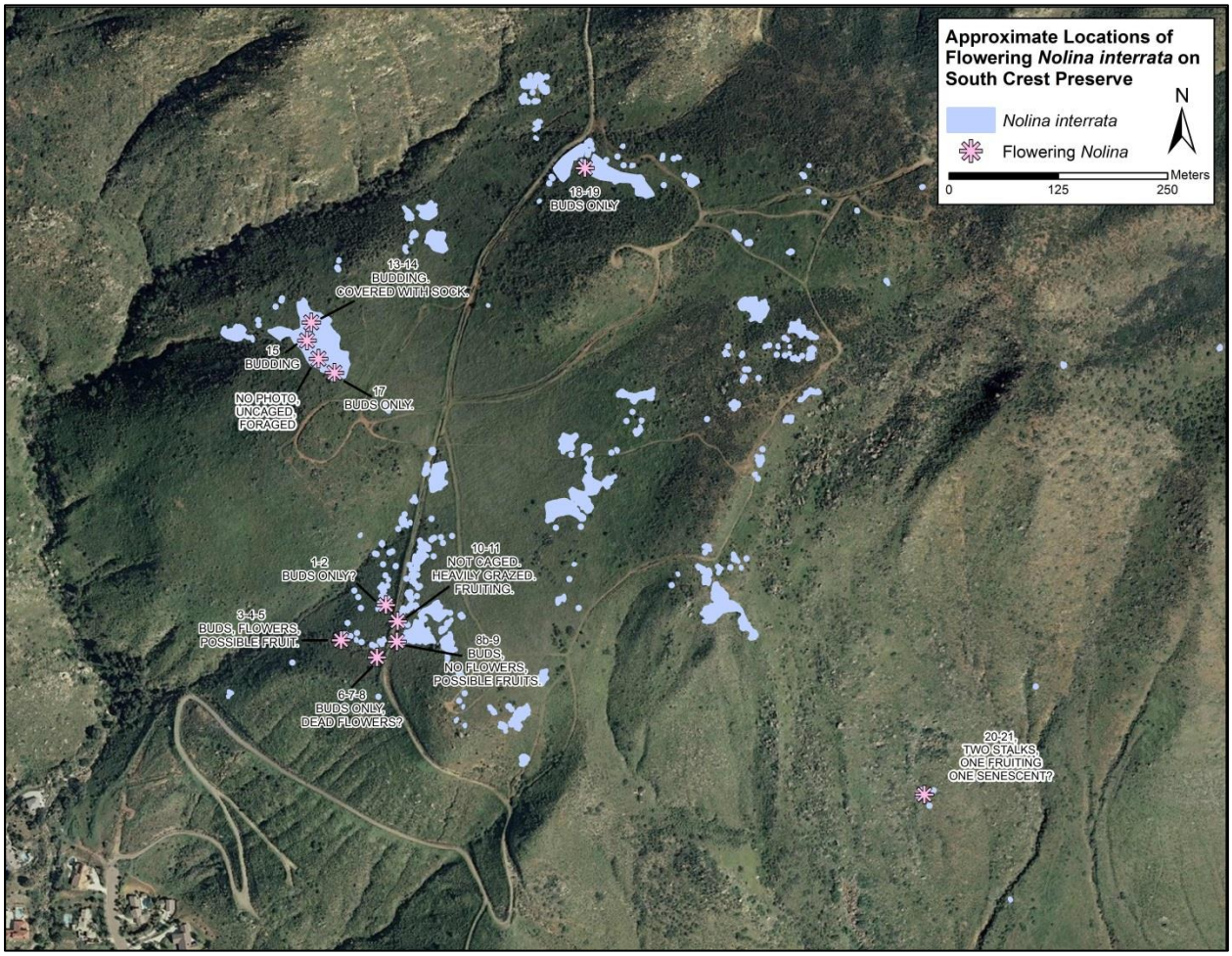

Figure 3. 2015 Flowering Dehesa Nolina Plants on the South Crest Preserve.

Plant Protection. CBI biologists protected all flowering plants on the preserve except for those few plants that were completely herbivorized or senesced early in the growing seasons. Protective measures varied by phenology and included wire cage installation, inflorescence protection (cheesecloth or organza bags), and pesticide application.

We protected emerging inflorescences from wood rat and mule deer herbivory by surrounding flowering plants with chicken wire cages (Figures 4a,b). We secured the wire cages by first installing wooden stakes into the ground and then attaching the wire to the wooden stakes. For taller flowering individuals, a second protective cage was attached to the top of the bottom cage.

Figure 4. Wire Cage Protection around Flowering Dehesa Nolina Plants: (a) wooden stakes with attached wire, (b) stacked protective cages.

We protected flowering individuals from insect herbivory to maximize seed production. In 2013, we wrapped and secured cheesecloth around some of the inflorescences to prevent insect damage. However, the cheesecloth held moisture and may have promoted mold formation on flowers and fruits. In 2014, the only flowering individual on the preserve was herbivorized completely before protection measures could be installed.

In 2015, we switched from cheesecloth to lightweight organza fabric bags to protect inflorescences and eliminate the mold issue. While we were able to purchase small and medium-sized organza bags that fit on inflorescence branches, we had to make (sew) large organza bags to protect entire inflorescences (Figures 5a, b). As part of this effort, one individual was bagged[3] early to protect developing flowers from insect predation, while inflorescences on the other

[3] CBI biologists prepared and installed 'pollination bags' on selected flowers/inflorescences. These bags prevented insect access to flowers but allowed sunlight and air circulation to ensure fruit development and inhibit mold or other disease that might adversely affect flower/fruit formation.

Figure 5. Pollination Protection Bags on Flowering Dehesa Nolina Plants: (a) large bag enclosing entire inflorescence, (b) small bag enclosing inflorescence branch.

flowering individuals were bagged after flowers opened and had a chance to be pollinated. Flowers on one individual appeared to be dead, possibly as a result of insect predation.

In 2013, we treated heavy beetle infestations on unprotected flowers with an organic pesticide spray. While dense beetle infestations are clearly destructive, these beetles may also be responsible for flower pollination.

Plant Monitoring. We monitored flowering Dehesa nolina plants between May-September 2013 and May-August 2015. CBI biologists took notes on inflorescence health, herbivory, and fruit formation during each monitoring visit, and photographed plants throughout the monitoring effort.

Seed Collection. Once fruit began to develop, we monitored capsule formation weekly or bi-weekly, and collected mature capsules once they were light brown in color, very dry (papery), and ready to drop off the plant on their own (Figure 6a,b). In 2013, we collected seeds up until

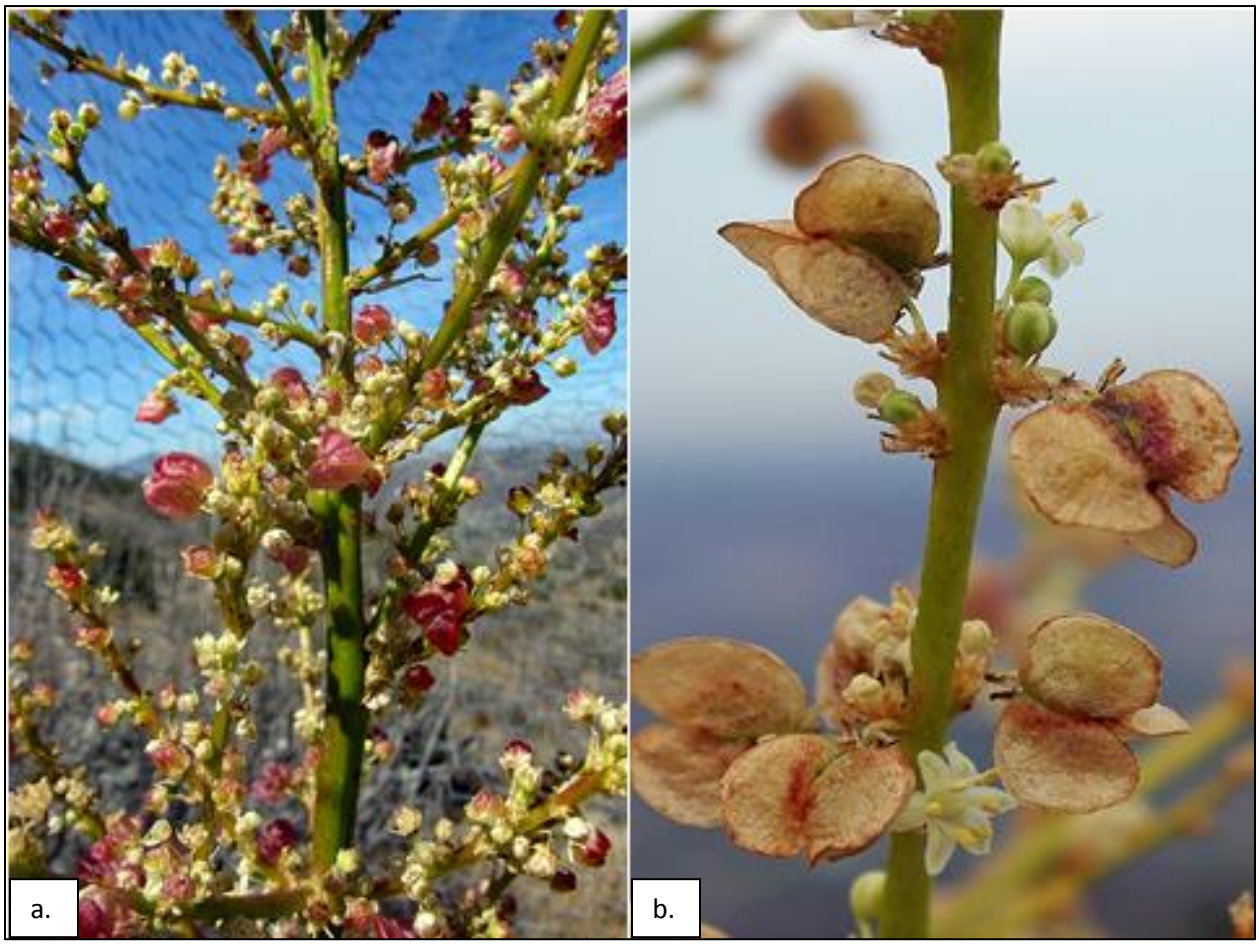

Figure 6. Dehesa Nolina Capsule (Fruit) Formation: (a) flowers and developing capsules, (b) nearly ripe capsules.

