Effects of Grazing and Climate on Greene's Mariposa Lily in the Cascade-Siskiyou National Monument



# 2012

# Final Report to the Bureau of Land Management Medford District

Report prepared by Carolyn Menke, Ian Pfingsten and Thomas N Kaye Institute for Applied Ecology



# PREFACE

This project is coordinated by the Institute for Applied Ecology (IAE) and is funded by the Bureau of Land Management. 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, and effective species, 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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**Cover photograph**: Greene's mariposa lily (Calochortus greenei) on the Cascade-Siskiyou National Monument. *Photo by R. Massatti.* 

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# Effects of Grazing and Climate on Greene's Mariposa Lily in the Cascade-Siskiyou National Monument

REPORT TO THE BUREAU OF LAND MANAGEMENT, MEDFORD DISTRICT

# INTRODUCTION

Calochortus greenei S. Wats., Greene's mariposa lily, is listed by the United States Fish and Wildlife Service (USFWS) as a federal species of concern, and is proposed for listing as a threatened species in Oregon. It is also a Bureau of Land Management (BLM) special status species.

Calochortus greenei occurs in grassland, shrubland and oak woodland habitats on both sides of the California-Oregon border. Its range also extends south into the Shasta Valley (Brock 1996). A portion of this area is included in the Cascade-Siskiyou National Monument (CSNM; Figure 1). The monument, which is primarily managed by the BLM, is an area of unique ecological diversity with species and influences from the Great Basin, Cascades, and Siskiyou Mountains.

Many areas supporting Calochortus greenei have been influenced by livestock, and are experiencing substantial invasion by non-native species. Many open areas within C. greenei habitat that were likely once dominated by native bunchgrasses including Roemer's fescue (Festuca roemeri ssp. klamathense) are now dominated by exotic grasses,



**Figure 1.** Cascade-Siskiyou National Monument in southern Oregon.

such as bulbus blue grass (Poa bulbosa) and medusa head (Taeniatherum caput-medusae). Substantial yellow starthistle (Centaurea solstitialis) populations occur on Agate Flat, one of the study areas for Calochortus greenei on the monument. Starthistle populations also line the I-5 corridor near the California border, which is adjacent to the Colestine study area, described later in this report. Calochortus greenei is an herbaceous perennial member of the Liliaceae (Lily family). Plants are 10-30 cm tall, with a large basal leaf up to 3 cm wide that is glaucus on both surfaces. Leaves typically begin to senesce as the plants flower. Flowers are large and showy, with pinkish-purplish petals, which are densely hairy on their inner surface. Flowers may be accompanied by small stem leaves. C. greenei flowers are likely pollinated by small native bees, honey bees and bumble bees, but may also be visited by butterflies, beetles and other insects (Brock 1988). Fruits develop in mid-summer, are approximately 2-2.5 cm long, and contain many 4-6 mm long seeds (Figure 2). Plants may also reproduce vegetatively through bulb offsets, but this has not been frequently observed or well documented.

Compared to associated species in the same plant communities, C. greenei blooms and remains green relatively late in the growing season. Leaves may be eaten by various organisms beginning in early spring, when its leaves emerge, through midsummer. Potential herbivores include insects, deer, rodents and livestock. Reproduction of C. greenei may be limited by intensive browsing of vegetative and reproductive structures. To develop management guidelines, and create future conservation strategies for this species, it is crucial to determine the long-term impacts of deer, rodent and cattle utilization on C. greenei. Since C. greenei has a complex life-history that includes dormancy, a multi-year demographic study utilizing permanent plots (some fenced and some unfenced) is necessary to assess the influence of herbivory on its population dynamics, and inform management actions in the Cascade-Siskiyou National Monument.



**Figure 2.** Mature Calochortus greenei capsules.

# **OBJECTIVES**

The objectives of this report are to:

- Describe population monitoring methods and plant community assessments for Calochortus greenei and associated plant communities on the Cascade-Siskiyou National Monument.
- Summarize population trends and plant community data over the 10 years of the study.
- > Evaluate influences of major herbivores on C. greenei and associated plant communities.
- Evaluate to potential effects of climate change on the species, and project future trajectory of the C. greenei populations on the Cascade-Siskiyou National Monument based on forecasts from climate change models.

