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Nipomo Lupine (*Lupinus nipomensis*) 2021-22 Year Outplanting and Assessment Studies

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During the 2019-20 hydrologic year several projects were initiated which were designed to evaluate questions resulting from the long-term Black Lake Ecological Area (BLEA) Nipomo lupine outplanting experiment. These include an assessment of soil conditions at various sites, an assessment of soil moisture at multiple sites and two additional experimental outplanting trials to evaluate the extent to which fog collection by cages could be more of a factor than herbivory-protection and to evaluate whether there are any benefits to lupine survival from growing ‘with’ versus ‘without’ other plants nearby. Results from those studies were compiled in the 2019-20 and 2020-21 reports after a second seeding of those experiments in 2020.

This report covers results from monitoring of the two experimental trials over the 2021-22 winter which was, again, very dry and reduced the number of seedlings which survived to produce seeds. This report also covers the 2021-22 augmentation effort which included 23 plots each seeded with 500 seeds established in the swales at BLEA (2014/15 trial swale and 2019 trial swale) and Kathleen Goddard Jones Overlook swale (KGJO). These plots included modest variation to further track the pros and cons of seed scarification and fencing/caging as well as parallel camera trap studies designed to document seed predation and plant herbivory.

Experimental Outplanting Trials:

Two questions came up in discussions with the recovery team regarding the results of the initial outplanting experiments from BLEA from 2014-15 and 2015-16 which were: 1. Could the uncaged plots where lupine seeds were vulnerable to herbivory have been negatively impacted by the fact that there was no mesh above the seedlings that could potentially capture fog moisture? 2. Some lupine seedlings have been observed germinating within the shade or immediately adjacent to veldt grass and other potential competitors. Could there be a facilitative function played by other plants for Nipomo lupine? To address these questions two trials were designed. The first is called “Fog versus Herbivory” which essentially creates an opportunity for lupine seedlings to receive potential additional of fog dripping off of a meshed enclosure (e.g. a cage with a mesh roof) but which also allows herbivory by lifting the cage above the ground by 5 inches. This project demonstrates whether the lack of seedlings in the uncaged treatment from earlier studies was due to herbivory or to reduced fog inputs and lower soil moisture conditions. The second experiment was designed to protect all plants from herbivory in cages but evaluates competition by placing seeds in completely

cleared/weeded plots and other seeds within a matrix of existing vegetation where weeds are not pulled. This allows us to address whether lupine germination, growth and seed production benefit from some intermittent clearing or disturbance to open the site and, alternatively, whether adjacent plants provide some reduced solar insolation or facilitate growth of the lupine plants.

The “fog versus herbivory” experiment was established at Black Lake Ecological Area (BLEA) and included five pairs of caged plots in three topographic positions (south facing, north facing and swale) in which 1 cage in each pair was lifted 5 inches off the ground to allow for small mammal/bird herbivory while retaining the cage and to potentially allow for fog drip from cage mesh tops (Figure 1, Map; Figure 2 photos).

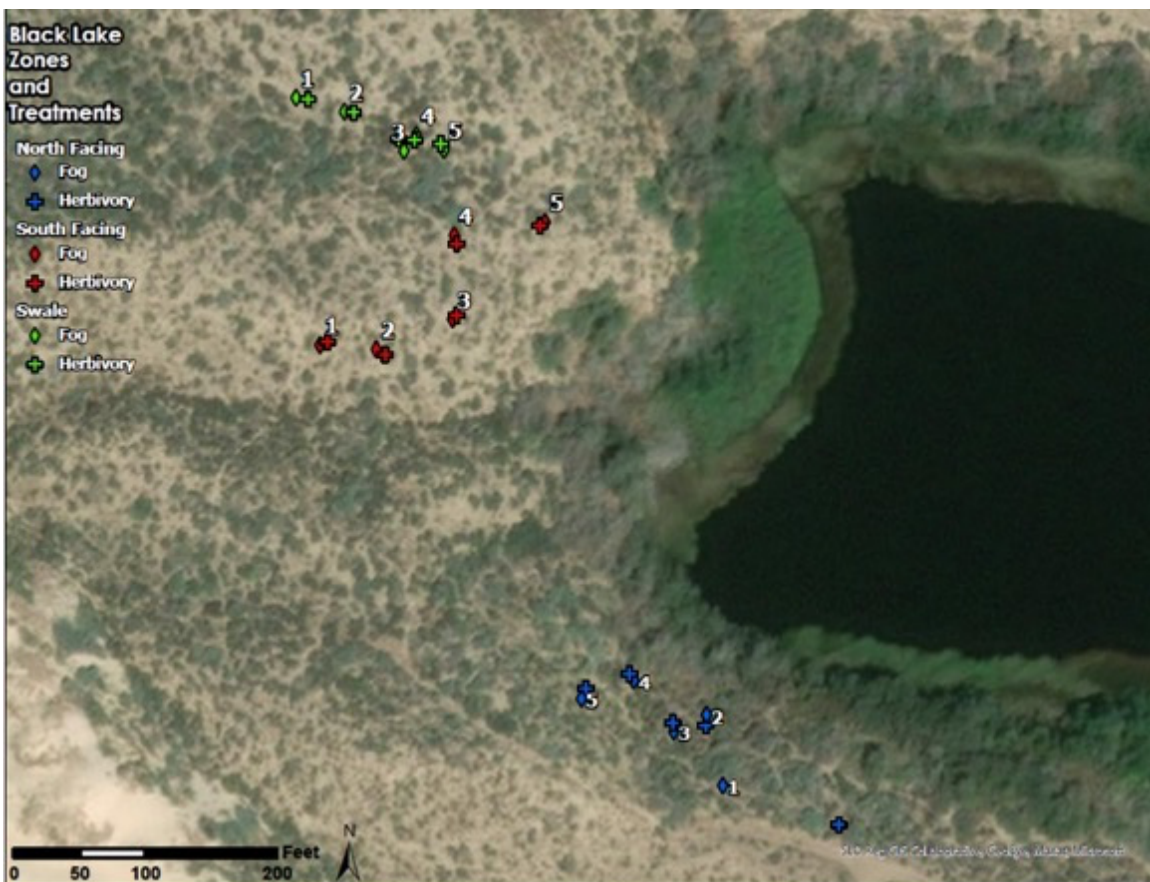


Figure 1. Map of Fog vs Herbivory Trial at Black Lake Ecological Reserve.