September 25th. No seeds were collected in 2014, when only one flowering nolina was detected on South Crest. Despite caging this plant and bagging the inflorescence once capsules began to form, this individual did not produce mature capsules or seed. In 2015, our latest seed collection date was August 31st. All seed collected on South Crest in 2013 was deposited at RNP for use in propagation.

Because the amount of seed collected in 2013 and 2014 was below target goals, CBI biologists contacted Rancho Santa Ana Botanic Garden (RSABG) regarding the possibility of acquiring Dehesa nolina seed for contract growing. We procured additional seeds from Evan Meyer, Seed Conservation Program Manager at RSABG in fall 2014 (Table 1); this seed sample was authorized by Cherilyn Burton of the California Department of Fish and Wildlife (CDFW) in an electronic email exchange (9/4/2014). In September 2014, RSABG sent 150 Dehesa nolina seeds to RNP. The RSABG seed was collected in 1990 in proximity to South Crest, and had been in long-term storage (RSABG lot 292, accession 16351). Although the provenance of this

Table 1. Rancho Santa Ana Botanic Garden Seed Information.

Attribute	Specific Collection Information
Taxon	*Nolina interrata*
Collection Date	1990
Collection Location (County)	San Diego
Regulatory Status	State Endangered/CNPS List 1B
Lot Number	292
Accession Number	16351
Seed Quantity	150

seed is not entirely clear, the two potential sources are either (1) McGinty Mountain or (2) the Dehesa Road population. Based on this information, the seed was determined appropriate for use onsite. Genetic studies conducted by James Heaney, a doctoral student at the University of Florida, demonstrated that the McGinty Mountain population of Dehesa nolina was genetically similar to plants on South Crest (J. Heaney pers. comm.). Furthermore, the Dehesa Road population is part of the larger Dehesa population that includes the plants on South Crest.

Floral Morphology and Sex Ratio

While examining plants in the field for flower production, we assessed floral morphology to determine sex of the flowering individuals. This study included collecting and dissecting a small number of flowers.

Propagation

On December 11, 2013, RNP propagated 25 of the 93 field-collected Dehesa nolina seeds by soaking in warm water for 24 hours to soften the seed coat, then placing on filter paper under a shade structure. Germinated seeds were sown into flats of native soil per the RNP established protocol for a related species, Bigelow's nolina (*Nolina bigelovii*). In late spring 2014, plants were moved to containers. The remaining 68 field-collected seeds were malformed and inviable.

On December 15, 2014, RNP propagated an additional 150 RSABG-acquired seeds. Seeds were soaked prior to planting and then sowed directly into D40 containers in a mixture containing 50/50 peat to perlite. The extra perlite helped with soil drainage; 2013 propagation efforts indicated Dehesa nolina is sensitive to excess moisture. RNP kept the soil moist during the seed and seedling stage, but once germination occurred, they allowed the soil to dry between watering events. RNP watered the plants in the nursery three times per week. Both 2013 and 2014-germinated plants were grown in the nursery until November 2015.

Outplanting

On November 2, 2015, Recon delivered all nursery-propagated Dehesa nolina plants to South Crest for outplanting into the field (Figure 7a,b). CBI biologists met with Recon biologist Mark Dodero, the Recon field crew and EHC director Michael Beck on South Crest to identify suitable planting sites and oversee the outplanting effort.

Figure 7. Nursery-grown Dehesa Nolina Plants for Outplanting onto South Crest: (a) 1-year old plant, (b) 2-year old plant.

The Recon field crew planted Dehesa nolina, created plant protectors, and watered in plants using a truck-mounted water tank and buckets. It rained lightly the night of the installation.

Planting holes were dug in suitable soils on South Crest in an area that had supported Dehesa nolina prior to the 2003 Cedar and Dehesa fires (Figures 8a). Watering basins around plants captured supplemental water and natural rainfall. Recon installed protective wire cages (chicken wire) around each Dehesa nolina plant; EDI and EHC subsequently installed plastic cones (treepees) around each plant inside the wire cage (Figure 8b).

Figure 8. Dehesa Nolina Planting: (a) planting holes, (b) wire cages around newly planted Dehesa nolina with treepees in background.

A one to two-inch gap was left between the bottom of the treepee and the soil surface to allow for air circulation. The treepees were intended to protect Dehesa nolina plants from the elements, while the wire cages protected plants from herbivores. Recon applied approximately 2 gallons of water per plant immediately following installation.

After planting, CBI established a supplemental watering schedule. We coordinated with EHC and EDI to ensure that plants were watered on a regular basis, at least during periods of low to no rainfall. EHC provided use of a mobile water tank to provide an adequate source of water. In general, each plant received approximately one to two gallons of water per watering period.

Monitoring

CBI established a monitoring schedule to track the health of outplanted Dehesa nolina; monitoring occurred approximately monthly between planting (November 2015) and June 2016,

and less frequently thereafter. During monitoring periods, we recorded survival and general health of the plants, photographed representative individuals, and weeded around planting basins.

Results

Seed Collection

We collected 98 Dehesa nolina seeds in 2013 and 14 seeds in 2015. A majority of the collected seeds were malformed (Figure 9, Tables 2 and 3). Of the 98 seeds collected in 2013, 93 were delivered to RNP (3 seeds were obviously inviable). RNP determined that only 25 of the 93 seeds (26%) were possibly viable. All of the 2015 seeds were malformed and inviable.

Figure 9. Dehesa Nolina Seed. Malformed seed from South Crest with viable seed in inset (viable seed photo: John McDonald, Rancho Santa Ana Botanic Garden Seed Image Database).

Table 2. 2013 Dehesa Nolina Seed Monitoring and Collection.

Nolina Plant #	2013 Monitoring Dates							
	August 9	August 15	August 22	August 31	September 6	September 11	September 19	September 25
1	Capsules healthy. Cheesecloth removed & replaced with one layer.	Collected 4 seeds (malformed).	Collected 3 seeds (still green). Seeds green and plump inside additional capsules.	---	Seeds green and plump, but drier and lighter color than 8/22.	Collected 2 seeds (1 healthy; 1 very small).	Collected 9 seeds (8 healthy and 1 very small). Capsules healthy; no mold.	Collected 28 seeds (healthy).
2	Capsules mostly healthy; some mold. Cheesecloth removed & replaced with one layer.	Collected 1 seed (malformed). Most inflated capsules have dropped; all contain small, shriveled (malformed) seeds.	---	---	Collected 16 seeds (malformed). Capsules moldy.	Collected 1 seed (malformed). Capsules moldy.	Collected 5 seeds (malformed). Capsules moldy.	Collected 5 seeds (malformed). Capsules moldy.
3	Capsules healthy. Cheesecloth removed, replaced with one layer. Inflorescence previously sprayed with pesticide.	Collected 5 seeds (malformed).	3 seeds collected (malformed).	13 seeds collected (malformed).	---	---	---	---

Table 2. 2013 Dehesa Nolina Seed Monitoring and Collection.

Nolina Plant #	2013 Monitoring Dates							
	August 9	August 15	August 22	August 31	September 6	September 11	September 19	September 25
4	Inflorescence dead; previously sprayed with pesticide.	---	---	---	---	---	---	---
5	Capsules healthy; flowers still present.	2 capsules present. Most flowers drying up and falling off.	---	---	---	---	---	---
6[1]	---	---	---	---	Collected 2 seeds (green). Seeds green and plump; some capsules falling off of inflorescence.	Collected 1 seed (malformed).	---	---

[1] Plant not located until September 6.

Table 3. 2015 Dehesa Nolina Seed Monitoring and Collection.

Nolina Plant #	2015 Monitoring Dates					
	June 9	June 22	July 3	July 16	August 4	August 31
1	Organza bags placed over some flowering racemes. Inflorescence previously sprayed with pesticide.	No capsules. All flowers drying up and falling off. No difference between protected and unprotected racemes.	---	---	Collected several capsules to determine if seed forming; no seed yet.	Many capsules formed but no seed formed in any capsules. All capsules very small, unlike capsules supporting seed. Most flowers dried up and fell off (lying in bottom of organza bag).
2	Some small capsules formed (protected with organza bag). Most flowers are dry, unopened, and falling off. Inflorescence previously sprayed with pesticide.	Capsules still present in the organza bag. All flowers are dry and falling off on both unprotected and protected racemes.	---	---	Collected several capsules to check on seed; seed not ready yet.	10 seeds collected (malformed).
3	Inflorescence previously sprayed with pesticide.	Almost completely herbivorized by insects (beetle and caterpillar located on racemes).	All flowers dry and falling off racemes. No capsules formed.	---	---	---

Table 3. 2015 Dehesa Nolina Seed Monitoring and Collection.