# **METHODS**

## **Study Areas**

Three study areas within the Cascade-Siskiyou National Monument were selected in 2003 to establish long-term monitoring and grazing study plots for Calochortus greenei and associated

plant communities: Agate Flat, Oregon Gulch, and Colestine (Appendix 1: Maps). These study areas contain the largest populations of C. greenei on the monument, and also span the range of recent cattle utilization intensity occurring on the monument, although livestock grazing ceased in 2009. Until 2009, the Colestine area was essentially ungrazed (except for stray cattle from California), Oregon Gulch experienced moderate to low utilization, and Agate flat received the highest intensity of utilization. Quantitative data regarding utilization is lacking. For directions to all plots, see Appendix 2.

# Sampling Design

In 2003, fifteen pairs of 2 m x 2 m large-mammal exclosures and controls were established in C. greenei populations, five in each of the three study areas: (Menke and Kaye 2003). Maps are included in Appendix 1. Plots were placed to contain as many C. greenei plants as possible and to maximize the similarity in microsite and plant community within plot pairs. Within the plot pairs, one plot was randomly assigned (by a coin toss) to be fenced in a 3 m x 3 m stockwire ( $\sim$ 2 in x 5 in grid) exclosure (Figure 3), and the other plot was left unfenced (Figure 4). All plots were marked with rebar pounded into the ground at the corners of the plot, tagged with a metal tag, positioned with a GPS unit (Table 1), and photographed.



**Figure 3.** 2 m x 2 m Calochortus greenei large-mammal exclosure, marked with rebar at the four corner posts, and fenced by a 3m x 3m exclosure (Oregon Gulch Walk in, Plot 13).



Figure 4. Unfenced (control) 2 m x 2 m C. greenei plot, marked with rebar and the four corner posts.

## **Plant Measurements and Mapping**

In each large-mammal exclosure and control, each individual C. greenei plant was mapped (Sample included in Appendix 3). A 1 m x 1 m quadrat frame with string/wire dividers was used to determine the coordinates of all C. greenei plants in each quarter of the 2 m x 2 m plots. We used a coordinate system in which the origin was in the southwest corner of the plot. To mark this corner in fenced plots, it was tagged on the exterior of the exclosure, and in control plots this corner was tagged on the rebar post.

From 2003 to 2012, we measured the length and width of each C. greenei leaf in all plots in the second week of June (early summer sampling). With reproductive plants, plant height and flower/bud number were also recorded. Plants were typically in bud, with no open flowers seen in the June sampling. We noted herbivory on leaves and flower buds. As possible, we classified herbivory as by mammals when plants were browsed in a clipped manner or as by insects when leaves or flowers had holes or other signs of herbivory that did not appear to be a result of larger animals. In some cases, it was difficult to differentiate between mammal and insect herbivory, and it was also generally impossible to separate mammal herbivory by deer, cattle or rodents. Herbivory to flower buds was problematic to assess, since we found some plants barely in bud, and others would not initiate buds until after the June sampling; for this reason, these data were not used in final analyses.

From 2004 to 2012, in the first few days of August (late summer sampling), we revisited all plots to determine how many C. greenei flower buds were actually matured into capsules. We relocated plants that were in bud on the earlier field visit, and counted matured (filled), aborted, and eaten or damaged capsules. In some cases, we found that plants had produced additional

buds/flowers after our first visit. Frequently we found that entire flowering stems had been removed, suggesting some sort of animal damage, but making it impossible to determine how many flowers were matured into capsules or aborted. In this case, we could only record that there had been some sort of reproductive structure and that it was damaged.

Calochortus tolmiei frequently co-occurs with C. greenei. Fresh specimens of the two species can be distinguished by their leaf surfaces; C. greenei is glaucus on both sides, whereas C. tolmiei is glaucus on only one side. Most mature C. tolmiei leaves are also narrower than those of C. greenei. In addition, the capsules of C. tolmiei tend to mature earlier than those of C. greenei, and their stems and capsules tend to nod, while C. greenei stems bearing capsules tend to remain upright. Because both species are frequently animal damaged or drying out at the time of sampling, it is often difficult to tell them apart. To avoid confusion between C. tolmiei and C. greenei when the two species occurred in the same plot, we mapped the locations of the C. tolmiei plants as well, and labeled them on the datasheets. Identifications were re-checked in each year of monitoring; some individual identifications remained unconfirmed and were omitted from analyses.



