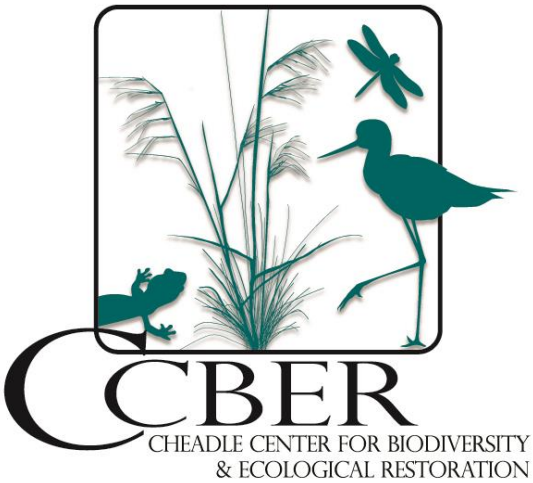
UC SANTA BARBARA



Seed Bulking of *Lupinus nipomensis*

September 2016

Catherine Reilly *Restoration Assistant*
Justin Luong *Research Affiliate*

SEED BULKING OF *LUPINUS NIPOMENSIS*

Prepared by: Cheadle Center for Biodiversity and Ecological Restoration; Catherine Reilly, Justin Luong

Prepared for: U.S. Fish and Wildlife Service
Ventura, California
Cooperative Agreement Award #F12AC00683
Project Officer: Connie Rutherford

September 2016

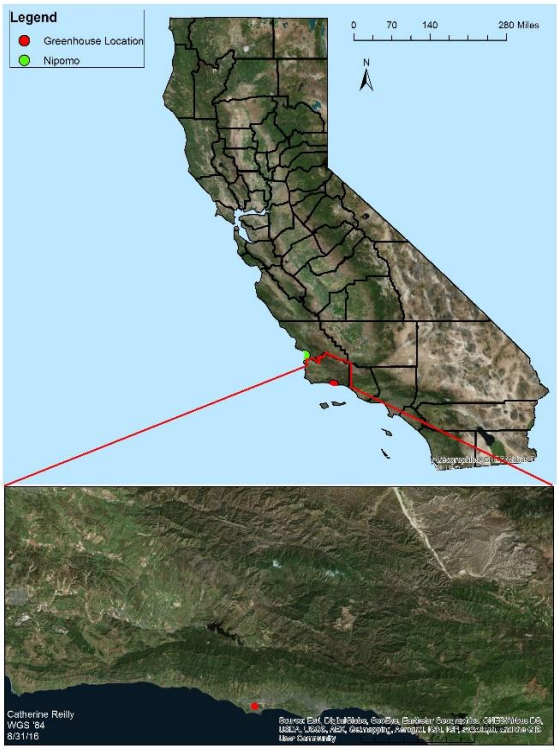


Figure 1. Pictured is California with an insert of the Santa Barbara region where the CCBER greenhouse is located in reference to the study area in Nipomo.

Introduction

The degradation and fragmentation of the habitat of the federally threatened Nipomo Mesa Lupine *(Lupinus nipomenis)* requires active conservation to preserve this species in the face of climate change. *L. nipomensis* is an annual forb with decumbent stems, standing 10 to 20 cm high, and producing small fruits generally containing between three to four seeds (Sholars, 2016). Found across a 2 mi^2 range in Central California, *L. nipomensis* is predominately found growing in sandy soils in the more stable back dune habitats where they receive less exposure to salt spray compared to plants living in fore dunes because they are further from the beach (Wilken, 2009; Hill, 2001).

L. nipomensis is threatened by a variety of anthropogenic forces and invasive species. One factor that heightens their vulnerability is their habitat. Dunes are especially sensitive to disturbance from recreation such as off road vehicles, development, and erosion due to sea level rise (Feagin, 2005). In addition to habitat loss, *L. nipomensis* is facing increasing competition from exotic species, such as Perennial Veldt Grass (*Erharta calycina*), resulting in its official listing as endangered in March of 2000 (Clark, 2000). In collaboration with U.S. Fish and Wildlife Services (USFWS), UC Santa Barbara's Cheadle Center for Biodiversity and Ecological Restoration (CCBER) works to identify environmental conditions that best supports the restoration of the species. An important component of this restoration effort is obtaining seeds for further research and out plantings. For this purpose, CCBER began a seed bulking project for *L. nipomensis* in November of 2015.