Nolina Plant #	2015 Monitoring Dates					
	June 9	June 22	July 3	July 16	August 4	August 31
4	Some small capsules formed (protected with organza bag). Most flowers are dry and falling off. Inflorescence previously sprayed with pesticide.	No capsules formed on unprotected racemes. Most flowers are dry and falling off.	---	1 healthy capsule. Others are dry and malformed. All flowers dry and falling off.	---	Many capsules formed, but only 3 seeds collected (malformed).
5	Inflorescence completely destroyed by insects. No flowers or capsules. Inflorescence previously sprayed with pesticide.	---	---	---	---	---
6	Buds present and some flowers open.	Capsules forming inside and outside of organza bags.	Capsules forming inside and outside of organza bags.	Capsules present, but not large and plump.	All capsules and flowers dry and dead.	---
7	Some flowers are open. Top portion of inflorescence covered and protected prior to flowering. Lower racemes left unprotected.	All flowers in protective bag are drying up and falling off. Unprotected racemes are flowering and insects (black beetles) were observed.	All flowers dried up and fell off in protected portion. No capsules formed.	---	No capsules produced either inside or outside of the protective bags.	---
8	Some flowers open, but already drying up and falling off. Protected some	Some very small capsules observed on protected racemes.	All flowers drying up and falling off.	No capsules present and inflorescence is senescing.	No capsules.	---

Table 3. 2015 Dehesa Nolina Seed Monitoring and Collection.

Nolina Plant #	2015 Monitoring Dates					
	June 9	June 22	July 3	July 16	August 4	August 31
	racemes with organza bags.					
9	Flower buds are drying up. Some small capsules located. Protected some racemes with organza bags.	Some capsules developing in both the protected and unprotected bags. Most flowers are unopened, dry and falling off.	---	Small capsules present.	---	Small capsules collected, but no seeds inside of capsules.
10	Some capsules formed.	Many large capsules formed. All appear large and healthy.	---	Covered inflorescence with protective bag.	Healthy capsules present.	Collected 10-15 medium-sized capsules but no seeds were found in any capsules. Capsules were smaller than capsules that contain viable seeds (based on photos and notes from 2013).
11	---	Inflorescences senesced. Dry flowers on ground and some still attached to racemes.	---	---	---	---
12	---	Capsules forming. Partially herbivorized.	2 healthy capsules present and many small capsules (without seed).	Protected healthy capsules with organza bag.	---	1 seed collected (malformed)

Most of the Dehesa nolina flowers on South Crest dried up and failed to form capsules. In 2013, two plants on South Crest produced copious amounts of capsules, but these inflorescences were completely herbivorized by deer before we could protect them with wire cages. We observed black and yellow striped beetles (possibly, western striped cucumber beetles) in large numbers and an undetermined species of caterpillar (Figure 10a) feeding on the flowers of nolina plants early in the season. In some cases, these insects completely destroyed early-flowering inflorescences (Figure 10b), but did not pose a problem for later-flowering individuals, likely because they had completed their life cycle and were absent or present in lower numbers later in the season. Heavy ant infestations early in the season also caused inflorescences to senesce prior to fruiting. Insects observed on Dehesa nolina later in the season, such as bordered plant bug (*Largus californicus*) (Figure 10c), did not appear to adversely impact flowers or capsules, possibly because of lower numbers.

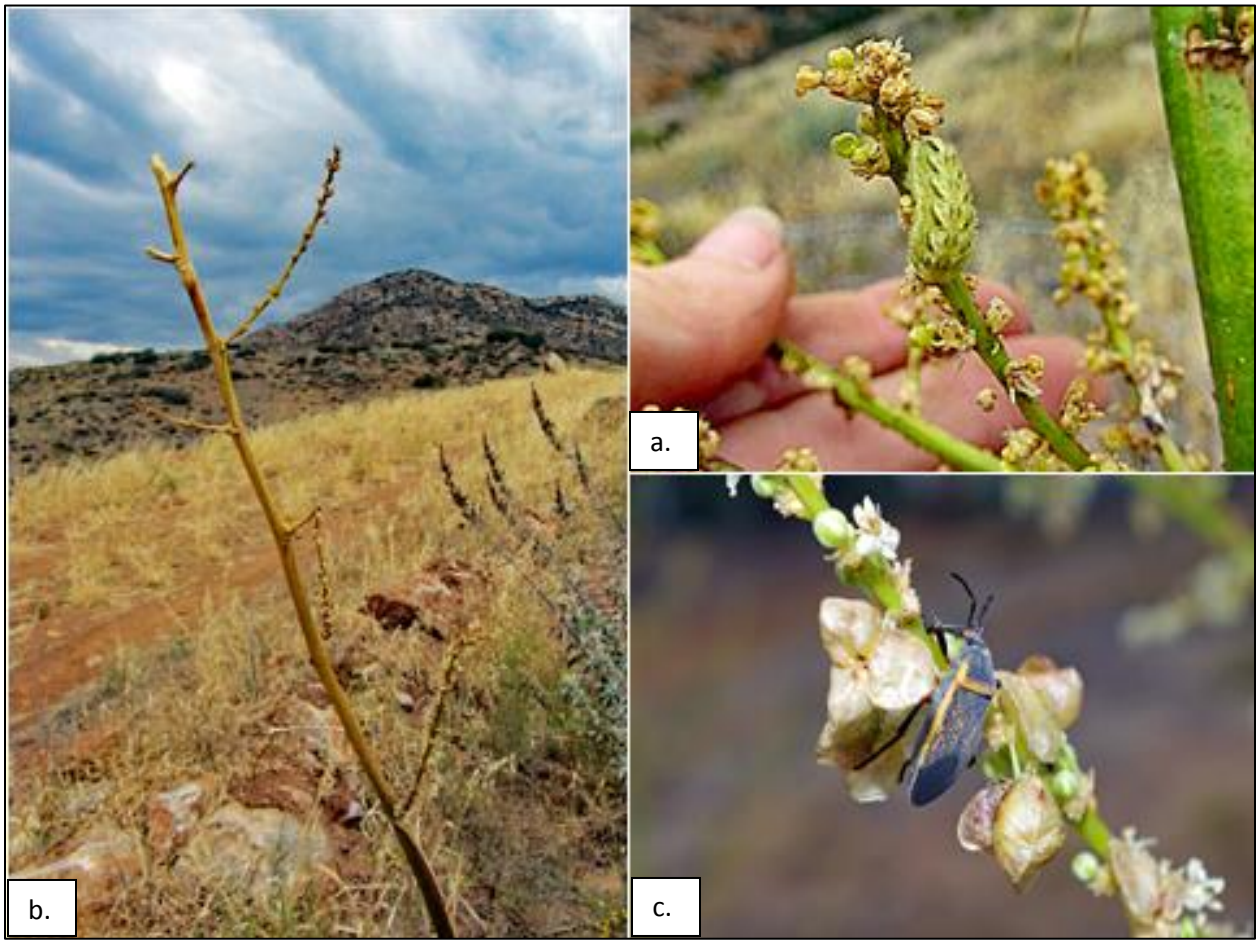

Figure 10. Insect Herbivory on Dehesa Nolina: (a) undetermined species of caterpillar, (b) inflorescence completely herbivorized by insects, (c) bordered plant bug late in season with mature capsules.

We presume that high levels of herbivory inhibited seed formation, although other factors likely played a role in both seed production and seed viability, as well. We noted some seed formation on inflorescences that survived beetle infestations, and lack of seed formation in some inflorescences that were completely protected from (presumably pollinating) insects.

In 2015, we observed a stand of Dehesa nolina on McGinty Mountain that produced multiple flowering inflorescences and capsules (Figures 11a,b). Eight inflorescences in the stand produced healthy capsules with well-formed seed. Conversely, three inflorescences produced no seed and flowers were drying and dropping from the plants (similar to plants on South Crest) (Figures 12a,b). This stand occurs within a fuel break adjacent to a residence. The homeowner had cleared habitat within the fuel break but did not remove the nolina plants. Upon closer inspection, we observed cut stems on plants that produced well-formed (presumably, viable) seed (Figure 13), but no cut stems on plants that produced no seed. Mechanical damage to the plants may have stimulated flowering and/or seed production. We saw no deer herbivory in this stand.