**Figure 5.** C. greenei demographic and plant community data collection in Oregon Gulch study area exclosures.

|             |                       | Plot # | Treatment | Tag # | GPS-   | 2004    |
|-------------|-----------------------|--------|-----------|-------|--------|---------|
|             | Powerline             | 1      | Exclosure | 544   | 550284 | 4652241 |
|             | Plots                 | 2      | Control   | 545   | 550312 | 4652193 |
| Q           |                       | 3      | Exclosure | 537   | 548804 | 465146  |
| Are         | Border                | 4      | Control   | 538   | 548799 | 4651442 |
| ά<br>λ      | Plots                 | 5      | Control   | 539   | 548774 | 4651462 |
| Stuc        |                       | 6      | Exclosure | 430*  | 548769 | 4651420 |
| đ           | Closed                | 7      | Exclosure | 535   | 549069 | 4652604 |
| Ē           | Road Plots            | 8      | Control   | 536   | 549095 | 4652614 |
| ate         | Intersection          | 9      | Control   | 542   | 549357 | 4651666 |
| Ag          | Plots                 | 10     | Exclosure | 541   | 549370 | 4651667 |
|             | •                     | 11     | Exclosure | 546   | 551576 | 4656190 |
| Ö           | Oregon                | 12     | Control   | 547   | 551571 | 4656213 |
| Are         |                       | 13     | Exclosure | 553   | 551451 | 4655894 |
| ٩٧          | in                    | 14     | Control   | 552   | 551453 | 4655898 |
| Stu         | Rosebud               | 15     | Control   | 554   | 551604 | 4657971 |
| -<br>C-     | Mtn.                  | 16     | Exclosure | 555   | 551625 | 4657980 |
| <u>Sul</u>  | Keane<br>Ridge        | 17     | Control   | 548   | 548238 | 4656685 |
| ) u         |                       | 18     | Exclosure | 549   | 548247 | 4656680 |
| 0<br>0<br>0 |                       | 19     | Exclosure | 550   | 548120 | 4656601 |
| ŏ           |                       | 20     | Control   | 551   | 548111 | 4656601 |
|             |                       | 21     | Exclosure | 527   | 532879 | 4653498 |
|             | Colestine             | 22     | Control   | 429** | 532890 | 4653522 |
|             | Freeway               | 23     | Control   | 526   | 532859 | 4653499 |
| ea          |                       | 24     | Exclosure | 525   | 532850 | 4653534 |
| Ar          |                       | 25     | Exclosure | 532   | 532146 | 4651736 |
| Арг         | Colestine<br>Overpass | 26     | Control   | 531   | 532149 | 4651768 |
| Sti         |                       | 27     | Exclosure | 530   | 532093 | 4651790 |
| ne          |                       | 28     | Control   | 529   | 532090 | 4651793 |
| esti        | Colestine             | 29     | Control   | 533   | 533624 | 4651633 |
| 0           | Corral                | 30     | Exclosure | 534   | 533681 | 4656126 |

**Table 1.** Calochortus greenei large-mammal exclosure and control locations, numbers, treatments and tag numbers.

 GPS coordinates are NAD 27, UTM Zone 10. Plots between horizontal lines are paired.

\* Tag lost (previously #540 and #591) and replaced in 2005 and 2006.

\*\* Tag lost (previously #528) and replaced in 2006.

# Plant Community Sampling

To describe the plant communities in which C. greenei occurs, and detect changes over time in fenced and unfenced plots, we collected data on cover and frequency of associated species in the C. greenei plots. In the southwest (tagged) quarter of each plot, we recorded the percent cover of each species, rock, bare soil, and plant litter using a 1 m x 1 m quadrat frame divided

into a grid of 25 20 cm x 20 cm squares. We used the quadrat frame in all four quarters of the plot to determine the percent frequency (out of a total of 100 20 cm X 20 cm squares) of the following functional groups: native perennial grasses, annual grasses (including *Poa bulbosa*, which is actually an exotic perennial), forbs, shrubs, bare soil and cow manure.