Figure 2a. Raised cage. Collects fog and allows for herbivory.



Figure 2b. Fully caged to collect fog and prevent herbivory.

The second 2019-20 experimental trial is the competition/facilitation experiment which was conducted in the swale at Kathleen Goddard Jones Overlook (KGJ Overlook) site where 5

pairs of cages were established at three topographic positions (south facing, north facing and swale sites). One of each pair of cages was fully weeded with a one-meter buffer around the cage (of either *Ehrharta calycina* (on south facing slope) or native plants (swale and north facing slopes) and the other was established within a matrix of non-native grass or native shrubs.

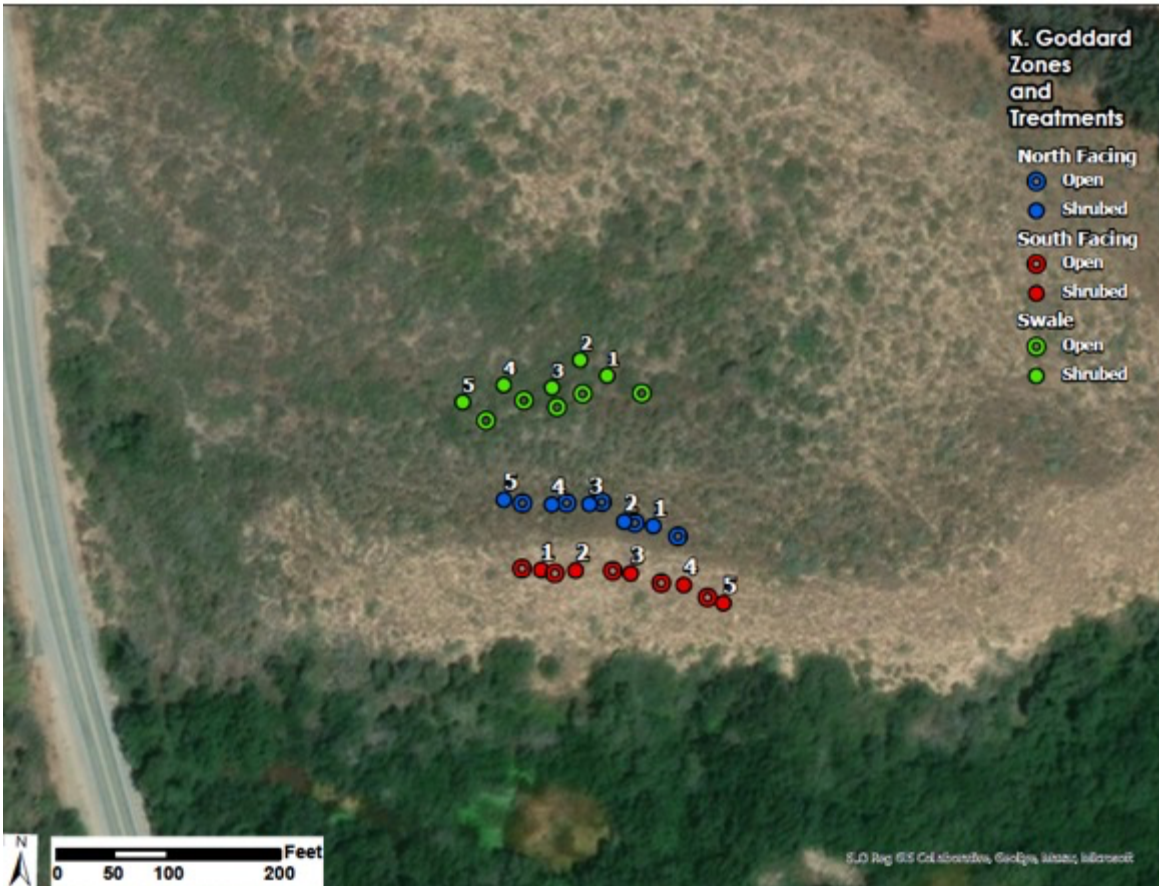


Figure 3. Experimental plots at Kathleen Goddard Jones Overlook comparing with vegetated, unweeded (+ competition, “shrubby”) with cleared and regularly weeded (- competition, “open”) treatments within caged plots.

The 2020-21 report on the Nipomo lupine Augmentation and Experimental Trials covers results from the 2019-20 and 2020-21 seeding and monitoring of these two trials. This report, which covers an additional very dry year, includes results for these trials and the augmentation effort.

Lessons learned in 2021-22 from Experimental Trials.

The fog and herbivory trial at Black Lake Ecological Area confirmed that herbivory is a factor in lupine germination and reproductive output (Figure 4). Despite both treatments (caged and lifted cages) having similar fog-catching capacity from the cages, the lifted cages had

marginally lower germination counts ($p = 0.099$) and significantly lower seed pod production (zero production, $p = 0.031$) compared to the average seed pod production of caged plots of 3.6 pods per plot. There was no significant difference in germination ($p = 0.46$) or seed pod production ($p = 0.363$) associated with topography. The caged plots had a higher proportion of germinants that reached reproductive maturity compared to lifted cages ($p = 0.005$).

