Propagation and reintroduction for Lupinus oreganus in the BLM, Roseburg District



2017 Report to the Bureau of Land Management, Roseburg District

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PREFACE

IAE is a non-profit organization whose mission is conservation of native ecosystems through restoration, research and education. IAE provides services to public and private agencies and individuals through development and communication of information on ecosystems, species, and effective management strategies. Restoration of habitats, with a concentration on rare and invasive species, is a primary focus. IAE conducts its work through partnerships with a diverse group of agencies, organizations and the private sector. IAE aims to link its community with native habitats through education and outreach.



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Propagation and reintroduction of Lupinus oreganus, Douglas County

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Cover photographs: *Lupinus oreganus* in the greenhouse, and outplanting at Loose Laces. (Photo credit: Denise Giles)

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EXECUTIVE SUMMARY

This document reports initial work from seed collection, propagation, and outplanting of *Lupinus oreganus* for the Roseburg District Bureau of Land Management. Sites managed by the Roseburg BLM represent the southernmost range of this threatened plant species. Three sites were outplanted including Callahan Meadows (~1,900 plants), Loose Laces (~1,075 plants), and Stout's Creek (~750 plants).

Seeds collected in 2005, 2011 and 2016 from four source populations were stratified and grown by IAE staff in the winter/spring of 2016-2017. Of the 6,920 seeds started in December 2016, approximately 3,725 were outplanted in April of 2017. Seeds were collected from China Ditch, Dickerson Heights, Loose Laces, and Stouts Creek.

Seeds were scarified and then placed in cold-wet stratification for 4 weeks. After cold stratification seeds were removed to warm greenhouse conditions to break dormancy (though nearly all seeds broke dormancy in cold).

Germinated seeds were inoculated with species specific rhizobium and potted into 10" Ray-Leach conetainers. After \sim 12-16 weeks in the greenhouse, plants were transferred outdoors to harden off for 1-2 weeks. Hardened individuals were then transferred to field sites and outplanted using both dibbles and hand tools.

A subset of *L*. oreganus at each site were outplanted into a grid to facilitate monitoring. Initial survivorship of these plants approximately eight weeks post-outplanting was above 90% at all three sites.

Information about mortality and survivorship from germination to outplanting will be valuable in determining plant material needs for future reintroduction efforts for *L. oreganus* in Douglas County.

Propagation and reintroduction for Lupinus oreganus in the BLM, Roseburg District

A REPORT THE BUREAU OF LAND MANAGEMENT, ROSEBURG DISTRICT

INTRODUCTION

This report documents reintroduction and propagation protocol activities conducted on *Lupinus* oreganus (nee *sulphureus* ssp. *kincaidii*, Kincaid's lupine; Figure 1), in the Roseburg District of Bureau of Land Management.

Species Status

L. oreganus, a member of the legume family (Fabaceae), is listed by the Oregon Department of Agriculture and the U.S. Fish and Wildlife Service as a threatened species. This species serves as an obligate host plant for larvae of Fender's blue butterfly (*Icaricia icarioides fenderi*), which is listed as an endangered species.

Background Information

L. oreganus is found in native prairie remnants in the Willamette Valley, southwestern Washington, and forest openings in Douglas County, Oregon. Only 161 sites are known to support this species and 94 of these cover less than one acre (USFWS 2010). The majority of the sites are on privately held land, which is exempt from protection(s) provided by state and federal listing, increasing the importance of management by state and federal agencies on public land. Only a limited number of remnant populations of *L.* oreganus exist in Douglas County. Of the extant populations in Douglas County, eight are located on BLM land, four are found on private land and one population is managed by the US Forest Service. **The 2006 Management and Recovery Plan proposes a goal of 5,000**



FIGURE 1. KINCAID'S LUPINE (LUPINUS OREGANUS).

 m^2 of foliar cover consisting of at least two meta-populations in Douglas County. It was estimated that in 2016 there was approximately 750m² of lupine cover in Douglas County (Ottombrino-Haworth et al. 2016).

Within the Willamette Valley, *L. oreganus* is a larval host plant for the endangered Fender's blue butterfly, making conservation of the *L. oreganus* important for the *L. oreganus* itself as well as the insect (Schultz et al. 2003). There are no known sightings of Fender's blue in Douglas County and it is not known whether the two species co-occurred historically in that area. Despite the apparent absence of Fender's in Douglas County, this southern population which shows tendencies towards being adapted to the shorter, and warmer springs of Douglas County could provide important genetic variability in future growout and seed increase projects in the face of changing climate. Work by IAE has shown that seeds from the southern range tend to do better in warmer microclimates, flowering before northern seeds sources, and germinating in cold stratification (Gray et al. 2013, Gray and Bahm 2017).