#### All-Mammal Exclosure Establishment



**Figure 6.** 1m x 1m all-mammal exclosure at Agate Flat.

In 2007, we established a total of 14 1 m x 1 m allmammal exclosures, with seven in Colestine and seven in Agate Flat (Table 2). Exclosures were placed to contain at least six C. greenei plants, and were located as close to existing fenced-unfenced pairs as possible. The all-mammal exclosures had sides constructed of hardware cloth (0.5 in grid) attached to 3/8 in rebar, and are approximately 2 ft high. The lids of the exclosures were constructed of 3 in x 2 in grid wire (Figure 6). Numbered metal tags were used to identify each exclosure in the southwest corner. The goal of the exclosures was to allow insect access while eliminating small (and large) mammal access to plants. This allowed us to evaluate the frequency and intensity of small mammal and insect impacts to C. greenei plants.

C. greenei plants in all-mammal exclosures were mapped, measured, and examined for herbivory damage starting in 2008. Plant community data were not taken in the all-mammal exclosures.

| Study<br>Area | Tag<br># | Location   | 2007 GPS |         |  |  |
|---------------|----------|--|----------|---------|--|--|
|               | 700      | By intersection near plots 9 & 10.                   | 549536   | 4651673 |  |  |
| -             | 699      | Adjacent to plots 9 & 10.                            | 549352   | 4651655 |  |  |
| Hat           | 698      | Adjacent to fenced plot 5.                           | 548776   | 4651449 |  |  |
| ate           | 697      | Downhill (south) from unfenced plot 5.               | 548779   | 4651438 |  |  |
| Age           | 696      | East of rodent exclosure 697, SE of unfenced plot 5. | 548788   | 4651436 |  |  |
|               | 695      | South west of and adjacent to fenced plot 3.         | 548805   | 4651452 |  |  |
|               | 694      | Adjacent to fenced plot 1.                           | 550277   | 4652220 |  |  |
|               | 693      | Upslope (S) from fenced plot 21.                     | 532883   | 4653469 |  |  |
|               | 692      | Downslope (N) from fenced plot 21.                   | 532883   | 4653497 |  |  |
| ine           | 691      | Adjacent to unfenced plot 23.                        | 532862   | 4653486 |  |  |
| lest          | 645      | Uphill from unfenced plot 26                         | 532152   | 4651751 |  |  |
| ပြီ           | 644      | In small opening towards freeway from plot 27 & 28.  | 532133   | 4651755 |  |  |
|               | 643      | Above fenced plot 25.                                | 532152   | 4651715 |  |  |
|               | 642      | Just uphill from unfenced plot 29.                   | 533629   | 4651627 |  |  |

 Table 2. Location of all-mammal exclosures. Coordinates are in NAD 27, UTM Zone 10.

# **Data Analysis**

Our goals for this portion of the final data analysis for this project were to evaluate relationships between the fencing treatments, study areas, and C. *greenei* plant performance and demographic trends. We also evaluated demographic trends over time as they relate to climate, fencing treatments and plant characteristics.

Several C. greenei variables (e.g., % plants flowering, % plants with vegetative herbivory, % plants with floral herbivory in August, % fruit set) and all plant community variables had nonnormal distributions. C. greenei variables were transformed using arcsine square root to meet normality assumptions for ANOVA analyses, and data displayed in graphs are back transformed. Due to frequent zero values we were unable to transform plant community data to meet normality assumptions. We completed the analysis without transformation, and results should be interpreted with the caveat that data did not meet normality assumptions.

The level of significance for all tests was set at 0.05; *P*-values of 0.05 or less were considered indicators of statistically significant differences between groups.

#### **Plant Abundance**

To determine whether the C. greenei plants in large-mammal exclosures increased over time or were more likely not to be dormant, we used repeated measures analysis of variance (ANOVA) with fencing (fixed) and site (random) as between subject (plot) factors and year as a within subject (plot) factor with data from 2003 through 2012. We used repeated measures ANOVA comparisons since plants in plots were measured repeatedly over 10 years, and thus samples were not independent between years; the repeated measures design also accounted for some of the variability between sample units (plots) through pooled estimates of variance.