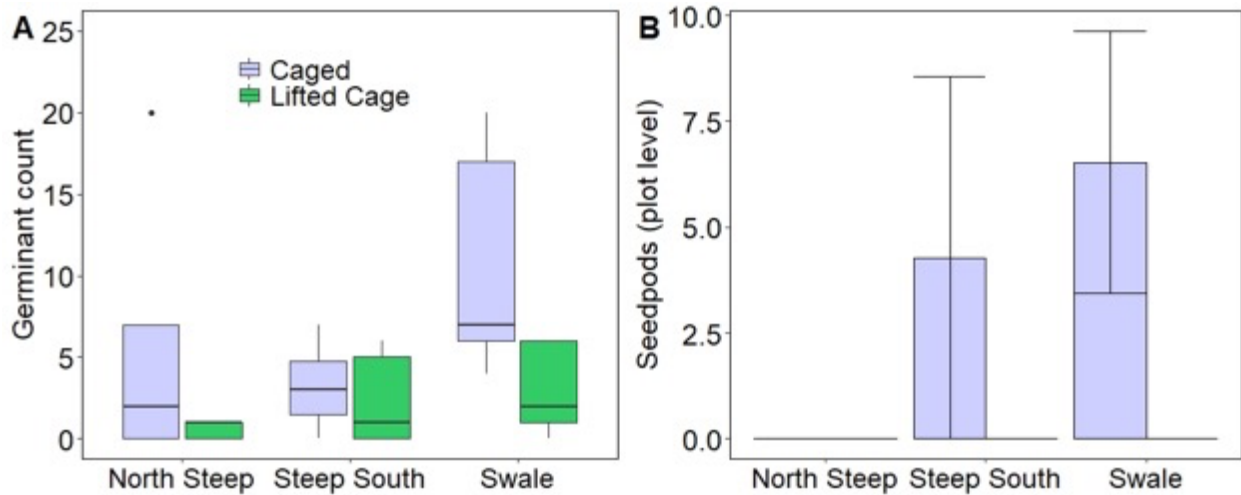


Figure 4. (A) Average germinant count and (B) average seedpod production by plot, topography and caging treatment from 2021-22 data.

We also monitored non-lupine plant cover in all plots and noted that the lifted cages showed evidence of herbivory by having significantly lower non-native cover ($p = 0.019$) and marginally lower native cover ($p = 0.076$) than the fully caged plots.

In the Kathleen Goddard Jones Overlook trial that compared caged cleared/weeded and vegetated/control control plots, we found that neither germination ($p = 0.994$) nor seed pod production ($p = 0.422$) by plot was different between cleared/weeded and vegetated/control plots (Figure 5). Topography, however, did produce significantly different results for both germination ($p = 0.037$) and seed pod production ($p = 0.089$). Swales had higher germination than the steep south-facing slopes ($p = 0.029$) and marginally higher germination than north facing slopes ($p = 0.074$). The significance of the effect of clearing may have been impacted by the overall low numbers of germinants and reduced numbers of mature plants in this dry year.

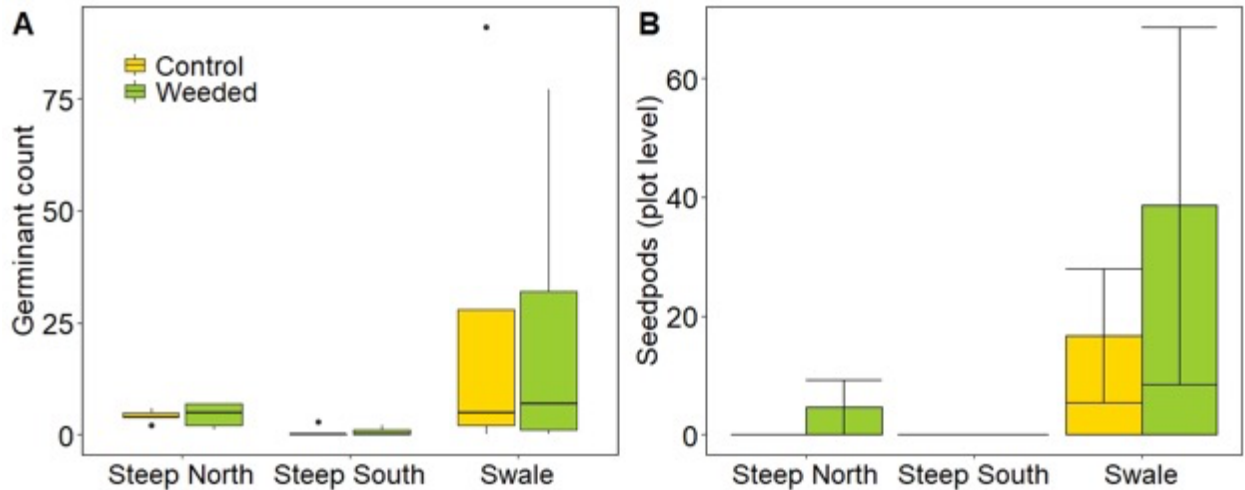


Figure 5. (A) Average germinant count and, (B) average seedpod production by plot, topography and weeding treatment from 2021-22 data from KGJO.

2021-22 Monitoring and Weeding Activities

The KGJO plots that were weeded/cleared were weeded throughout the growing season while the vegetated/control plots were not weeded at all. At Black Lake only Veldt grass and slender iceplant were pulled from the plots.