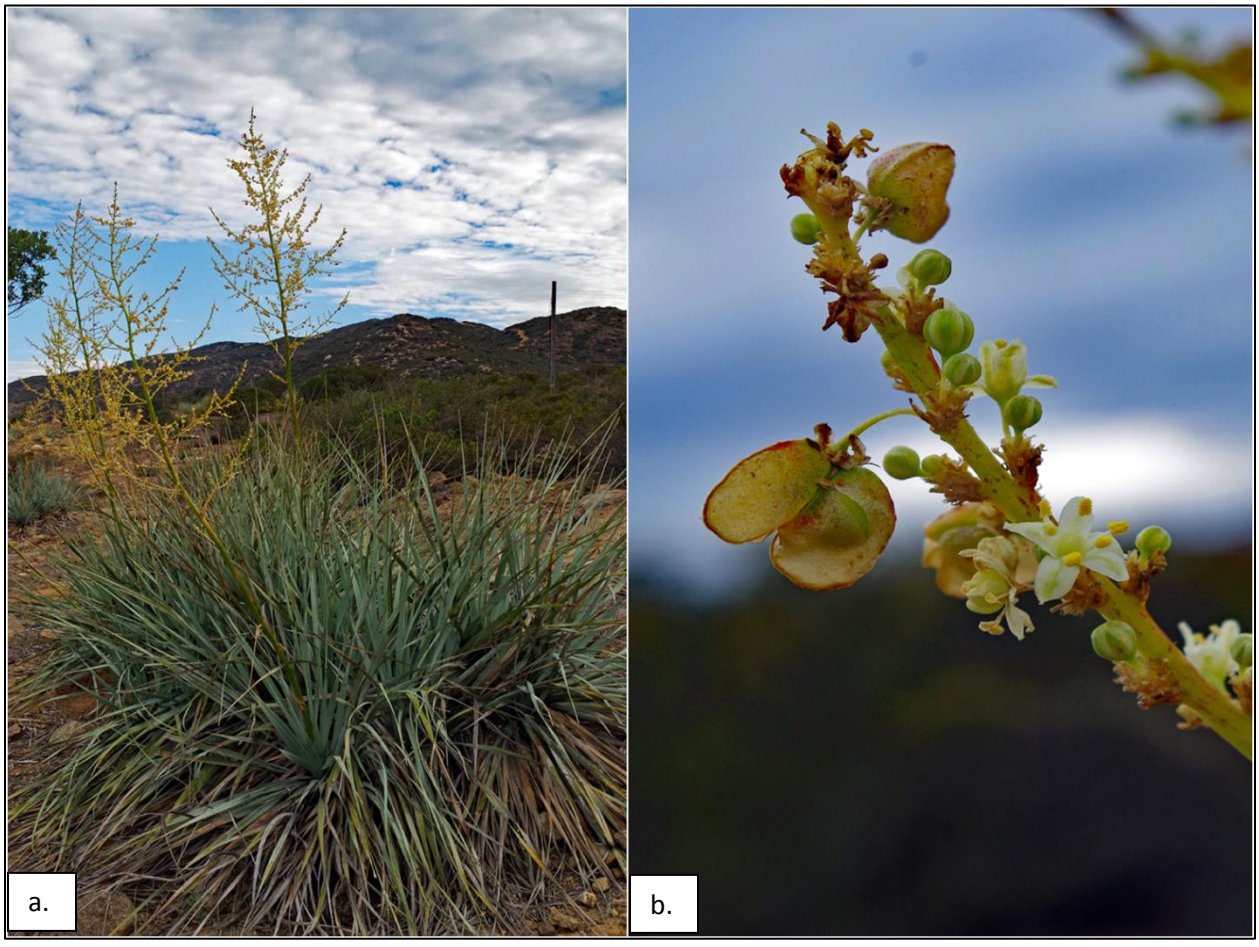

Figure 11. Dehesa Nolina Plants on McGinty Mountain: (a) flowering inflorescences, (b) maturing capsules.

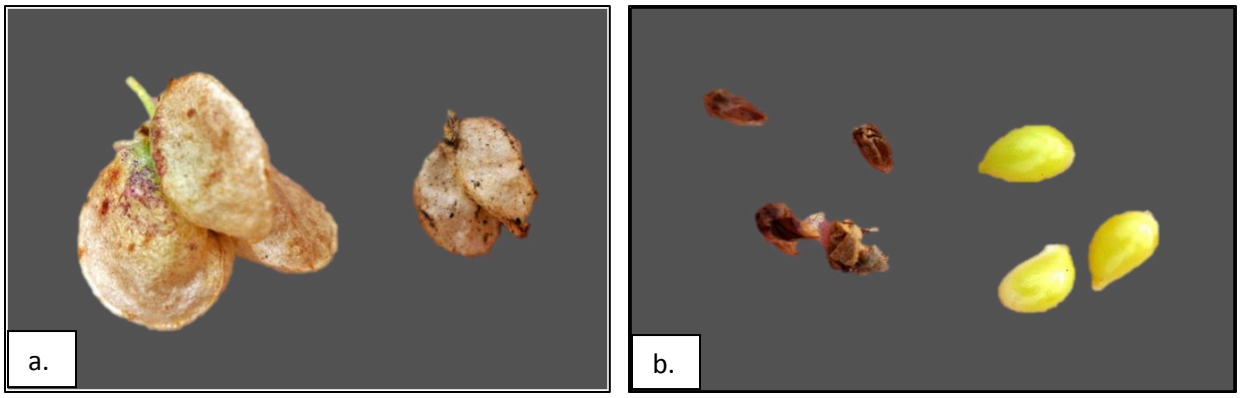

Figure 12. Dehesa Nolina Capsules and Seed: (a) viable (left) and aborted (right) capsules, (b) aborted seed (left) and viable seed (right).

Figure 13. Flowering Dehesa Nolina with Cut Stalk (arrow), McGinty Mountain.

Floral Morphology and Sex Ratio

In 2015, we collected and dissected flowers from 5 Dehesa nolina plants on South Crest (Table 4). Four plants were determined to be female based on the presence of pistils and staminodes, while the fifth plant was male based on the presence of both stamens with pollen-producing anthers and a reduced pistil (Figure 14). Female plant 10, growing at a distance from other flowering nolina plants, produced capsules but no seed. Plants 6-9 were adjacent to each other, but females produced no viable seed despite proximity to the male plant.

Table 4. Dehesa Nolina Floral Morphology.

Nolina Plant #	Plant Sex	Flower Dissection
7	Male	• Large stamens with copious amounts of pollen. • Pistil reduced; no obvious styles.
6	Female	• Staminodes present; no pollen. • Pistil not reduced; stigmas (3) obvious.
8		
9		
10		

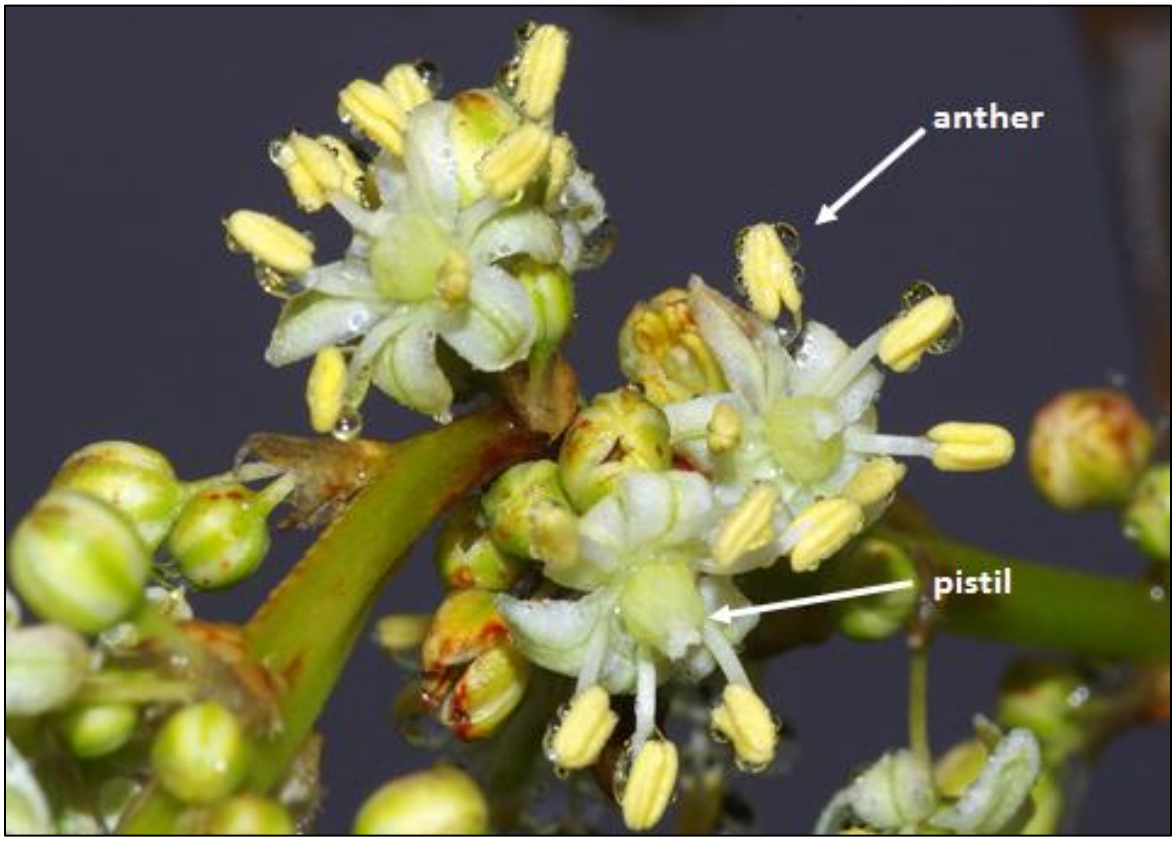

Figure 14. Dehesa Nolina Flowers with Pollen-bearing Anthers and Reduced Pistils.

Propagation

Of the 25 nolina seeds that were pre-treated for germination in December 2013, 6 germinated and 5 survived the first few months in the nursery. The five individuals are all from the same plant. The 2013 germinated plants did well in the nursery setting, but were very slow-growing and temperamental with respect to moisture. Therefore, RNP maintained them on the drier side to minimize problems with disease, although providing less water also slowed their growth rate.