#### Herbivory

To evaluate the effectiveness of exclosures at reducing herbivory to vegetative and reproductive plant structures, we used repeated measures ANOVA with large-mammal exclosure and control plot data from 2003 through 2012. Reproductive structure herbivory data were not collected in 2003, so this portion of the analyses used data from 2004-2012. We also used repeated measures ANOVA with control, all-mammal and large-mammal exclosure data from 2008-2012. The all-mammal exclosures were established after sampling in 2007. In both analyses, fencing and site were between subject factors and year was a within subject factor. We used one way ANOVA for single year comparisons.

#### **Plant Size and Reproductive Effort**

To examine the effects of excluding different types of herbivores on C. greenei plant size and reproductive effort, we compared leaf width, flower number, % plants flowering and fruit set between large-mammal exclosures and controls using repeated measures ANOVA with data from 2003 through 2012. Fruit set data were not collected in 2003, so this portion of the analyses used data from 2004-2012. We also used repeated measures ANOVA on the same variables from the control plots, all-mammal and large-mammal exclosures from 2008-2012. In both

analyses, fencing and site were between subject factors and year was a within subject factor. We used one way ANOVA for single year comparisons.

#### **Plant community**

We evaluated the effect of large-mammal exclosures on functional group (native perennial grass, annual grass, forb, shrub), bare ground and cow manure frequency and the cover of native and exotic forbs and grasses using repeated measures ANOVA with fencing and site as between subject factors and year as a within subject factor. We used one way ANOVA to compare large-mammal exclosures and controls in individual years.

#### **Population Viability Analyses**

#### POPULATION DEMOGRAPHICS

We examined the population demography of C. greenei in the large-mammal exclosures and control plots from 2003-2012 by conducting a population viability analysis. Individual plant leaf widths (mm) were chosen as the indicator of population vital rates (growth, survival, and fecundity). Multiple previously-observed plants were absent in our plots, only to return after one to five years. We considered these individuals as dormant during those absent years, and we included this vital rate in our viability analysis. Dormancy could only be assessed retroactively after plant reemergence, and plant survival is linked to dormancy. Thus dormancy and survival rates were absent for the final monitoring year (2012). Seedlings were likewise difficult to observe in plots due to their small size (<1 mm wide). We estimated seedlings from new, non-flowering plants the following year that had leaf widths <10 mm. These individuals were considered one-year-old plants, but true seedling numbers were unknown in our plots. Due to this uncertainty and low overall annual recruitment, we estimated variation in seedling establishment due to years, but not sites or treatments.

#### DEMOGRAPHIC MODELS

We classified population growth, survival, dormancy, and fecundity rates by individual leaf widths in an integral projection model (IPM) of annual population growth (Easterling et al. 2000, Ellner & Rees 2006). Each IPM consists of coupled vital rate functions, which were modeled independently, but from the same data set. Growth was modeled as a continuous linear function of individual leaf widths from year t to year t+1. The residual error (variance) around the growth function was modeled as a continuous linear function of squared residuals around predicted leaf widths. Survival was modeled as a continuous quadratic function of leafs widths from year t to individual presence in year t+1. Dormancy was modeled in three parts: (1) a continuous linear function of leaf widths in year t to probability of absence, but not mortality (dormancy) in year t+1, (2) a discrete probability of individuals remaining dormant from year t to t+1, and (3) a continuous Gaussian distribution function of leaf widths in year t+1 from individuals dormant in year t. Fecundity was modeled in four parts: (1) a continuous linear function of leaf widths in year t to probability of flowering in year t, (2) a discrete probability of viable capsule production in year  $t_{i}$  (3) a continuous log-linear function of number of viable capsules produced per individual in year t from leaf widths in year t, and (4) a discrete probability of seedlings established per capsule in year t+1.

We estimated dormancy and survival function coefficients in 2012 as the average from all other years. We likewise did this for capsule production probability and capsule number functions in 2003 due to missing capsule data from the initial monitoring year. Seedling numbers were also estimated for 2004 and 2012 this way due to the assumption that they were missed for the first year of emergence.