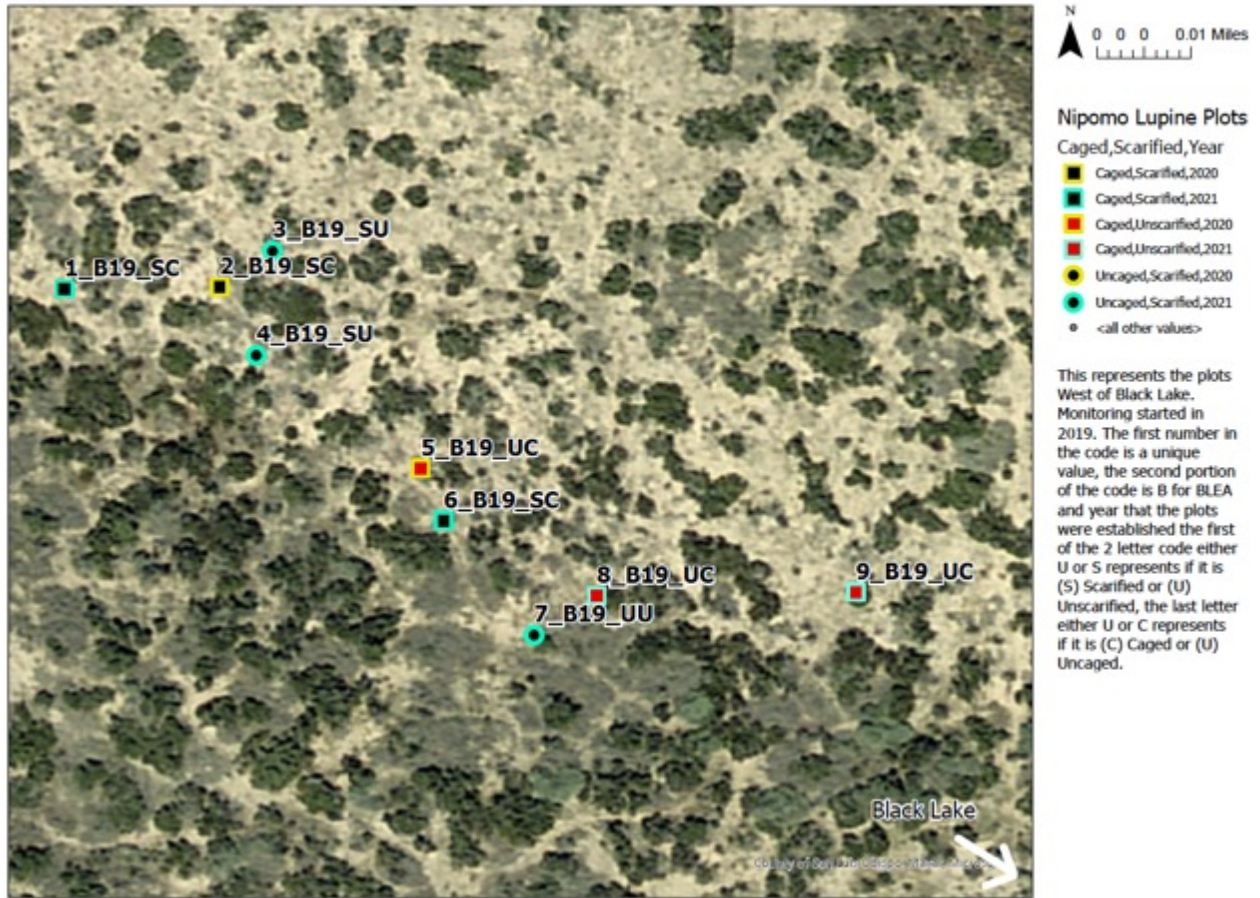
All plots, except the competitive KG Jones plots, were fully cleared during the fall 2021 to provide an open surface for lupine germination.

All trials were monitored in the 2021-22 year every two weeks for lupine germination, survivorship and reproductive output. Late in the growing season, but before plants dry up, cover and average height of all non-lupine species were estimated for each Black Lake plot.

Augmentation Plots 2021-22

In early December 2022 we established 23 ten by ten-foot plots in the swales at BLEA (18) and KGJO (4) as well as one plot at the overlook area at KGJO. Each plot was seeded with 500 seeds. Experimental components included 1) comparing scarified and unscarified seeds within cages (8 plots of each treatment); 2) comparing caged and uncaged plots with scarified seeds (6 of each in the BLEA swales). We also compared performance between the KGJO swale and the BLEA swales (both the 2014/15 trial swale and the 2019 trial swale) with 9 plots in each of the BLEA swale areas and 4 plots in the KGJO swale (See maps A, B and C).

Nipomo Lupine BLEA 2019 plots



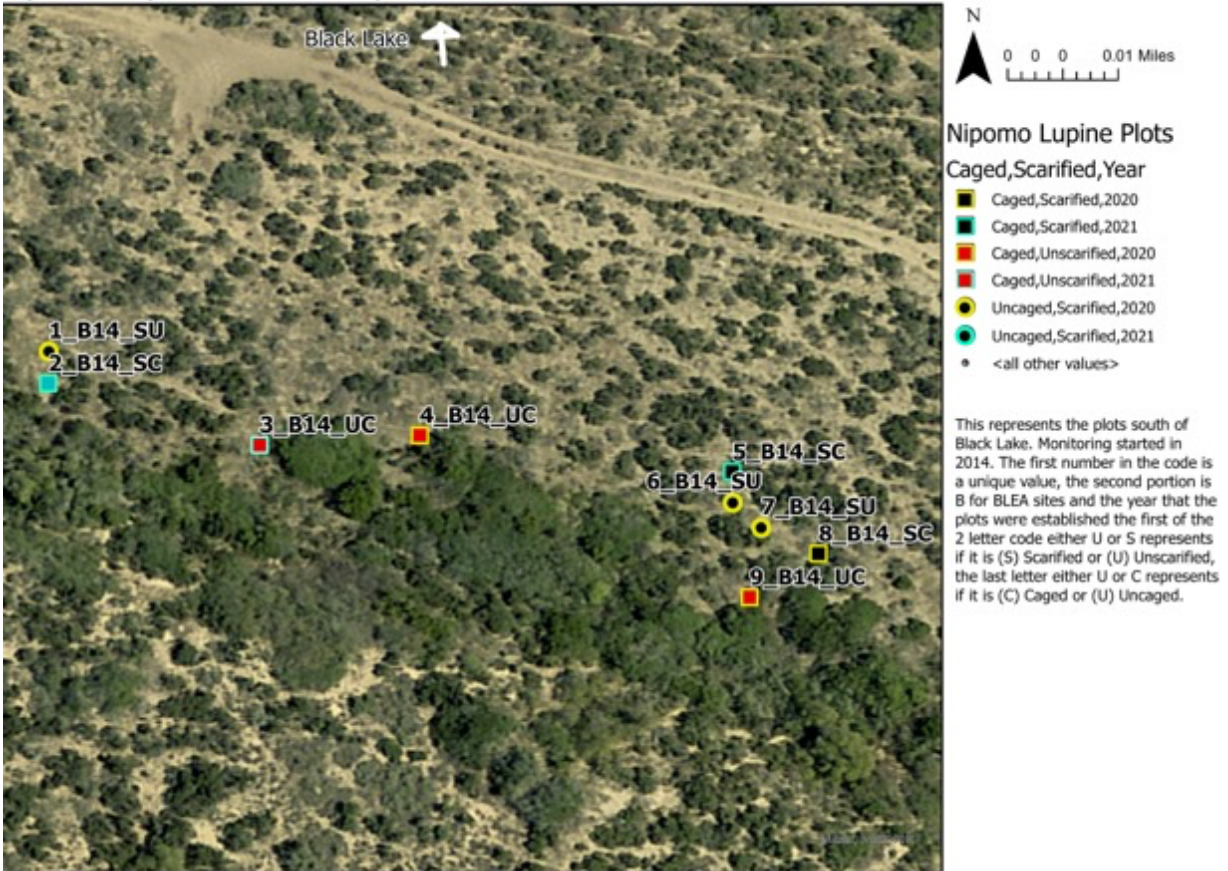
Map A. Augmentation plots at BLEA 2019 swale (9 plots)

Nipomo Lupine KGJO plots



Map B. Augmentation plots at KGJO swale (4 plots).