Of the 150 Dehesa nolina seeds obtained from RSABG and sown directly into D40 containers in December 2014, 135 (90%) germinated in January 2015 and were about 1 inch tall by January 31, 2015. The nursery experienced issues with aphids and root rot in February 2015. Seedlings were managed closely to reduce losses, which stabilized by late March 2015 with 69 surviving plants (51% of the germinated plants). One more plant was lost between April and June, and two plants were lost between June and September due to extreme heat and humidity in the nursery. The remaining plants (5 from 2013, 66 from 2014) were healthy and thriving in the nursery setting (Figure 15).

Figure 15. Dehesa Nolina Plants Growing at RNP Nursery. Large plants in back left from 2013 field-collected seed; other plants from RSABG seed planted in 2014.

Outplanting

A total of 71 Dehesa nolina plants were delivered to South Crest on November 2, 2015 for outplanting into the field. This included 5 2-year plants and 66 1-year plants. Table 5 presents results of the Dehesa nolina outplanting effort with respect to plant survivorship in the first 10 months following planting. Over this period, 72% of the plants survived, including 100% of the 2-year old plants and 70% of the 1-year old plants. Nearly a third of all plant mortality (30%) occurred within a few weeks of planting, and may have been due to transplant shock and/or immature root systems. Another 25% of plant mortality occurred prior to the onset of supplemental watering. Six plants were lost during the driest, hottest part of the year (June-August), and one of these losses was ascribed to herbivory by mice. Survivorship was assessed based on the above-ground portion of individual plants; therefore, it is possible that some plants counted as dead may sprout vegetatively, depending on climatic conditions.

Table 5. Dehesa Nolina Outplanting Survivorship.

Date	Clusters[1]								Total[2]
	1	2	3	4	5	6	7	8	
11/2/15[3]	6	8	7	5	17	9	10	9	71
11/20/15	5	7	7	5	14	9	10	8	65
12/2/15	5	7	7	5	14	9	10	8	65
12/30/15	5	6	7	5	14	9	9	8	63
1/13/16	5	7	7	5	12	9	10	7	62
2/13/16	5	7	7	5	11	7	9	6	57
3/8/16	5	7	7	5	11	6	9	7	57
4/4/16	5	7	7	5	11	6	9	7	57
4/23/16	5	7	7	5	11	6	9	7	57
5/31/16	5	6	7	5	11	5	9	7	55
6/16/16	5	6	7	5	11	5	8	7	54
8/9/16	5	5	6	5	11	5	8	6	51

[1] Clusters = discrete planting locations (1-8 in cluster subheading), each of which support several Dehesa nolina plants; numbers in table = number of plants per cluster.

[2] Total = Total number of plants in all clusters at given dates.

[3] Indicates initial outplanting date.

Figure 16 depicts the growth of one of the 1-year plants during the 2016 monitoring period, while Figure 17 depicts the growth of one of the 2-year plants during this same period.

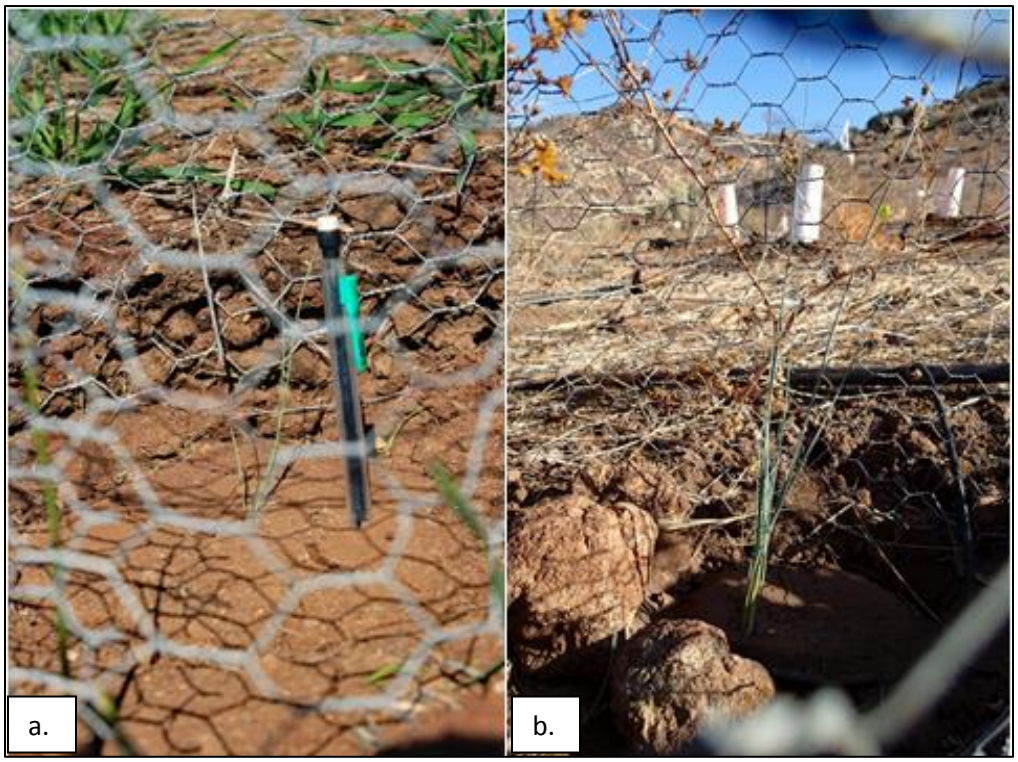

Figure 16. One-year Old Dehesa Nolina Plant: (a) spring 2016, (b) late summer 2016.

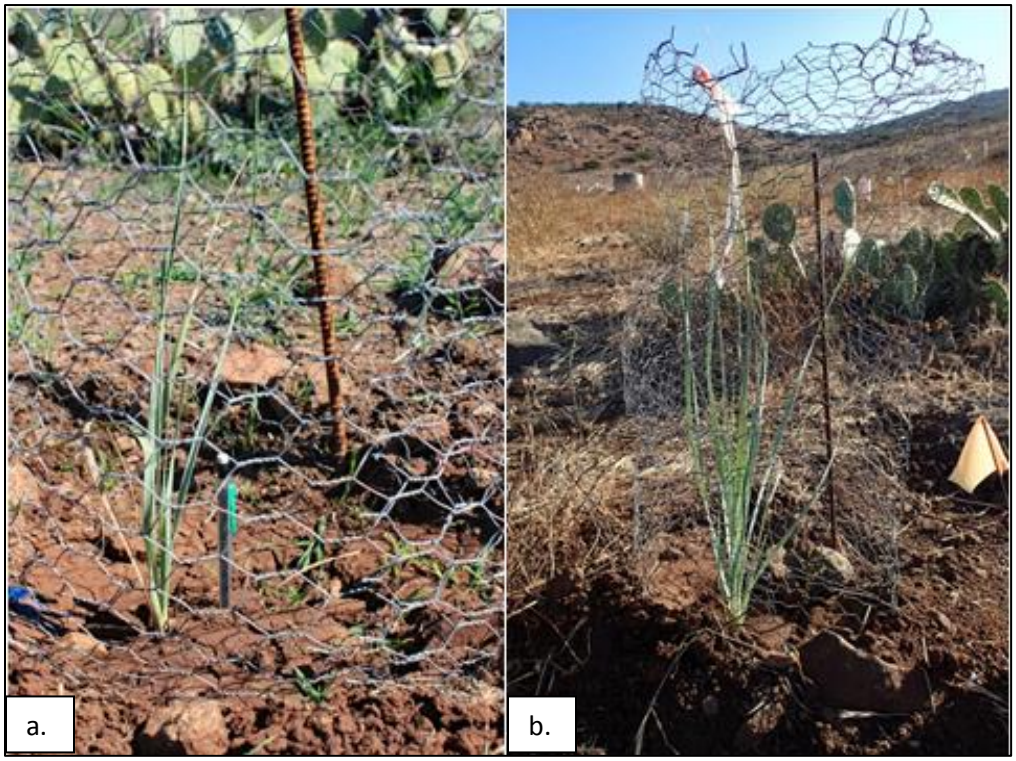

Figure 17. Two-year Old Dehesa Nolina Plant: (a) spring 2016, (b) late summer 2016.

Discussion

Biological Threats

Several animals threatened emerging Dehesa nolina inflorescences throughout the growing season. During early emergence, wood rats chewed off the thick inflorescences prior to flowering. Deer also consumed entire flowering and fruiting inflorescences, although in some cases, a smaller inflorescence emerged post-deer herbivory.

It appears that some level of insect (black and yellow beetles) visitation is needed for pollination, healthy capsule formation, and viable seed development. Early-blooming inflorescences receive high levels of insect visitation which can destroy all flowers. Later-blooming inflorescences also receive insect visitation, but insect numbers appear to be lower later in the season, which may allow for inflorescence survival while still effecting pollination.

Caging Dehesa nolina plants and bagging inflorescences were effective measures to reduce herbivory. We found that stacking two cages to attain a height of approximately 6 feet was necessary to protect plants from deer. Organza bags allowed for air flow and eliminated the mold problem associated with cheesecloth bags. Timing of bagging is an important consideration. When flowers are bagged too early (i.e., prior to pollination), no capsules (fruit) formed. Conversely, heavy insect infestation prior to bagging can destroy inflorescences. We used an organic pesticide spray to control the level of insect infestation prior to bagging. However, pesticide should be used sparingly as the flowers may dry up and die post-application. In addition, the pesticide may kill effective pollinators.