We built 54 separate IPMs for individual plants in each treatment (excluding all mammal exclusions) (n=2), site (n=3), and year transition (n=9) using linear mixed-effects model regressions to estimate variation in regression coefficients due to year, site, and treatment differences in our vital rate functions (Rees & Ellner 2009). All functions, except for growth variance, had different intercepts for each year, site, and treatment, but constant slopes. We then computed deterministic ( $\lambda$ ) and stochastic population growth rates ( $\lambda_s$ ) as a measure of current and long-term population viability, respectively (Caswell 2001), for each IPM, where values of  $\lambda > 1$  indicated the population was increasing at a rate of  $\lambda$ . We tested for a significant difference among site and treatment  $\lambda$  using repeated measures ANOVA.  $\lambda_s$  was simulated by randomly selecting IPMs from years after the first two of the study (2003 & 2004) due to potential treatment effect lag. This simulation ran for 1,000 iterations for each site by treatment.

#### CLIMATE-DRIVEN POPULATION MODELS

To assess the effect of local climate on population viability, we conducted a population viability analysis using climate drivers to predict vital rates in our IPMs. We assessed climate-driven viability across years for the control and large-mammal exclosures without site-specific effects, for a total of 18 IPMs. First, we examined the correlations between vital rate function coefficients (intercepts and slopes) and weather station data during the observed period (2002-2012), aggregated into seasons. We collected weather station data from the Buckhorn Springs, OR Remote Automatic Weather Station (42° 07' 11", 122° 33' 48", 2,780 ft ASL). We chose a single climate driver for each vital rate function, based on the highest correlation coefficient from a subset with P-values <0.1, to use in linear regression models to predict the vital rate function coefficients. We used drivers selected from the fenced treatment for both the control and fenced treatment climate-driven viability assessment. We then projected our vital rate functions, and thus our IPMs, into the future (2000-2099) with climate forecasts using climate data generated by 16 general circulation models (GCMs) from the World Climate Research Programme's (WCRP's) Coupled Model Intercomparison Project phase 3 (CMIP3) multi-model dataset, downscaled and bias-corrected (Reclamation 2011). We chose climate projections from the SRES A2 scenario (SRES 2000) as it is more appropriate than other SRES scenarios, which project more optimistic realities than what is currently observed.

We looked at 32 candidate climate drivers from the weather station data and GCMs, which were total precipitation (mm), total Kimberly-Penman reference evapotranspiration (Wright 1982) (mm), and average maximum and minimum temperature (°C) aggregated across four biologically-relevant seasons: dry growing (Jun 1 – Jul 31), dry dormant (Aug 1 – Oct 31), wet dormant (Nov 1 – Feb 28), and wet growing (Mar 1 – May 31). We also staggered each driver by one year back (*t*-1) to compare one-year lagged climate effects on vital rates, which gave us a 2002-2011 and 2003-2012 time frame for the set of drivers.

We simulated climate driven population sizes and 50% extinction probabilities (Caswell 2001) into the future (2000-2099) for both treatments by averaging 100 repetitions of IPMs projected from each GCM (16 GCMs X 100 reps), starting from the rounded average observed population size of 900 plants (including dormants). We compared these simulations to population sizes and extinction probabilities from randomly-selected, current year IPMs (considered a stable-climate future) by iterating 1,000 projections during the same time period. We used R 3.0 (R Core Team 2013) for all demographic analyses.

## RESULTS

#### Plant Abundance

#### LARGE-MAMMAL EXCLOSURES

At the start of the project in 2003, we mapped a total of 656 Calochortus greenei plants in the large-mammal exclosures and controls, and in 2012 the total number of plants tracked had increased to 973 (Figure 7). The number of plants present per plot ranged from a minimum of five to a maximum of 86. Changes in total number of plants over the course of the project may relate to plants emerging from dormancy, initial sampling error as plants were mapped the first years



**Figure 7.** Total number C. greenei plants in largemammal exclosures, all mammal exclosures and unfenced controls in 2003-2012.

of the project, and seedling recruitment. Plant abundance from 2003 and 2012 was not significantly influenced by the large-mammal exclosures (P=0.95).

#### ALL-MAMMAL EXCLOSURES

In 2008, we mapped and measured a total of 126 C. greenei plants in the all-mammal exclosures, and in 2012, this number had increased to a total of 144 plants (Figure 7). Two all-mammal exclosures, one in Colestine (#642) and one in Agate Flat (#700), had clear evidence of small mammal activity and are excluded from data summary and all analysis. The number of plants per exclosure ranged from of 3 to 31.