Seed bulking is important for conservation because even if the species is declining in the wild, seed bulking allows for a stock of seed to be available for future conservation efforts such as out plantings. Seed bulking is the process of collecting seed from a desired species and growing out individuals to collect more seed from the next generation, with the goal of increasing the stock of that seed (Ritchie, 1996). In the case of *L. nipomensis*, this seed is valuable due to the scarcity of the species and the threat of extinction. The goal of this effort is to determine if *L. nipomensis* can be bulked in a greenhouse setting and, if so, which nursery practices best support their success.

Methods

Seed Collection Site

All seeds were sourced from the Guadalupe-Nipomo Mesa in southern San Luis Obispo County by Santa Barbara Botanic Garden (SBBG) in 2005. There is one existing population with nine known meta-populations. Out of those nine two of these occurrences are on Conoco-Phillips gas property, two occur in the protected reserve, and the remainder occur on private property. The Guadalupe-Nipomo Mesa area receives approximately 13 inches of rainfall per year with an average high of 68.8°F and an average low of 45.9°F (Western Regional Climate Center, 2016). Approximately ninety miles south-

east of the Guadalupe-Nipomo Mesa, the city of Goleta, where CCBER is located, experiences a similar Mediterranean climate with mild wet winters and warm dry summers (Heusser, 1978). Due to its Mediterranean climate, during the summer the primary water source along the California coast is from fog drip. The fog can also create a shading effect decreasing water loss through evaporation (Fischer et al, 2008). The seeds collected in 2005 at the Guadalupe-Nipomo Mesa were the seeds used in trial A. However in 2013, using the original seed collected from 2005, another bulking effort was performed at the CCBER greenhouses. Seeds for trial B were sourced from this later bulking effort.

Greenhouse Seed Bulking Effort

All plants were grown at the nursery managed by CCBER near the coastal city of Goleta, CA (Figure 1). Prior to planting, each trial received a different pre-treatment with Trial A seeds receiving a cold stratification treatment, and trial B seeds receiving a hot water stratification treatment. Before sowing, all the seeds were scarified by dragging the seed across approximately thirteen centimeters of 400 grit sandpaper (Table 1). In each trial, three seeds were planted in a regularly spaced pattern in a 1.76-gallon pot. A total of 30 seeds were planted in Trial B and 150 seeds were planted in Trial A. Plants were watered every other day with approximately 200 milliliters of water per plant, using potable water from the hoses at the greenhouse supplied by the Goleta Water District. Goleta's water has a considerable amount of calcium carbonate present per liter of water (Goleta Water District, 2003). Plants were housed in a raised enclosure covered in half-inch hardware cloth to prevent herbivory by organisms such as rodents and birds. The enclosure was located under a shade cloth structure, lessening the plants exposure to direct sunlight throughout the day.

Trial	Treatment	Source Seed Collection Year	Greenhouse Collection Period
A	- Scarified, 400 grit sandpaper - Cold stratified at 3°C - Jars and seeds cleaned with hydrogen peroxide first and seeds placed in 5mL of water	2005	05/11/16-07/08/16
B	- Scarified, 400 grit sandpaper - Soaked in hot water (91° C) and 15mL of native Guadalupe sand for ½ day	2013	04/01/16-06/03/16

Table 1. A summary of both trials looking at treatment types, collection year, and time of year plants were grown out.

Once planted, pots were monitored every morning for the germination of new individuals, the development of seed pods, and the presence of insects. Seed collection for trial B ran from April-June and seed collection for trial A ran from May-July. Once seed pods began to mature and desiccate, they were removed using scissors and allowed to dry prior to long term storage.

Results

Together, trial A and trial B both produced a total of 66 mature individuals and 6620 seeds in the bulking efforts. Trial B resulted in 20 plants (a 67% germination rate) and 5020 seeds while trial A resulted in 46 plants (a 31% germination rate) and 1600 seeds (Table 2). During storage 308 seeds were lost to molding which resulted in a total of 6312 viable seeds. A Welch t-Test was performed ($t= 6.867$, $p=1.089x10^{-6}$) indicating that the difference in amount of seed production between plants from trial A and plants from trial B was significant with individuals from trial B producing 660% more seed than individuals from trial A.

Table 2. Summary of results from the 2016 seed bulking effort.