Seed Collection

Based on results from South Crest, a small amount of viable seed may be produced in any given year in the absence of fire or other damage to plants. Later-blooming inflorescences may have a better chance at producing some viable seed due to fewer damaging insects, although lower insect levels may also result in lack of pollination. In the absence of pollination, plants may produce capsules but will not produce viable seed.

Mechanical damage to plants may stimulate flowering and increase viable seed production, as was noted in plants on McGinty Mountain. Plant damage (leaf clipping) increased flowering significantly in *Xanthorrhoea australis*, an Australian monocot that mass flowers after fire and has a number of morphological and ecological similarities to *Nolina*. In this case, the stimulus may be ethylene, which is reportedly contained in smoke and released from plants after tissue injury (Gill and Ingwerson [1976] *in* Dice [1988]).

Floral Morphology and Sex Ratio

While some sources indicate that Dehesa nolina is dioecious (e.g., USFWS 1995), many of the plants we observed had flowers with both stamens and pistils. In male plants, the pistils were somewhat reduced. In female plants, the stamens were also reduced or present as staminodes, which are rudimentary, sterile, or abortive stamens. Thus, it might be more accurate to refer to Dehesa nolina floral morphology as functionally staminate or functionally pistillate, rather than strictly dioecious. This is consistent with other observations for the species or genus indicating that 'stamens are greatly reduced and non-functional in female flowers' (Dice 1988), 'flowers on one plant may be unisexual or bisexual (Baldwin et al. 2012), and 'flowers are functionally unisexual, i.e., pistillate flowers with staminodes and staminate flowers with reduced pistils (Hess 2008). Because we sampled only a small number of flowering individuals, it is not known whether this morphology is consistent throughout the species.

In the absence of fire, only a small number of Dehesa nolina plants flower in a given year. On South Crest, the sex ratio in our very small sample size was four female plants to one male plant. In addition, one of the female plants was not in proximity to the male plant. Without knowing effective pollinators and the distance these pollinators can travel, we cannot assess the adequacy of these ratios. Because flowers bagged prior to pollination did not form viable seed, we presume that some level of outcrossing is necessary for production of viable seed and that successful recruitment from seed will require both male and female plants in a population.

Propagation and Outplanting

Despite some issues in the greenhouse with insects and root rot, this project demonstrated that it is feasible to propagate Dehesa nolina in a nursery setting and introduce it successfully into the field. Greenhouse mortality issues were stabilized with modified nursery practices, and field mortality was reduced with plant protection and supplemental watering.

Recommendations

While we were able to collect a small amount of viable seed in the field and propagate plants from this seed in the nursery, seed collection in the absence of fire or another event that stimulates mass-flowering is not the most effective strategy to augment or restore Dehesa nolina populations. The majority of seed used in our propagation program – and the majority of outplanted individuals – came from 25-year old stored seed that nonetheless had a 90% viability rate.

Therefore, we recommend developing a permanent Dehesa nolina seed source (seed bank) that consists of both conservation and propagation collections. The conservation collection should be held in long-term storage to preserve genetic diversity and provide a seed source in the event of

catastrophic disturbance, thus providing a 'hedge' against extinction or extirpation. This collection may also function as source material for management-oriented research. The propagation collection would provide genetically appropriate source material for seed bulking or outplanting to augment extant populations or create new populations in the future. Seed should be collected during mass-flowering events (i.e., post-fire). It will be important to identify potential seed collection locations and protocols, and secure funding for seed collection prior to such an event. Seed banking should include the following elements:

- Follow existing Best Management Practices (BMPs) for seed collection (RBGK 2001, Wall 2009) and maximize genetic variability by collecting over multiple years and across populations and sampling from multiple habitats and ecological niches.
- Collect seed from all conserved populations of sufficient size to accommodate harvest or that possess a unique genetic structure.
- Maintain detailed records for all collected seed to document donor and receptor sites, collection dates, and amounts collected; submit data to the SC-MTX website portal and regional seed bank database.
- Store seeds at a qualified seed bank by population, date, and for small populations (<1,000 plants), along maternal lines. Test seed for viability upon accession and regularly thereafter to assess seed viability over time.
- Structure the seed testing program to obtain additional information through the testing process, such as dormancy factors and germination rates.

There is some evidence that minor damage to plants, such as removing a rosette at the caudex or cutting leaves, might stimulate production of viable seed. Although we do not advocate damaging healthy plants, this method of seed production might be worth testing if augmentation is necessary and another seed source is not available.

Little is known about the reproductive biology of this species, including the mating system, effective pollinators, and sex ratios. Therefore, we recommend a separate study that assesses these factors across multiple populations. This study should be conducted during mass-flowering events to ensure adequate sample size of inflorescences/flowers. Key questions might include:

- Is Dehesa nolina strictly outcrossing or does it possess some level of self-reproduction (selfing)?
- What are the effective pollinators and pollinator distances?
- Do high insect levels cause premature inflorescence mortality and/or impact viable seed production in mass-flowering populations? Are there specific insects that are harmful to

flowering Dehesa nolina? Is timing of insect infestation a factor in viable seed production in mass-flowering populations?

- Do populations possess skewed sex ratios that could adversely affect viable seed production?

Our study demonstrated that Dehesa nolina can be successfully grown in a nursery setting and outplanted into the field. In the nursery, we had a 50% post-germination success rate, which could be improved by controlling insects and watering regimes. Our best outplanting success was achieved with 2-year old plants. Therefore, future propagation programs for this species should produce plants that are at least 2 years old prior to outplanting. Further, outplanting programs should include both plant protection and supplemental watering on a consistent schedule (e.g., weekly or bi-weekly) to improve post-planting success, at least in the first year following planting.

Finally, we recommend that EHC continue weeding, plant protection, and supplemental watering for outplanted Dehesa nolina on South Crest through 2017, as needed, and then re-assess the need for continued management of these plants. Specific recommendations include:

1. Maintain watering basins for outplanted Dehesa nolina plants. If it becomes necessary to build up watering basins, try to avoid introducing nonnative seeds into the soil.
2. Maintain current levels of supplemental watering for Dehesa nolina plants for at least one more year (2017), since most plants will need another year to fully establish. As plants mature, increase the interval between watering gradually in 2018 (or terminate during periods of sufficient rainfall). We do not anticipate supplemental watering by 2019 except perhaps under severe drought conditions.
3. Maintain treepees around outplanted Dehesa nolina plants and keep in place until plants outgrow them. Although some disturbance to treepees may occur during weeding, minimize disturbance to the degree feasible to avoid damaging Dehesa nolina plants.
4. Continue weeding in and around Dehesa nolina planting basins. Remove nonnative species as well as allelopathic native species inside basins (e.g., fascicled tarplant, *Deinandra fasciculata*). Rake nonnative thatch and/or seeds away from basins after weeding and dispose of on- or offsite but away from planting basins.

Variegated Dudleya Augmentation

Methods

Seed Collection

In spring 2014 and 2015, CBI biologists identified and marked flowering variegated dudleya plants on South Crest and adjacent EHC-owned conserved lands with pin flagging. We monitored these plants for several months to determine the optimal time for seed collection. CBI biologists collected variegated dudleya seed from South Crest in June 2014 and 2015 (Figure 18). This seed was sent to RNP for use in nursery propagation for outplanting back onto South Crest.

