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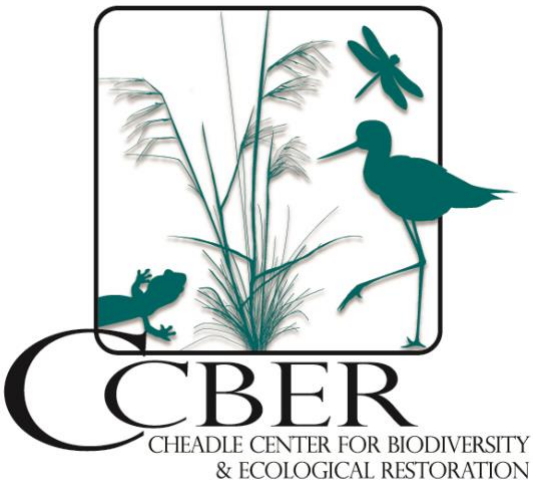

Lupinus nipomensis First Season Seed Bulking Report, 2012 – 2013

July 1, 2013

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Nipomo Lupine Seed Bulking Report

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Introduction

The following is a summary of CCBER's Nipomo lupine seed bulking trials from the 2012-2013 growing season. This interim report includes the dates, treatments, soils, and accessions used. Also included are the number of seeds harvested to date, and the accession numbers of the seed source for those plants which produced seed.

Sowing treatments:

Batch A: Tuesday Dec 13, 2012
After construction of a 108 1 gallon pot capacity enclosure, 128 seeds of *Lupinus nipomensis* were sown on Dec 13, 2012. The enclosure is outdoors, and exposed to more or less full sun.

Seed treatment for all seeds: soaking in hot water until cool before sowing.

Soil mix for all seeds: 39% CCBER-made compost/47% AGROMIN potting soil no fertilizer added/13% parts sharp sand. Additionally, 1% native sand from location mixed into the top ½" of soil for bacterial inoculation.

Seeds used from each accession:
-2012- no accession "A" /35.0362 120.6021- (SE of Jack's Lake) **50 seeds**, 1 per pot.

-2012- no accession "B"/ 35.0455 120.5901- (SE of Calendar RR, just S of Hwy 1) **6 seeds,** 1 per pot (2/8 seeds were non viable, slightly disfigured and easily crushed upon squeezing between fingers.)

-SBBG 2007-359- **15 seeds**, 1 per pot. These seeds were larger, and yellower than the others after soaking

-SBBG 2005-186- **10 seeds**, 1 per pot.

-SBBG 2005-185- **10 seeds**, 1 per pot

-SBBG 2005-184- **10 seeds**, 1 per pot

-SBBG 2004-194- **25 seeds**, 5 per pot (1985 seed)

Batch B: January 10 2013

Due to the late arrival of material ordered for the previous lupine enclosure, and the exceptionally cold conditions this fall and winter, germination on the first batch stands now at around just +/- 10 plants.
Soil mix was the same, except that the top 1" of soil was replaced with sunshine #5 organic plug mix, with a small amount of native sand sprinkled on the plug mix as inoculant.

These plants will also be started in a greenhouse, rather than outside which has warmer temperatures, and is already pest free. As the plants develop they will be moved into a similar outdoor full sun enclosure.

The greenhouse conditions are probably not necessary in most years, but the persistent frost has made the greenhouse more suitable and conducive to better germination counts.

Our second attempt involves another 100 pots, and 100 seeds, **50 from each 2012 "NO Accession", and 2007-259, respectively.** These were treated with warm very/warm water (not hot or boiling), briefly before sowing.

Batch C: Feb 25, 2013
A third sowing was tried for the growing season of 2012/2013. As persistent cold temperatures were the rule this winter, a water heater was installed in the greenhouse to assist with what was perceived as a cold problem. Therefore, on this trial, the seeds were not soaked in hot water before sowing, but rather, were watered in with warm water.

For this treatment, **100 seeds from "2012 not accessioned east of Jack's Lake"** were used**.**

Soil was 70% Sunshine #5 organic, 20% washed river sand, 10% sifted compost. All pots had 1% inoculum sand, sprinkled and mixed in center of pots to a depth of around .5".

Charts

Sowing Trail #	**Trial 1**	**Trial 2**	**Trial 3**
Initiation date	**12/13/12**	**1/10/13**	**2/25/13**
Soil Mixture %:			
Compost-	**39**	**37**	**10**
Potting Soil-	**47**	**45**	**0**
Sharp Sand-	**13**	**12**	**19**
Sunshine Plug Mix -	**0**	**5**	**70**

Inoculum Sand-	**1**	**1**	**1**
# Of Seeds Used	**116**	**100**	**100**
# Of Plants That Produced Seed or Fruits	**3**	**NA**	**6**
# Of Seed Colletcted	**260**	**0**	**8**
Seed Pre-Treatment	**1 Hour Hot water Soak**	**1 Hour Hot water Soak**	**No Treatment/Watered In With Warm Water**

Seed Producing Plant	Accession #	# Of Seeds Collected
#1	**SBBG 2005-184**	**55**
#2	**SBBG 2012 "A" NA**	**205**
#5	**2012 NA E of Jack's Lake**	**4**
#7	**2012 NA E of Jack's Lake**	**2**
#9	**2012 NA E of Jack's Lake**	**2**

Overview of Results

Germination
In short, both germination, and survival were low. Germination hovered at around 10% for all treatments. Initially, the cold/freezing temperatures were thought to be to blame for low germination, but the eventual release of winter's grip and the installation of a water heater in the greenhouse still yielded poor germination.