Reproduction and Population Biology

L. oreganus is an herbaceous, perennial that reproduces by seed. Plants form clumps of basal leaves and eventually produce one or more flowering stems. This species also spreads vegetatively, though it is unknown to what extent vegetative growth might result in the formation of physiologically distinct clones. L. oreganus requires insects for successful fertilization and seed formation (Kaye, 1999).

Objectives

- Collect seed from populations in Douglas County including China Ditch, Dickerson Heights, Loose Laces, (Letitia Creek), and Stouts Creek.
- Propagate Lupinus oreganus seed collected in Douglas County
- Grow individuals for outplanting
- Outplant L. oreganus at sites in Douglas County.
- Monitor and assess initial success of outplanting efforts

METHODS

Seed Collection

In June of 2005, 2011, and 2016 seeds were collected from 4 sites in Douglas County; China Ditch, Dickerson Heights, Loose Laces, and Stouts Creek. In 2016, approximately 30 breathable, mesh bags were placed over maturing fruits. One to five mature stems were gathered into each bag and tied with a 10" length of hemp or cotton 1/8" string. Bags were left in place until the seed pods had fully matured. Mature fruits were collected in early July. Bagged stems were cut with scissors or broken by hand and placed into paper bags for temporary storage and subsequent cleaning.

Cord to tie the bags was weather resistant and selected to not stretch or shrink significantly with changes in temperature or humidity. Care was taken when tying bags to exclude stems with obvious insect infestations. Other species, particularly weeds, were also excluded when tying bags. Care when placing collection bags saved cleaning time, and decreases predation risk for these valuable collections.

Seed Cleaning

Collected plant materials were cleaned within one week to two months of collection. At the time of collection, many seeds had already dehisced into the collection bags. For cleaning, the dehisced seeds, as well as the mature, dry stems were removed from pollination bags; pods which had not already opened, were opened by hand. Mature, clean seed was evaluated based on plumpness of the mature seed as well as presence/absence of insect grazing or apparent presence of other diseases, including mold. Seeds with evidence of predation, or that were apparently unfilled, diseased, or immature were not included in our germination trials. Seeds were kept separate by year of collection and site.

Seed Preparation and Germination

Cleaned seeds, were sorted into envelopes of 50-150 seeds by seed source and year of collection. Seeds from 2005 and 2011 had been stored in paper envelopes at room temperature. Seed from 2016 was stored in the same manner for a shorter period of time.

In December 2016, all stored seeds were scarified for 3 mins at 25 psi, using a pneumatic seed scarifier at the Oregon State University Seed Laboratory. Scarified seeds were used immediately.

After scarification, seeds were placed into germination boxes with damp seed germination paper. All germination boxes and tools were sterilized prior to use. Blotter paper was wetted with distilled water.

Groups of 150 scarified seeds were placed into 6.5" x 9.5" germination boxes, and sprayed with distilled water. Filled boxes were placed into cold, $5^{\circ}C$ ($41^{\circ}F$), dark stratification for 4 weeks. Boxes were checked daily in the first week, and then moved to a weekly monitoring schedule. Moldy seeds were removed, and boxes were sprayed with distilled water to keep seeds and blotter paper moist (but not soaking). In the first days of stratification, the seeds required extra water as they imbibed (nearly doubling in size) and began the germination process.



FIGURE 2. LUPINUS OREGANUS FROM SEED TO PROPAGULE.

Propagation

Soil preparation

Sungro[®] LA4PC potting soil was used for potting germinants, this mix contains 55-65% sphagnum, with the remainder perlite, pumice and dolomite. Potting soil was amended with; a slow release fertilizer for container plants (Apex[®] 14:14:14 at a medium rate of 7lbs per cubic yard), a micronutrient supplement (Micromax[®] micronutrients at a rate of 1.25 lbs per cubic yard), and a biological fungicide (Actinovate[®] at 2 oz/cubic yard of soil). Mixed and wetted soil was loaded into RayLeach SC-10 cone-tainers, and

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wetted again. Pots and trays were steam sterilized prior to use. Trays were filled to allow 1/8" -1/4" head space.

Potted plants were treated with predatory nematodes at the time of potting to prevent fungus gnats. When plants were one to two weeks old, and had begun to form true leaves, a thin layer of sand was spread on the top of each conteainer to prevent gnats from ovipositing into the soil. Dead or dying plants were removed promptly from the greenhouse to prevent the spread of disease. These preventative measures are recommended to reduce greenhouse pest issues.