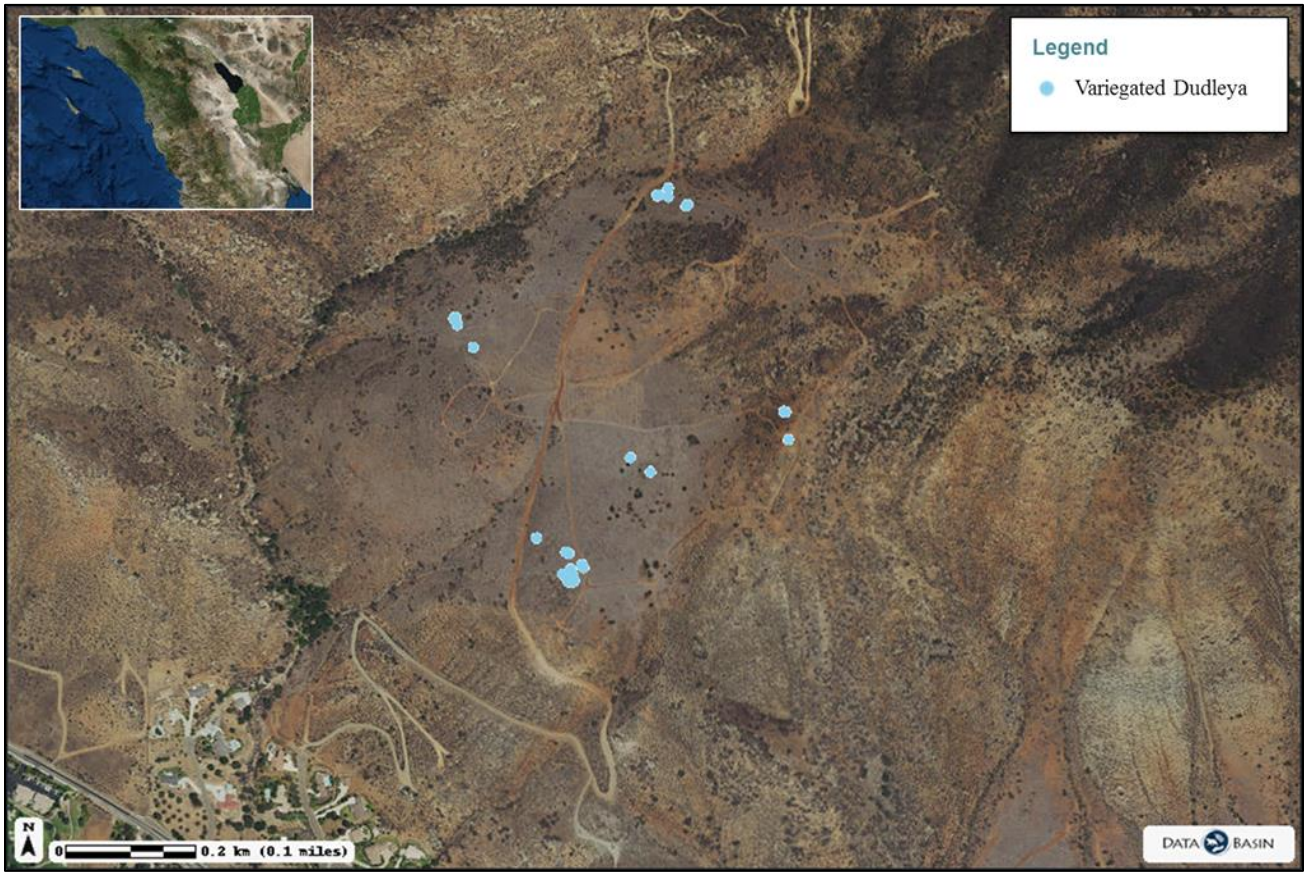

Figure 18. Variegated Dudleya Seed Collection Locations on South Crest.

Propagation

RECON field crews salvaged clay soil from South Crest to use for variegated dudleya propagation. Seeds were sown into flats on December 11, 2013 and December 15, 2014,

respectively, per RNP established protocol for this species. The 2013 seeds were sown directly into flats with native soil, while the 2014 seeds were sown initially into flats with potting soil and transplanted subsequently to native soil in flats once they reached sufficient size.

Outplanting

CBI biologists met with Recon biologist Mark Dodero, the Recon five-man field crew and EHC director Michael Beck on the South Crest Preserve on November 2, 2015 to identify suitable sites for planting and oversee the outplanting effort.

The Recon field crew planted variegated dudleya flats in four discrete locations and created protective cages around these plantings. Variegated dudleya plants were not watered after planting. We relied on natural rainfall to stimulate growth from the underground corm. It rained lightly the night of the installation, and this natural precipitation was sufficient to stimulate variegated dudleya growth.

During the planting process, a "Dudleya sandwich" was created to protect variegated dudleya corms from herbivores, including gophers and rabbits. Mr. Dodero developed the Dudleya sandwich and has used this method successfully in other variegated dudleya outplanting projects. The method is described below and depicted in Figure 19:

- Dig a shallow hole that is roughly the same shape and slightly larger in size than the variegated dudleya flat.
- Line the bottom and sides of this shallow hole with chicken wire. The chicken wire should rise several inches higher than the sides of the shallow hole. The wire prevents gophers from eating variegated dudleya corms from below.
- Carefully cut off the sides of the plastic variegated dudleya flat. Carefully slide the flat (soil with corms) into the shallow hole. Try to avoid breaking the soil, but if breaks do occur, carefully put the pieces back together. Fill in any cracks or holes with native soil and then backfill around the flat with native soil. Pack the soil around the flat.
- Cut another piece of chicken wire to secure to the wire in the shallow soil. Only the sides of the chicken wire should be visible since the rest is underneath the variegated dudleya and native soil. Secure the new piece of chicken wire to the chicken wire that is visible along the sides of the variegated dudleya flat so that the flat is completely enclosed in chicken wire.

Figure 19. Variegated Dudleya Sandwich: (a) laying protective wire mesh in planting hole, (b) placing variegated dudleya flat on wire mesh, (c) building up sides of cage, (d) securing chicken wire to top of cage.

Monitoring

CBI established a monitoring schedule to track the health of outplanted variegated dudleya; monitoring occurred at approximately 2-4 week intervals from November 2015 to August 2016. During monitoring periods, we recorded survival and general health of the plants, photographed the phenological progression of plants, and weeded around and within planting cages.

Results

Seed Collection

In 2013, CBI collected an estimated 700 variegated dudleya seeds from South Crest. Because this was below projected estimates, we collected additional seed in 2014. We do not have a count of 2014 seed, but the number was somewhat less than was collected in 2014.

Propagation

RNP produced 7 flats supporting an estimated 50 variegated dudleya plants/flat (350+ plants) over the 2-year growing period in the nursery (Figure 20). Plants were irrigated in the nursery through spring 2014 and 2015, and then taken off irrigation and allowed to go dormant during the summer seasons. Plants re-emerged in fall in response to rains. Germinated plants were healthy and thriving in the nursery, and there were no significant plant losses reported.

RNP finished growing variegated dudleya at their nursery facilities and delivered the 7 flats of one- and two-year old plants to South Crest on November 2, 2015 for outplanting into the field. The plants were dormant at the time of planting.

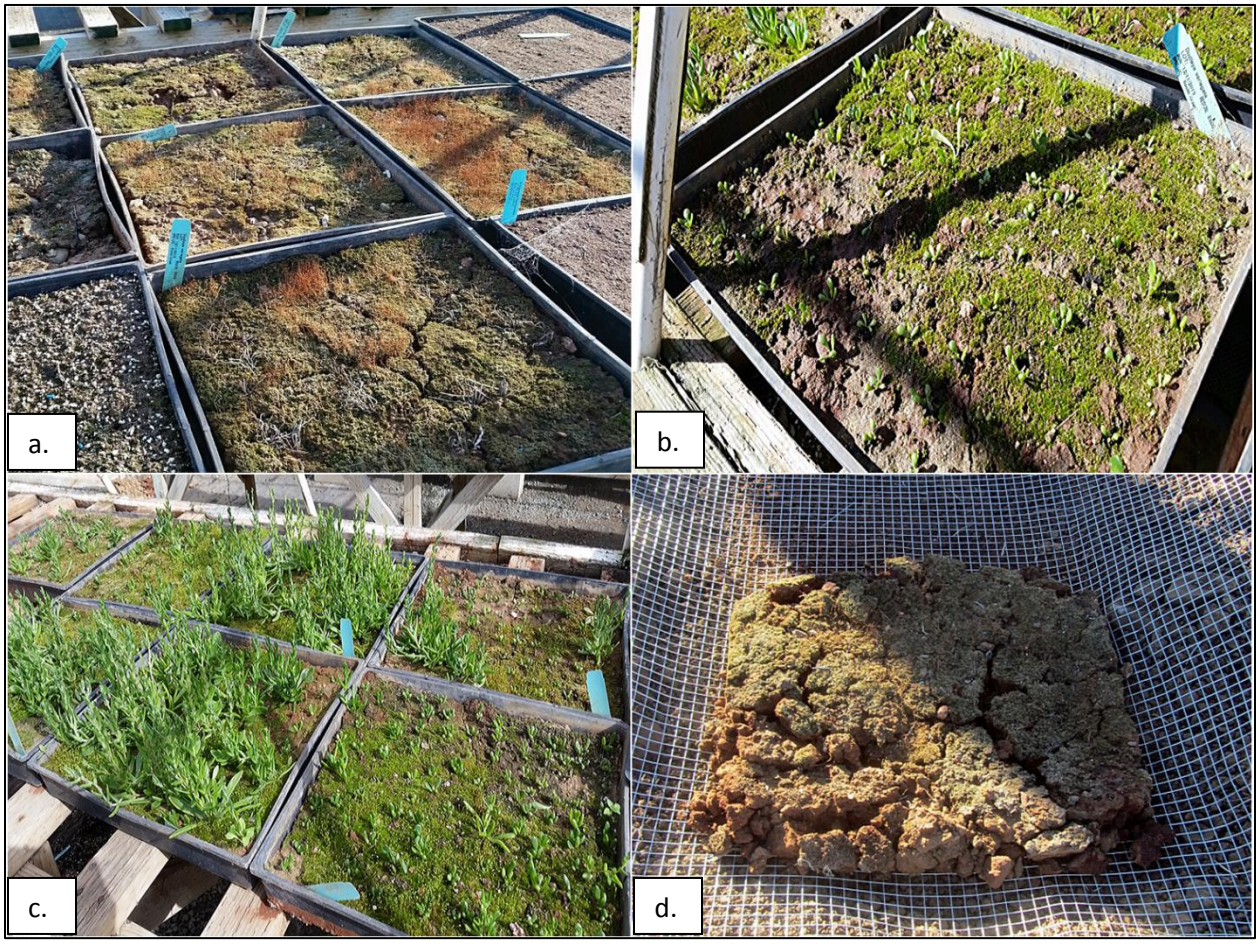

Figure 20. Variegated Dudleya Flats in Nursery: (a) dormant plants in September 2014, (b) emerging plants in March 2015, (c) vigorously growing plants in April 2015, (d) dormant plants in November 2015 during planting.