Nipomo Lupine BLEA 2014 plots



Map C. Augmentation Plots at BLEA 2014/15 swale (9 plots)

Plots were seeded just before or within the early rains of December: KGJO swale (12/1/22), BLEA 14 and 19 swales (caged plots: 12/10/22; and uncaged plots 12/21/22). The majority of the rainfall fell between 12/22/22 and 12/27/22 (Figure 6).

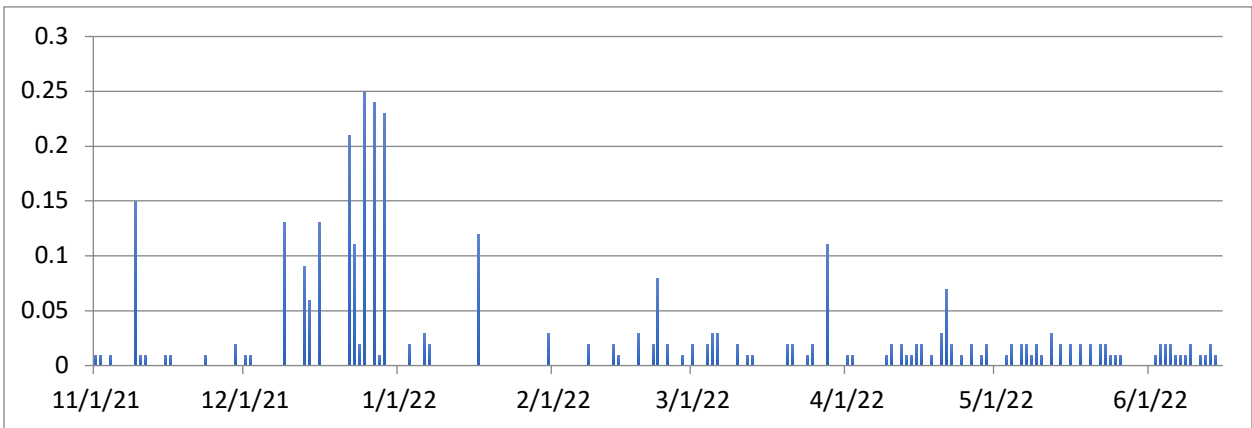


Figure 6. Rainfall (inches) in 2021-22 rainfall year which was overall very dry with minimal rainfall after the December rains.

2022 Augmentation Plot Results

Germination was significantly higher in caged (17%) compared to uncaged (1.5%) plots ($p < 0.001$) (Figure 7). Scarified seeds germinated at a significantly higher rate (17%) compared to unscarified seeds (8%), where scarification involved dragging individual seeds across a sheet of sand paper (Figure 8).

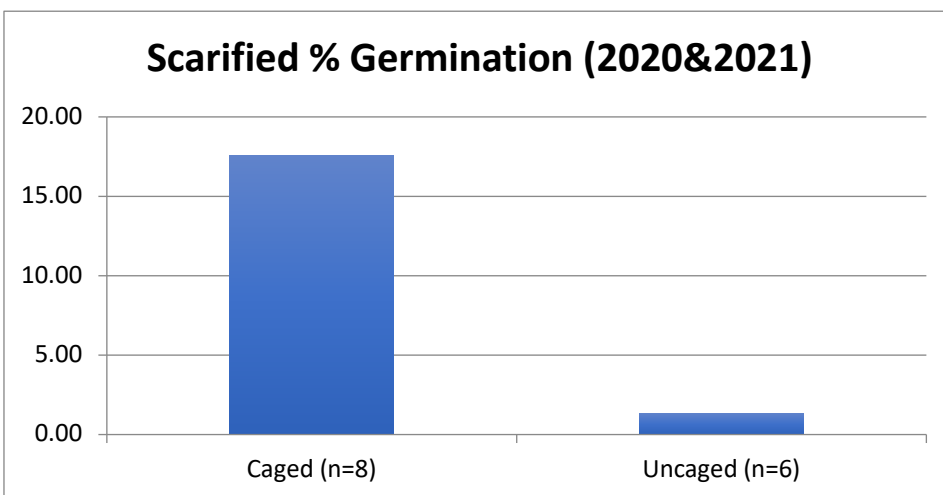


Figure 7. Scarified seed germination within and outside of caged plots.

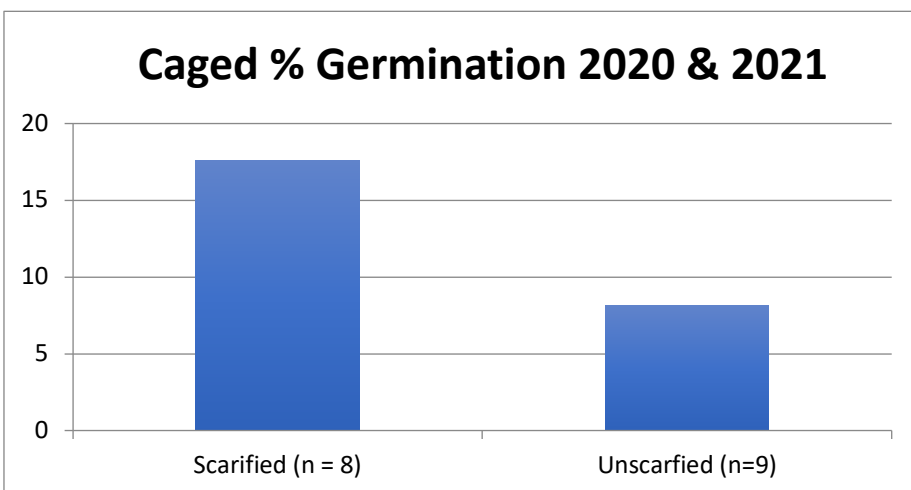


Figure 8. Scarified and unscarified seed germination within cages.