Potting germinated seeds

Germinated seeds were removed from cold stratification and allowed to sit for 0-3 days in the greenhouse which was maintained with 21°C (70°F) days and 15.6° C (60°F) nights. Many seeds had already germinated while in cold stratification, and had radicles 1-8cm long (Figure 2).

Germinated seeds were tossed in an inoculant which included a variety of rhizobium species, these beneficial bacterial live in the roots of the plants, fixing nitrogen from the air in the form of ammonium making this important element available for plant growth. Inoculant was prepared by Dr. Thomas Wacek, using a *Bradyrhizobia* culture isolated in 2014 from nodules harvested from *Lupinus* oreganus in a production field managed by the USDA Plant Materials Center in Corvallis, Oregon. A mix of other more generalized inoculant for annual and perennial lupine species was also included in the inoculant mix. Generalist mixes of inoculant are available commercially, and have been used successfully in the past when growing *L*. oreganus.



FIGURE 3. LUPINUS OREGANUS PROPAGULE READY TO BE OUTPLANTED. NOTE THE NODULES ON THE ROOTS (CIRCLED IN BLUE).

After each germinated seed was tossed in inoculant, tweezers or bare fingers were used to guide the radicle into the prepared conetainer. The entire radicle was placed below the soil surface.

Greenhouse maintenance

Greenhouse temperatures were maintained with 70°F days and 60°F nights. Supplemental lighting was not utilized. Lupine in the greenhouse, have a tendency to develop mold and fungal diseases. Propagules were watered twice weekly, allowing pots to dry between waterings. Watering of propagules occurred in the morning; this helped to keep humidity down, and allowed the leaves of the plants to dry throughout the day.

Outplanting

Plants were removed to an outdoor space to harden off two weeks prior to outplanting.

At the time of outplanting plants were removed from conetainers on site and planted into prepared holes. Nodules were noted on the roots of many of the propagules (Figure 3). Dibbles and hand tools were used to create holes for outplanting. Each outplanted propagule was covered with native soil. Plants were outplanted with a spacing of roughly 0.5-1.0m between plants, dependent upon site conditions.

Areas were selected for outplanting based on similarity to existing habitat at each site. At each of the three sites, a portion of the outplanted individuals were planted in a grid in order to facilitate monitoring of survivorship and vigor. The remaining plants at each site were not planted in a grid, thus the number of outplanted individuals into each area is approximate.

Approximately 3,725 plants were outplanted at three sites on April 11-13, 2017 including Callahan Meadows (~1,900 plants), Loose Laces (~1,075 plants), and Stout's Creek (~750 plants) (Table 1).

TABLE 1. NUMBER OF PROPAGULES OUTPLANTED INTO PATCHES AT EACH SITE. BOLD INDICATES AREAS OUTPLANTED INTO A GRID. REMAINING VALUES ARE APPROXIMATE NUMBER OF PLANTS OUTPLANTED INTO EACH AREA.

	Area Designation	Lupine present in			
Site Name	Designation	area prior to outplanting?	Description	Number outplanted	Total
Loose Laces	А	Y	Cutbank- grid	125	
	В	Ν	Above cut bank in grid	100	
	С	Ν	Above Cutbank - amorphous	450	
	D	Ν	Kendig's Patch - above cutbank	400	
					~1075
Stouts Creek	A	Y	Cutbank m126-139 – damaged by road maintenance	100	
	В	Y	Cutbank m60-90 – damaged by		
	C	V	road maintenance	150	
	C	ř	Above Cutbank – amorphous – damaged by road maintenance	400	
	D	Ν	Above cutbank - 5m x 5m grid (L. oreganus in vicinity, but none in plot	400	
			prior to outplanting)	100	
					~750
Callahan	А	N			
Meadows			Near to existing plot - grid	440	
	В	Ν	Amorphous central area	460	
	С	Ν	Rectangle- within this space	1000	
					~1900

TOTAL OUTPLANTED

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~3725

Site Specific Outplantings 2017

CALLAHAN MEADOWS

Three areas were outplanted at Callahan Meadows with a total of 1,900 plants (Figure 4). Plants were outplanted into currently unoccupied habitat located along the treeline between the existing monitoring plots at the site. 440 plants were planted in a 20mx12m grid, labeled "A" (Figure 4). The remaining plants were outplanted into areas "B" (460 plants) and "C" (1,000 plants) with an approximate spacing of 0.5-1.0m. At the time of outplanting, no other lupine were found to be present in any of the areas outplanted.

Due to site access issues, we were not able to assess the initial survivorship at this site.