Survival
Seedling survival was also a difficult issue, with few of the successfully germinated seeds showing proper vigor as seedlings. Most seedlings developed pale foliage, wilting, and eventually died. This was difficult to diagnose, but temperature extremes, and under/overwatering were ruled out. Apparently the soils used had some toxic affect on the plants. Several dead seedlings were brought to plant pathologist Heather Sheck of the Santa Barbara agricultural commissioner's office, and yielded no signs of any pathogen by either culture or immunoassay.

Figure 1. Typical deceased seedling. These were delivered to the SB Ag commissioner's office and yielded no pathogens by either culture or immunoassay.

Curiously, all non-desirable plants that germinated in these trials, both native and non-native, thrived in the same soils. This included several species of natives that were introduced in the sand inoculum sand. An attempt was made to transplant 23 of the seedlings from "Batch A" into what was deemed to be more suitable soils on Feb 25, 2013 (the same, refined soil mix being used for the "Batch C" trial on the same date). Only one of these transplanted individuals was observed to have some active rhizobia on the roots, despite all having inoculum sand. Perhaps the bacteria/fungi in the compost interfered with the rhizobia in the sand connecting with the seedlings.

Figure 2. A healthier plant in flower

Comments/Recommendations for Future Trials

Lupinus nipomensis has proven to be a very peculiar and challenging plant to grow. This is the first plant I have experience with that has displayed these interesting characteristics and appears to be adversely affected by compost. Here are a few thoughts on how to improve germination and survival of the species.

Germination
Seeing how there was little to no difference in germination across the age spectrum of the seeds, or the treatments, a different approach in the future is warranted. Here are some potential different approaches:
-**A longer hot water soak.** In trial A and B, seeds were soaked in hot water until the water cooled (+/- 1 hour). A Subsequent conversation with Dieter Wilken indicated that soaking the seeds overnight, and sowing in the morning could improve germination.
-Scarification of the seeds. Also per conversation with Dieter, scarification was discussed. Some anecdotal evidence in the refuge indicates that the presence of *Lupinus nipomensis* is sometimes clustered around roads. This could indicate that some seed scarification occurs as a result of vehicle operation in the sand. Therefore, light scarification of the seeds should be implemented in future trials.
-Soil changes. This relates mainly to seedling survival, but possibly also to the successful germination of seeds in the soils used. For whatever reason, the organics used in our soils mixes may have been pathogenic to developing plants. This may be affecting germinating seedlings beneath the surface of the soil that cannot be observed. Therefore, I would recommend that all organics be dropped from the soils (with the exception, likely, of some sphagnum moss). Feeding should apparently be done chemically.

Survival
Due to the peculiar distaste this species shows for compost, a new soil type(s) should be implemented that omits the use of compost or any added organics that would generally be added to provide nutrition for the plants. Any feeding should be done chemically. We would like to do further soil tests in active population areas to asses a number of soil traits, of particular interest to me is salinity. We have had success keeping the seedlings of other fickle coastal species alive by light spraying with pure seawater (ie. *Suaeda*). This seemed to prevent damping off and other problems, as well as increasing seedling vigor, and may be beneficial for the Nipomo lupine.

Figure. 3. Plant displaying aborted flowers.; alive, but not producing seed.

Figure 4. Note both healthy fruits and an aborted flower on this plant. The aborted flower is visible just outside my index finger.

Additional Comments

The plants appear to be self-fertile. No pollinators were observed on the plants in their outdoor enclosure, but that does not rule out possible pollination. One

individual that flowered first, when the plants were still kept in the greenhouse due to the cold, set some seed (green pods) inside, where any insect pollination would have been very unlikely.

Figure 5. Numerous species thrived in the same soils the lupine struggled in. Among the genera included were *Artemisia, Camissoniopsis, Cardamine , Castilleja, Claytonia, Cyperus, Crassula, Erodium, Ehrharta, Plagiobothrys, and Stellaria* to name a few. All species showed no aversion to the soils, except *L. nipomensis.*

Lupinus nipomensis is a decidedly fickle plant to grow, much more so than other difficult dune species we have worked with. The fact that lupines associate with rhizobia of specific species (and potentially even genotypes) indicates that the bacterial community in the soil may be of high importance. According to Dieter's experience, the plants in his trial grow yielded no visible rhizobia on the roots. So, the species does not appear need to have a symbiotic relationship with its bacterial partner as long as it gets its nutrient needs met (although this symbiosis is likely desirable). However, it appears that the presence of an otherwise beneficial diverse bacterial/fungal community in the soil is detrimental to this species. This may offer

some insights into why the species thrives where it does, although it may be difficult to test for.

As of June 27, 2013, 8 plants remain alive, all but one is either in the process of finishing seeding, or has nearly finished. One plant, a late germinator, is in bud and has yet to flower. To date 268 seeds have been harvested, and +/-54 pods have yet to finish. One plant has just begun to flower and appears healthy. The number of seeds harvested should continue throughout the summer as this and other plants mature.