Overall germination at KGJO was lower than at BLEA with caged scarified seeds germinating at a rate of 3.7% at KGJO and 22% at BLEA. But caged, unscarified seeds germinated equally at the two sites at a rate of 2% at KGJO and BLEA. There were no uncaged plots at KGJO to compare with BLEA. There were also no plants at KGJO which survived to set seed, while at Black Lake significantly more seed pods were produced: 9% of

scarified, caged germinants produced seed and 7.5% of caged, unscarified germinants set seed ($p = 0.015$). There were a total off 11 average scarified germinants that set seed per plot and 3 unscarified germinants that set seed per plot at BLEA. There were essentially no uncaged, scarified plants that produced seed at BLEA ($p < 0.001$)

Because of the extremely dry winter (Figure 9) more than 50% of the germinating seedlings in most plots died before February and another 50% before March (Figure 10).

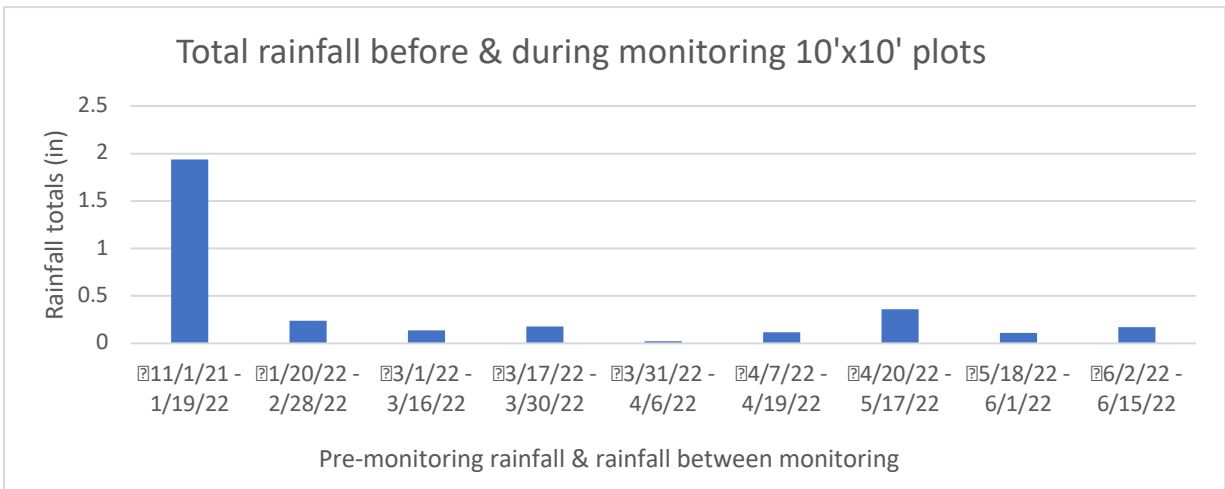


Figure 9. Rainfall totals between monitoring periods in Nipomo.

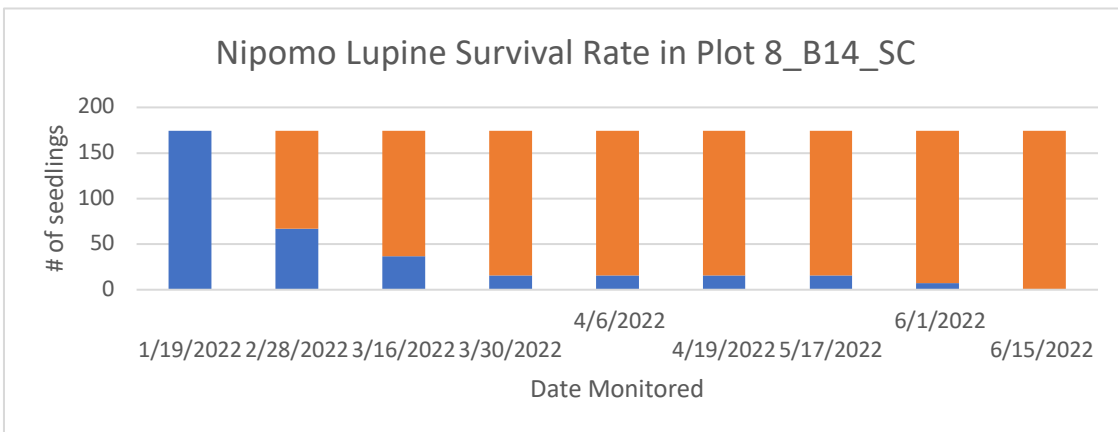


Figure 10. Example of caged scarified plot germination and survivorship over time.

We estimated the total seedbank that could be in each of the plots by taking the original 500 seeds, subtracting the total number of germinants and adding in the estimated seeds produced based on multiplying the counted number of seed pods per plot by an estimated 2 seeds per pod. The caged and scarified plots have the largest seed bank (avg of 795) and the uncaged plots have the lowest seed bank (avg of 485) with the unscarified plots fall in-between (avg 625) (Figures 11-13). This does not include the potential impacts of seed predation by rodents and birds which we documented through a series of camera traps focused on seed

feeding stations.

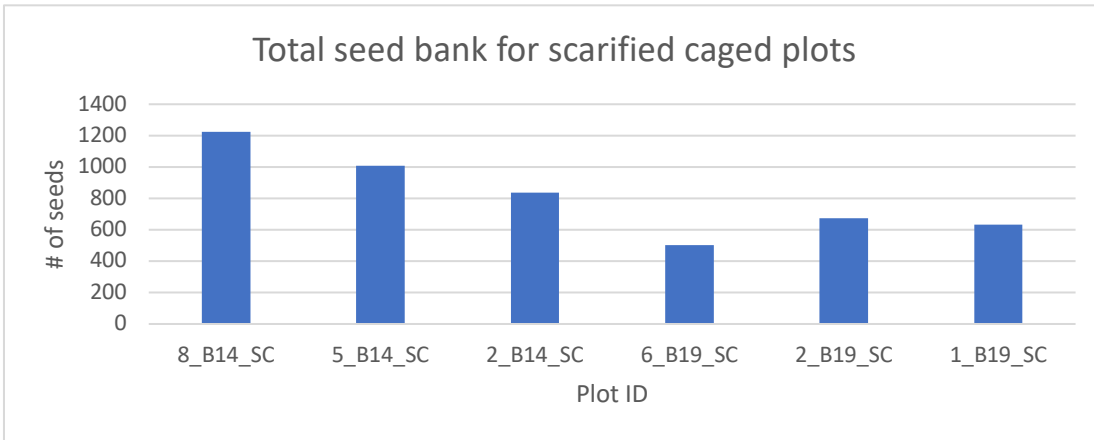


Figure 11. Estimated seed bank for caged and scarified seed plots at BLEA (795).