Area A:

This area contains 440 plants outplanted in 20m x 12m grid. The grid extends from the existing monitoring plot, with a 22m gap between plot edges. The southern corners are marked with concrete markers placed flush with the ground, the northwest corner is marked with a piece of PVC in the ground, and the northeast corner is not marked. Along the long axis, plants are spaced every 0.5m and on the short axis, plants were outplanted every 1.0m. Rows at meters 0,2,4,6,8,10, and 12 are planted on the ".25m" and ".75m"; odd numbered rows at meters 1,3,5,9, and 11 were planted on the ".5m" and ".0m". (Row 7 was skipped.) No *L*. oreganus were noted in the plot boundaries at the time of outplanting.

Area B:

This area, to the east of A, contains approximately 460 plants. Boundaries of the area outplanted can be seen in Figure 4. (No plot markers define this area.) No *L*. oreganus were noted in the plot boundaries at the time of outplanting.

Area C:

This 13x 20m area contains approximately 1,000 lupine plants. The corners of the area outplanted are marked with concrete markers placed flush with the ground. Plants were outplanted within this rectangle with an approximate, and not systematic, spacing of 0.25-1.0 m between plants.

LOOSE LACES

At Loose Laces, four areas were outplanted with a total of 1,075 plants in areas labeled A-D (Figure 6). Areas A and B are planted in a grid pattern, with 0.5m spacing. Areas C and D contain approximately 475 and 450 plants respectively, with an approximate spacing of 0.5-1.0m in the areas defined.

Area A:

125 plants were planted into Sub-population 3 which is found along the roadcut. Three, 20m long rows, have plants placed every 0.5m; plants are staggered such that the first and third row plants are placed on the ".25m" and ".75m", and the central row is planted on the ".5m" and ".0m". The central row is marked with a concrete marker placed flush with the ground at the head and end of the central transect. These transects were established at roughly 60-80m along the existing monitoring transects for Loose Laces Sub-population 3.

Area B:

100 plants were planted into a $5m \times 5m$ grid located approximately 10m north of rebar tagged #514 and #517 as part of the Sub-population 4 monitoring plot. The four corners are marked with concrete markers placed flush with the ground. No *L*. oreganus were present in the grid area prior to outplanting.

Area C:

Approximately 450 plants were outplanted into appropriate habitat on the bank above sub-population 3, and to the North of sub-population 4. At the time of outplanting, lupine was not present in this area.

Area D:

Approximately 400 plants were outplanted into Sub-population 2, "Kendig's Patch". The majority of these plants (\sim 350) were placed on the upper portion of the bank located to the northeast of Sub-population 2, while \sim 50 plants were placed into the skid road extending the population to the north. The skid road was found to be very compacted and difficult to plant into. All *L. oreganus* were planted outside of the existing plot boundaries at the site.

STOUT'S CREEK

At Stout's Creek, four areas were outplanted. Two areas were outplanted into the cut-bank which includes monitoring transect for the road-side portion of Sub-population 2. Plants were placed at m60-m90 (Area A) and again at roughly m126-m139 (Area B). These areas were selected because road maintenance at the site had removed much of the topsoil and plants associated with these sections of the roadside transect (Giles and Bahm 2017).

An additional 500 plants were placed on the bank above the roadcut. 100 were placed in a 5m x 5m grid with plants placed every 0.5m; this grid is marked with concrete markers placed flush with the ground. No lupine was in the planted grid, though naturally occurring *L*. oreganus was present near the grid boundaries. The remaining 400 *L*. oreganus were placed along the bank into appropriate habitat with naturally occurring individuals; the approximate area planted can be seen in Figure 5.



FIGURE 4. AREAS OUTPLANTED WITH LUPINUS OREGANUS AT CALLAHAN MEADOWS IN APRIL 2017.



FIGURE 5. AREAS OUTPLANTED WITH LUPINUS OREGANUS AT STOUT'S CREEK IN APRIL 2017.



FIGURE 6. AREAS OUTPLANTED WITH LUPINUS OREGANUS AT LOOSE LACES IN APRIL 2017.

RESULTS

Seed Collection and Germination

A total of 6,920 seeds were utilized for growout from four different sites from collections in three years. A summary of collections is listed in Table 2.

Year of Collection	China Ditch	Dickerson Heights	Loose Laces	Stout's Creek	TOTAL
2005	257	251	56	52	616
2011	1294	545	0	686	2525
2016	1504	852	839	584	3779
Total Seeds Collected	3055	1648	895	1322	6920
Total Number of germinants	2949	1313	739	1057	6058
% Germination	92 %	88%	81%	79 %	87%
from each seed source Number of seeds started	1753	1155	507	798	4213
per propagule outplanted	1.7	1.4	1.8	1.7	1.6
rercentage seeds outplanted as propagules	57%	70%	57%	60%	61%

TABLE 2. NUMBER OF L. OREGANUS SEEDS COLLECTED IN 2005, 2011, 2016.