#### Herbivory

Relative to the controls, the large mammal exclosures reduced vegetative herbivory (P = 0.027; Figure 8). The difference became statistically significant the second year of the study (2004), the first growing season after the exclosures were established. More than 50% of plants were still grazed inside the large-mammal exclosures.

Herbivory to reproductive structures was extremely variable, and did not differ statistically between exclosures and controls in the repeated measures analysis (P=0.23). Single year

comparisons found the frequency of reproductive structure herbivory differed between the control and large-mammal exclosures only in 2012 (P=0.05).

The repeated measures analysis of all three plot types found a significant treatment effect (P=0.031) on vegetative herbivory while the all-mammal exclosures were in place (2008-2012). Single year comparisons found three of the five years (2008, 2011, 2012) had more frequent vegetative herbivory in the controls than the all-mammal or large-mammal exclosures (P=0.04, 0.008 and 0.02, respectively), but frequencies were similar between treatments in 2009 and 2010 (P=0.07 and 0.25, respectively). The all-mammal and large-mammal exclosures did not differ in any years. There was not a significant treatment by year or treatment by site interaction (both P>0.07).



**Figure 8.** Frequency <u>+</u> SE (%) of leaf herbivory (from 2003 through 2012) and August floral herbivory (from 2004 through 2012) in control and large-mammal exlcosures. Columns of points below a star are statistically different ( $P \le 0.05$ ) in single year ANOVA comparisons.

In the period from 2008 to 2012 the exclosures significantly reduced damage to Calochortus flower and fruiting structures; repeated measures ANOVA analysis found a significant treatment effect on reproductive structure herbivory (P=0.033; Figure 9). Single year comparisons found the controls had more reproductive structure damage than both exclosure types in 2008 (P=0.001) and more than the all-mammal exclosures in 2010 (P=0.027). There was not a significant treatment by year or treatment by site interaction (both P>0.70).



**Figure 9.** Frequency <u>+</u>SE bars (%) of leaf herbivory and August floral herbivory (from 2008 through 2012) in control, large-mammal exclosures and all-mammal exclosures. Data include Agate Flat and Colestine. Points within a year with different letters are statistically different ( $P \le 0.05$ ) in single year ANOVA comparisons.

## **Plant Size and Reproductive Effort**

#### Comparisons between Controls and Large-Mammal Exclosures (2003-2012)

Beginning the second growing season after large mammals were excluded as herbivores, plants in the large-mammal exclosures had wider leaves and more flowers than plants in control plots (Figure 10). For both variables the repeated measures of the treatment (fencing) factor was not statistically significant (P=0.07 and 0.09, respectively), however the treatment by year interaction was statistically significant (P=0.01 and 0.05, respectively), indicating that the effect of fencing depended on the year of observation. The treatment effect became apparent in the third year (2005) of the study. Across all sites, plants in large-mammal exclosures also tended to flower more frequently (P=0.05) and have greater fruit set than plants in control plots (P=0.04; Figure 17); there were no significant study area by treatment interactions. The increase in rates of flowering became statistically significant in 2006 (exclosures in place for three years), while the increase in fruit set began the first growing season after exclosures were in place (2004).

# Comparisons between Controls, All-Mammal and Large-Mammal Exclosures (2008-2012)

Excluding animals as herbivores resulted in plants that were larger than in controls, but we did not see differences between the two types of exclosures (Figure 11). In three way comparisons among controls, large-mammal and all-mammal exclosures, we found the fencing had significant effects on leaf width, the frequency of flowering, number of flowers per plant and the frequency of fruit set (all repeated measures P<0.032; Figure 18). In repeated measures ANOVA, there were no significant treatment by year interactions (all P>0.10). Single year ANOVA comparisons found the all-mammal exclosures produced the most consistent difference from controls (wider leaves, more flowers, greater flowering frequency and greater fruit set).



Figure 10. Plant size and reproductive output (means  $\pm$  SE) in controls and large-mammal exclosures from 2003 to 2012 (fruit set data 2004-2012), including all study areas. Points in columns below a star are significantly different (P $\leq$  0.05) in single year ANOVA comparisons.