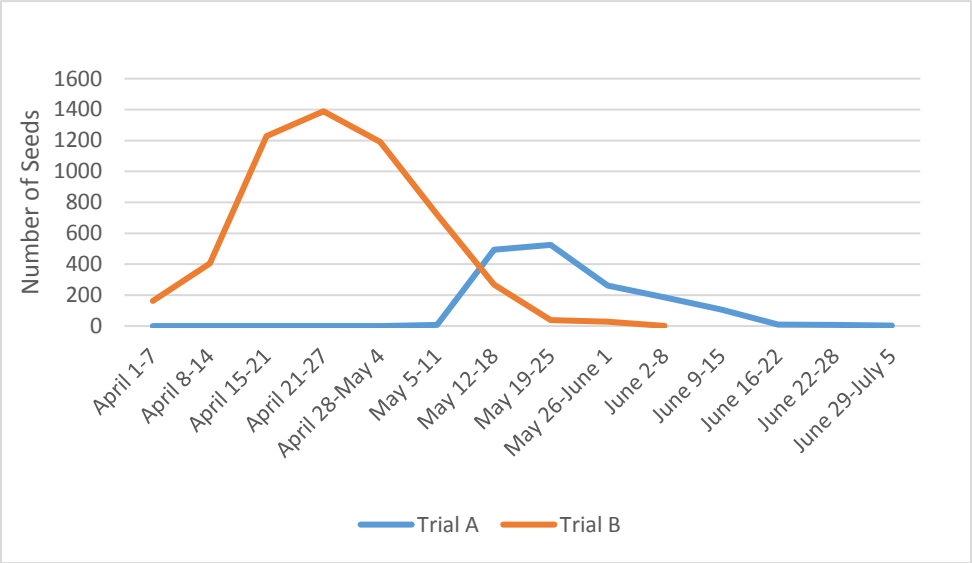
Trial	Percent Germination	Total Seeds Collected	Average Number of Seeds per Plant
A	67%	1600	38
B	31%	5020	251

Discussion

Overall it was found that *Lupinus nipomensis* can successfully be bulked in a greenhouse setting. Based on total number of seeds produced and average number of seeds produced per plant, Trial B was more successful than Trial A. In the end, Trial B produced 3,420 more seeds than Trial A with each plant in the earlier trial producing on average almost 7x more seed than the later trial. Our results suggest that to maximize seed output it is beneficial and to apply a hot water treatment, to plant earlier in the season, and to plant newer seeds. While previous studies have shown that legume seeds in temperate regions experience higher germination rates after being chilled and then experiencing fluctuating temperatures (Van Assche et al., 2003), our finding that a hot water stratification was more beneficial is not unprecedented. One study on the species *Senne multijuga (*also a member of the Fabaceae family) found that exposure to hot water was able to break physical dormancy (Rodrigues-Junior et al., 2014). These treatments would not directly affect seed production rates of individuals, but could have affected germination rates. Our results suggest that *L. nipomensis* should be seed bulked earlier in the season from April-June. One possibility for the benefit of earlier season growth is the temperature and precipitation differences experienced by each trial. Trial B was planted in December and trial A was planted in January. Seeds that were started in December experienced temperatures that were on average 2.1 °F cooler and received 5.49 less inches of precipitation in the month of their initial planting (it rained 0.3 inches in December and 5.79 inches in January). Trial B began seeding in April and trial A began seeding in May. Seeds that began seeding in April experienced temperatures that were on average 1.3°F warmer, and received 0.53 more inches of rain in the month of their initial seeding (National Weather Service Forecast Office, 2016). Additionally, while collecting seeds from trial A, it was observed that small black and yellow striped caterpillars belonging to the Noctuidae family were heavily consuming stems and foliage from individual plants. Although trial A produced significantly less seed, it did have over two times the amount of germination which could be explained by the heavy herbivory experienced by trial A plants by the Noctuid caterpillar. Each day that seeds were collected, anywhere in the range of ten to thirty caterpillars were observed on stems, leaves, flowers, and on the enclosure. Previous studies have shown that damage to fruits and flowers due to herbivory can decrease seed output of *Lupinus arboreous* by 80% (Harrison & Maron, 1995). At the CCBER greenhouse caterpillars consumed the stems of the plants which hindered development of any seeds. Even if seeds had begun developing on that stem, it was observed that often they would not

fully mature, severely decreasing the number of viable seeds collected from trial A. Lastly, although all the seed used was originally sourced from the same location, seeds from trial A are from 2005, and seeds from trial B are from 2012. Trial A, using older seed from 2005, had higher germination rates, but more seed was collected from trial B, using newer seed from 2013. *L. nipomensis* belongs to the Fabaceae family, a family of plants known to have long seed longevity due to the hardness of their seed coats (Spira et al. 1983). Therefore, seed age may not be as significant in regards to differences in germination rates.

Figure 2. Amount of seed collected for trial A and trial B over a three month period.