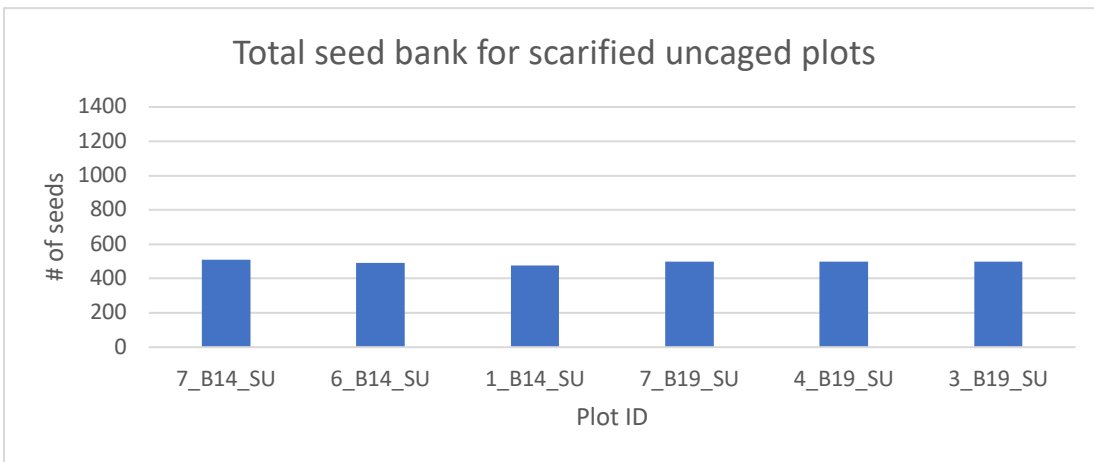


Figure 12. Estimated seed bank for uncaged, scarified seed plots at BLEA (485).

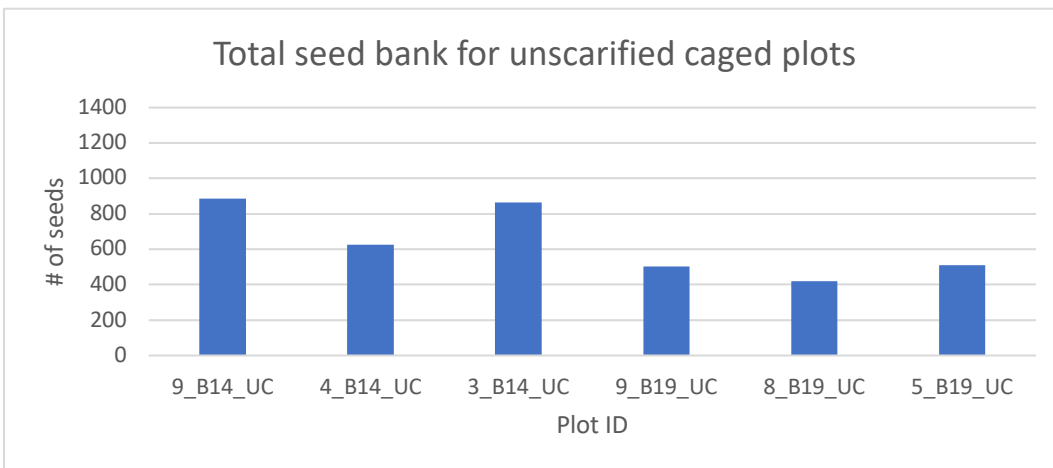


Figure 13. Estimated seedbank for caged but unscarified seed plots at BLEA (625).

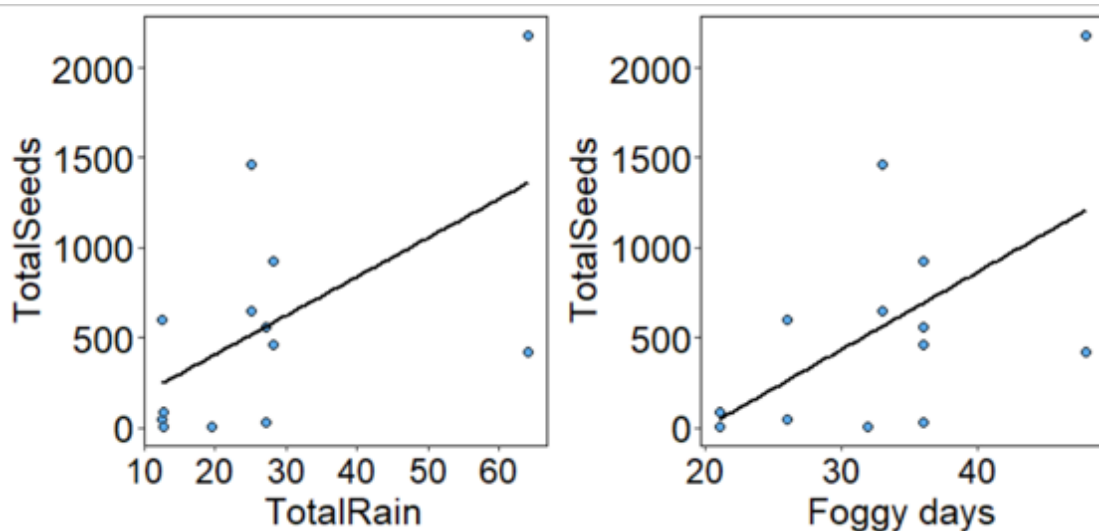
Initial Assessment of Climate Variables and the 8 years of BLEA 14/15 trial results