Outplanting

No supplemental irrigation was provided to the outplanted variegated dudleya plants. However, a light rain occurred immediately after planting. Dormant plants began to emerge by late November 2015 and most plants had emerged by late December 2015. The plants grew steadily through the spring, flowered and set seed in early to mid-summer, and were dormant again by late summer 2016 (Table 6, Figure 21). Plants relied on rainfall for growth, which was sufficient in spring 2016 to stimulate flowering.

Because of the growth form of this species, it can be difficult to distinguish between individual plants, particularly when plants are small and growing close together. Therefore, counts of individuals varied somewhat through the year, but these differences were not necessarily related to survivorship. We observed some plant losses when small plants were outcompeted by larger plants and were not able to survive the higher temperatures of late spring and early summer. The nonnative grasses *Brachypodium* and wild oats (*Avena* sp.) were present in and around planting cages and were weeded periodically to reduce competition to variegated dudleya plants.

Discussion

Seed Collection

Variegated dudleya seed was relatively easy to collect, although marking flowering plants was important for re-locating fruiting plants. We were hindered in the amount of seed collected only by the dudleya population size on South Crest. In order to adhere to standard seed collection guidelines and not decimate the extant population by collecting all available seed, we spread our seed collection efforts over two seasons. Collected seed appeared to be well-formed and viable.

Propagation

RNP had prior experience growing variegated dudleya in a nursery setting, and applied their BMPs to growing variegated dudleya for this project. As a result, plants grew well in the nursery setting and there were no incidents of plant loss due to disease, insects, or propagation practices.

Outplanting

Recon had prior experience outplanting variegated dudleya and had, in fact, pioneered the method used in this project which included wire caging to protect corms and emerging plants from above- and below-ground herbivory. Outplanting success was enhanced by growing conditions during the 2016 season, which included adequate rainfall and relatively cool temperatures through the growing season. The effects were apparent in the number of plants that emerged, flowered, and set seed. Nonnative grasses threatened to out-compete variegated

Table 6. Variegated Dudleya Outplanting Survivorship.

Date	Location[1]				Total[2]	Notes
	1	2	3	4		
11/20/2015	9	4	1	17	31	Weeded nonnative grass seedlings within and adjacent to cages.
12/2/2015	40	43	10	48	141	No weeding necessary.
12/30/2015	56	101	52	102	311	Weeded #3 only.
1/13/2016	64	109	56	112	341	Weeded all locations.
2/13/2016	48	105	44	100	297	No dead individuals, but some previously counted plants may have been part of a larger plant, so counts adjusted downward. Most individuals are healthy; a few are drying or sunburnt. Weeded #3 and 4.
3/8/2016	56	103	51	107	317	Cage 2 had been stepped on and 1 individual killed, with 2 others damaged.
4/5/2016	56	96	44	86	282	Many plants developing flowering inflorescences.
4/23/2016	48	93	44	80	265	Many plants are close to flowering and have grown through top of cage. Weeds are less problematic; weeds within cages can't be removed without disturbing variegated dudleya plants. Individual plants more distinguishable at this stage.
5/4/2016	---	---	37	60	97	Incomplete count using MSP rare plant monitoring protocol.

Table 6. Variegated Dudleya Outplanting Survivorship.

Date	Location[1]				Total[2]	Notes
	1	2	3	4		
5/31/2016	33	55	30	53	171	100% flowering. Individual plants more distinguishable at this stage and some smaller individuals have been lost; thus, numbers reflect current status.
6/16/2016	33	50	27	48	158	All plants starting to fruit; about 20% still have some flowers.
8/9/2016	0	0	0	0	0	All plants have seeded and gone dormant.

[1] Location = planting locations (1-4 in cluster subheading; 7 flats of plants were planted in 4 discrete locations); numbers in table = estimated number of plants per location.

[2] Total = Total number of plants at location on given dates.

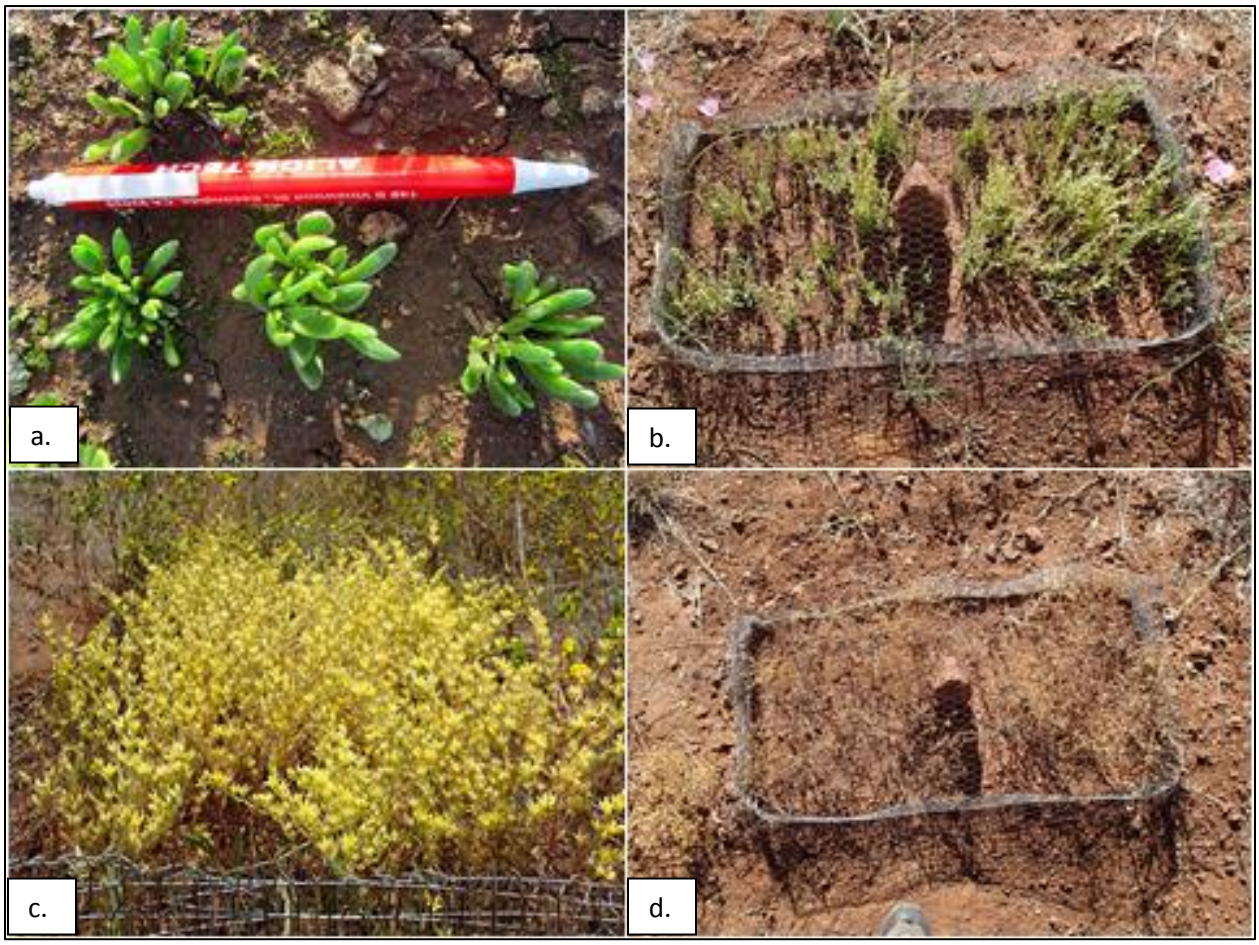

Figure 21. Outplanted Variegated Dudleya Plants: (a) emergent plants in February 2016, (b) vigorously-growing plants in April 2016, (c), flowering plants in May 2016, (d) dormant plants in August 2016.

dudleya plants early in the season; thus, we hand-weeded around and within planting cages to remove these nonnatives. The weeded variegated dudleya plants were, on average, four times larger than naturally-occurring variegated dudleya plants onsite that were not weeded. Larger plant size potentially translates to more flowers and fruits, as well as underground corms with greater capacity to survive less-than-optimal conditions.

Although the total number of plants reintroduced onto South Crest was less than targeted initially, the outplanted individuals are currently healthy and appear to have a relatively high survival rate. The focus is to maintain several locations on South Crest where variegated dudleya stands will stabilize and expand under suitable growing conditions, and will reproduce successfully and contribute to the soil seedbank.

Recommendations

Variegated dudleya plants outplanted onto South Crest were healthy but dormant at the end of the 2016 growing season, and now include two- and three-year old corms. For the next five years, we recommend the following measures to ensure continued success:

4. Maintain caging around variegated dudleya plants but elevate the cage ceiling to allow for flowering and modify the top of the cage to facilitate weeding (e.g., allow top of cage to slip over the buried cage with a loose fit and use garden staples to keep cage top in place).
5. Continue hand-weeding nonnative grasses and other emergent invasive species within and around planting cages. Hand-weeding should begin in late November or early December of each year, depending on nonnative grass germination, and continue monthly during the growing season, as needed. As demonstrated in this project, early control of nonnative species will result in less maintenance later in the year.
6. Provide supplemental irrigation during drought or low rainfall years only for plants that emerge naturally to allow them to survive. Supplemental irrigation is not recommended where rainfall is insufficient for natural emergence.
7. Depending on resources and funding, consider enhancing the small, naturally-occurring variegated dudleya population on the western slope of Skeleton Flats with nursery-grown plants. Enhancement should occur in conjunction with a larger weed control program.

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