Older seeds had lower germination rates than new seed though germination rates were relatively high independent of year of collection (range: 45%-95%, average 87%, Table 3). Seeds from China Ditch and Dickerson Heights consistently had higher germination than seeds collected at Loose Laces or Stout's Creek (92% and 88% vs 81% and 79% respectively) (Table 3). Some germination boxes had high numbers of moldy seeds that were not suitable to plant. While these moldy seeds were not suitable for propagation, they are included in the germination totals below.

Survivorship of germinated seeds to the time of outplanting was 61% (range: 57%-70%). This includes 20% mortality due to moldy seed – (which were not potted), as well as attrition in the greenhouse (Table 1).

J					
Year of Collection	China Ditch	Dickerson Heights	Loose Laces	Stout's Creek	Average
2005	71% (±9%)	69% (±8%)	45%	67%	66% (±9%)
2011	95% (±2%)	94% (±1%)		79% (±6%)	90% (±4%)
2016	94% (±3%)	90% (±2%)	87% (±4%)	82% (±12%)	90% (±3%)
Total	92% (±3%)	88% (±6%)	81% (±12%)	79% (±6%)	87% (±3%)

TABLE 3. AVERAGE PERCENT GERMINATION OF SEEDS BY SITE AND YEAR, THESE TOTALS INCLUDE SEED THAT GERMINATED BUT WAS NOT POTTED DUE TO FUNGAL INFECTION. VALUES IN () REPRESENT 95% CONFIDENCE INTERVALS WHERE N>1.

Initial Outplanting Survivorship

Average Percent Germination

Survivorship at Loose Laces and Stout's Creek eight weeks post-planting was greater than 90% in the representative grids. At Loose Laces, 97 out of 105 plants were found in the roadside grid (Area A), and 99 out of 103 in the grid on the bank above (Area B). At Stout's Creek, 92 out of the 95 outplanted were present at the time of monitoring. It is anticipated that survivorship will be lower in subsequent years; longer term survivorship will inform plant material needs to reach recovery goals into the future.

CONCLUSIONS AND RECOMMENDATIONS

In order to reach recovery goals for *L*. oreganus in Douglas County, foliar cover will need to be increased from the current ~750m² to more than 5,000m². In order to achieve these goals, augmentation, reintroduction and habitat maintenance will be necessary. Monitoring of existing populations should continue, and decreases in cover and raceme counts should trigger management actions. Monitoring of outplanted areas, should include survivorship (and foliar cover) of plants within the outplanted grids (at Callahan-Area A, Loose Laces- Areas A and B and Stout's Creek- Area D). In most of the areas outplanted, no *L*. oreganus were present, thus any increases in foliar cover in those areas can be attributed to augmentation and reintroduction efforts.

Germination of seeds from three different seed collection years ranged from 45-95%. Mortality of germinants due to mold in germination boxes was approximately 20% (range: 4%-35%). Historically, seed set has been higher at China Ditch and Dickerson Heights (Giles and Bahm 2017); these two sites also had the highest germination and percent survivorship of propagules in the greenhouse.

From germination to initial outplanting, it took approximately 1.6 *L*. oreganus seeds for every propagule outplanted. This number does not take into consideration anticipated outplanting mortality, which could significantly increase the number of seeds needed per propagule. In a common garden study, propagule survivorship one year post outplanting ranged from 20-50% (Gray et al. 2013). In a separate effort, survivorship five years after lupine seed was direct seeded was 4% (Gray and Bahm 2017). This information on seedling and propagule survivorship can be used as a guideline when anticipating plant material needs for augmentation.

Anthracnose infection in germination boxes resulted in the loss of ~20% of germinated seeds. Care should be taken to prevent the infection, and spread of this fungus through both seed cleaning prior to scarification, and rigorous greenhouse cleanliness. Cleanliness at all stages of propagation, rapid response, and immediate quarantine of potential pests can help to keep greenhouse mortality low. In order to prevent infection of seeds, they should be treated with hydrogen peroxide or a dilute bleach solution. It is recommended that seed be allowed to soak for ~30seconds to 1 minute in a dilute bleach solution (3Tbs/ 1 gallon of water) or a 3% hydrogen peroxide solution, rinsed with distilled water, and allowed to dry prior to scarification. This process should decrease the transfer of fungus or other pathogens, including anthracnose infection from the seed coat to the embryo during scarification and subsequent stratification.

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