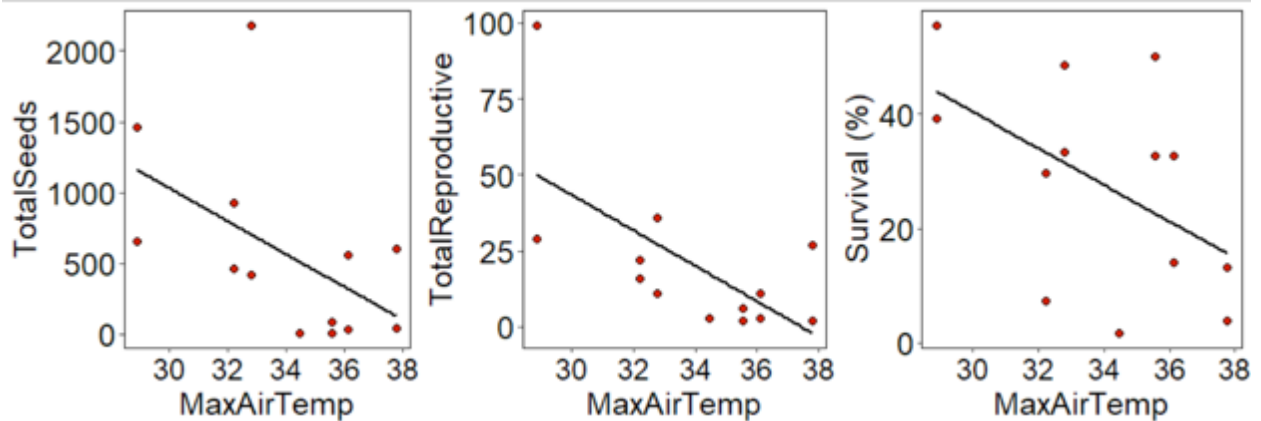
Justin Luong, recent doctoral graduate of UCSC and associated researcher with the Cheadle Center analyzed climate data and the 8 years of the BLEA 2014 project (2014-2022) lupine data to evaluate the effect of Total rain, early rain (October – January), late rain (February to May), rain in the previous year, Max air temperature, Average Air temperature, vapor pressure deficit, solar radiation intensity and “fog” based on the number of days with lower than 60% of ‘normal’ solar insolation (250). The measurements looked at the relationship of those variables with Total per plot germination, total seed pods, total reproductive individuals, survival (reproductive/germination), average germination per plot, average seed pods and average reproductive individuals. Because the data were mostly normal, a Generalized Linear Model was used

Total seed production was significantly correlated with total, early and late rain and with fog (p between 0.036 and 0.045) and R^2 between 0.28 and 0.26. Max air temperature was marginally (p = 0.61) significant.

Total reproductive individuals was significantly affected by Max Air Temp (p = 0.015), R^2 = 0.22. The proportion of seedlings that survived to become reproductive was marginally significantly correlated with Max Air temperature (p = 0.071) and Average air temperature (p = 0.085).

These data can be visualized in the graphs below.





Precipitation (especially earlier season) seems to be positively related to total seed production for the season whereas maximum air temperature has a negative effect on number of seeds, reproductive individuals, and survival. This may indicate that precipitation and water can help improve seed production but *Lupinus nipomensis* appears to be sensitive to warmer maximum temperatures. Similar trends were seen marginally for average air temperature for some fitness measurements.

Seed predation Assessment

While there is no prediction that the 2022-23 year will have higher rainfall and results for the experimental trials and augmentation trials appear to have demonstrated the primary conclusions regarding scarification and caging increasing germination and seed set, there is still the opportunity for on-going analysis of weather on germination and seed set data from these trials by continuing to monitor these plots over time. In addition, given the differences in the estimated seed bank between the various augmentation plots, it should be possible to assess the potential impact of seed predation in and out of cages in this trial by assessing total germination by plot next year to see if there is a correlation with the estimated seed bank. These results will help determine whether it makes sense to continue to bulk seed and attempt to establish new populations in the field under ambient weather conditions and whether such efforts should include caging. From our greenhouse work, we are relatively certain that irrigation of plants during dry periods after germination would benefit the population, but is that kind of supplemental management appropriate for an annual species?

During the 2021-22 year more than 10,000 hours of camera trap time were collected by Mary Cadogan, UCSB undergraduate, with 4.3 of those hours including wildlife observations. Overall, deer mice, kangaroo rats and several seed-eating birds (primarily California towhee and white-crowned sparrow) were observed directly foraging where the seeds were placed. Rodent numbers were significantly higher in uncaged bait station areas adjacent to vegetation and bird observations were higher within caged and uncaged plots further from vegetation.

The proximity of vegetation appears to affect seed predation and, hence, seed bank persistence in this species. This predation study will be conducted again with less-obscured seed that are not buried in sand so that observations are clearer and over shorter time periods so that seed consumption can be more easily counted without factors such as wind scouring seed out of the bait boxes between observation periods.

Proposed 2022-23 Activities

BLEA 2014/15 plots – These plots will be assessed after rain events and only those plots with germinants will be monitored long term to quantify seed set.

BLEA 2019/20 plots – These plots will be monitored.

KGJO 2019/20 plots – The swale and north facing plots will be monitored

Augmentation plots –

2021-22 – The 23 plots (16 caged, 6 uncaged and 1 KGJO overlook plot) will be monitored in 2022-23.

2022-23 – 10 new caged plots in recently cleared areas at P66 will be established and seeded with scarified seeds (500 per plot) in November 2022 before the first rain.

- 6 new caged plots will be established at BLEA 2014/15 swale (3) and 2019/20 swale (3) and seeded with 500 scarified seeds before the first rains in November 2022.

These plots will be monitored.

Monitored plots – All seedlings will be marked with plastic tags and documented in a data base, total number of surviving seedlings will be tallied every 2-3 weeks and total number of seedlings surviving to become reproductive will be documented per plot and each plant's total seed pod count will be assessed.

Seed predation-

A follow-up seed predation study will be conducted with a UCSB undergraduate and wildlife camera traps in the fall and early winter with seed trays dispersed adjacent to and far from Veldt grass and in and out of cages with seeds being counted over various time frames (3 days to 2 weeks) to get a better estimate of seed predation rates. Camera traps will be deployed and analyzed to further document wildlife visitation rates.