Overall due to the unexpected infestation by the Noctuid caterpillar it is difficult to isolate which conditions or combination of conditions are the most beneficial to bulking *L. nipomensis*. However, as it stands, our projects demonstrate that *L. nipomensis* can be successfully seed bulked in a nursery setting with our efforts producing over 6600 seeds. That being said, there is more to learn to improve our methods in order to protect this endangered species. In the future, additional experiments exploring how time of year impacts the probability of insect infestations should be undertaken as well as exploring if there is an impact of time in the growing season with the efficacy of pre-treatments. Overall, our results suggest that seeds exposed to hot water stratification prior to sowing and grown earlier in the year will produce the most seeds.

References

Clark, J. R. March 14 2000. Endangered and Threatened Wildlife and Plants; Final Rule for Endangered Status for Four Plants from South Central Coast California. Federal Register Vol. 65, No. 54.
< http://ecos.fws.gov/docs/federal_register/fr3546.pdf>

Feagin, Rusty A., Sherman, Douglas J., & Grant, William E. 2005. Coastal erosion, global sea-level rise, and the loss of sand dune plant habitats. Frontiers in Ecology and the Environment 3(7): 359-364.

Fischer, Douglas T., Still, Christopher J., & Williams, A. Park. 2008. Significance of summer fog and overcast for drought stress and ecological functioning of coastal California endemic plant species. Journal of Biogeography 36(4):783-799.

Goleta Water District. 2003. Frequently Asked Questions About Water Quality.
<http://www.goletawater.com/assets/documents/water_quality/Goleta_CCR_6_18_03.pdf>

Harrison, S. & Maron, J.L.. 1995. Impacts of defoliation by tussock moths (*Orgyia vetusta*) on the growth and reproduction of bush lupine (*Lupinus arboreus*). Ecological Entomology 20: 223-229.

Heusser, L. 1978. Pollen in Santa Barbara Basin, California: A 12,000-yr record. Geological Society of American Bulletin 89: 673-678.
< http://gsabulletin.gsapubs.org/content/89/5/673.full.pdf+html>

Hill, K. 2001. Dune Habitats. Smithsonian Marine Station. <
http://www.sms.si.edu/irlspec/Dunes.htm>

National Weather Service Forecast Office. September 2016. Observed Weather Reports-Santa Barbara.
< http://w2.weather.gov/climate/index.php?wfo=lox>

Ritchie, G.A. 1996. Operational Use of Vegetative Propagation in Forestry: World Overview of Cloning and Bulking. In: Landis, T. 0.; South, D. B, tech. coords. National Proceedings, Forest and Conservation Nursery Associations. Gen. Tech. Rep. PNW-GTR-389. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station: 192-197.
< http://www.fcanet.org/proceedings/1996/ritchie.pdf>

Rodrigues-Junior, Ailton G., Faria, Jose M. R., Vaz, Tatiana A. A., Nakamura, Adriana T., & Jose, Anderson C. 2014. Physical dormancy in Senna multijuga (Fabaceae: Caesalpinioideae) seeds: the role of seed structures in water uptake. Seed Science Research 24(2):147-157.

Sholars, Theresa. 2016. *Lupinus nipomensis*, in Jepson Flora Project (eds.) *Jepson eFlora*, http://ucjeps.berkeley.edu/cgi-bin/get_IJM.pl?tid=31836, accessed on September 15, 2016.

Spira, T.P. & Wagner, L.K.. 1983. Viability of Seeds Up to 211 Years Old Extrated from Adobe Brick Buildings of California and Northern Mexico. American Journal of Botany 70(2): 303-307.

Sungro Horticulture. 2013. Professional Growing Products.
< http://www.sungro.com/files/catalogues/SunshineMMTechno_Catalogue_2013.pdf>

Van Assche, Josef A., Debucquoy, Katrien L.A., & Rommens, Wouter A. F. 2003. Seasonal cycles in the germination capcacity of buried seed of some Leguminosae (Fabaceae) 158(2): 315-323.

Weber, J., Karczewska, A., Drozd, J., Licznar, M., Licznar, S., Jamroz, E., Kocowicz. 2007. Agricultural and ecological aspects of sandy soil as affected by the application of municipal solid waste composts. Soils Biology and Biochemistry 39(6): 1294-1302.
http://www.sciencedirect.com/science/article/pii/S0038071706004974

Western Regional Climate Center. 2016. Period of Record Monthly Climate Summary.

<http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?ca7940>

Wilken, D. 2009. *Lupinus nipomensis* (Nipomo Lupine) 5 Year Review: Summary and Evaluation. U.S. Fish and Wildlife Service, Ventura, CA. <
<https://ecos.fws.gov/docs/five_year_review/doc3219.pdf>