**Figure 11.** Plant size and reproductive output (means  $\pm$  SE) in controls, large-mammal and all-mammal exclosures from 2008 to 2012, including Agate Flat and Colestine study areas. Points within a year with different letters are significantly different from each other (P $\leq$  0.05) in single year ANOVA comparisons.

#### **Plant Community**

Placing plant communities within exclosures for 10 years did not result in changes in plant community composition or structure as measured by functional group frequency (native perennial grass, annual grass, forbs, shrubs), bare ground, or cover of native grasses, exotic grasses, native forbs and exotic forbs. The plant communities in Agate Flat, Colestine, and Oregon Gulch differed strongly from each other, but repeated measures ANOVA found no significant treatment (fencing effect) on functional group or cow manure frequency or functional group cover (all P>0.26). Bare soil frequency was lower in large-mammal exclosures overall (repeated measures P=0.045), but single year ANOVA comparisons found no significant differences (all P>0.29). The study area by treatment interaction was significant only for native grass frequency (P=0.035) and cover (P=0.008), both of which were higher in the large-mammal exclosures from the start of the study in Colestine and Oregon Gulch, and largely absent in Agate Flat. All other study area by treatment interactions were not significant (all P>0.17). Species cover and frequency data from each study area are included in Figure 12 and Figure 13. The major difference between study areas remained that Agate Flat had much lower perennial grass and bare soil frequency, but much higher annual grass frequency than Colestine and Oregon Gulch.



**Figure 12.** Mean frequency (% +/- SE) of functional groups and bare ground in ten plots in 3 study areas in 2003-2012. Bare ground frequency from 2012 are unavailable. Large-mammal exclosures and controls not differentiated in graphs.



**Figure 13.** Functional group cover ( $\% \pm SE$ ) between 2003 and 2012 at Agate Flat, Oregon Gulch and Colestine study areas. Large-mammal exclosures and controls not differentiated in graphs.

# **Population Viability Analyses**

#### POPULATION DEMOGRAPHICS

Leaf widths from all plants (n=1576) in our demographic study ranged from 1-93 mm, and the average and median leaf widths were 17 and 16 mm, respectively. Leaf widths above 50 mm were relatively rare (n=15). Therefore we considered leaf widths >50 mm as outliers and removed them from analysis. In 2003, 1,208 plants were alive, including 481 dormant plants. By 2011, 1,234 plants were alive, and of those, 100 plants were considered dormant. The estimated number of new recruits in 2003 was 86, while in 2011, the number dropped to 37. Seedlings ranged in number from only 2 in 2006 to 146 in 2010, while capsules ranged from 107 in 2004 to 394 in 2008.

#### DEMOGRAPHIC MODELS

The vital rate functions produced general trends important to understanding the life history of C. greenei. The growth function predicted positive annual growth with variance around the prediction increasing with leaf width. The survival function predicted a high, but hump-shaped, survival probability, with peak survival (~100%) from 20-30 mm leaf widths. The dormancy function

predicted a decreasing probability of entering dormancy with increasing leaf widths, although this probability never reached higher than 30% in a year. The fecundity functions predicted a general increase in flowering and fruit production with leaf widths. Flowering probability peaked (~100%) around 40-50 mm leaf widths, with a steady increase from 10-40 mm. Viable capsule production probability varied greatly across years, sites, and treatments, but not with plant size. However, the number of viable capsules increased with leaf widths, with a maximum observed capsule number of 7 for leaf widths above 35 mm.

Individual year ( $\lambda$ ) and long-term population growth ( $\lambda_s$ ) varied mostly across years and treatments, with only Agate Flat differing from the other sites (Table 3). The first two years had similar growth rates for all sites and treatments, as expected from a lag in treatment effect. However, the third year also had similar growth rates across treatments, but afterwards growth rates were generally lower in the control treatment and in Colestine and Oregon Gulch, but only marginally significantly so (P=0.07). The long-term growth rates were likewise lowest in the control treatments and Colestine and Oregon Gulch, with an over 4% decrease per year in the control plots and less than 1% increase per year in the fenced plots (Table 3). Agate Flat had the highest long-term growth in both treatments, with a 2% decrease per year in the control plots and an almost 9% increase per year in the fenced plots.

|               |            | Control   | Fen          | iced (Large M | ammal)    |              |
|---------------|------------|-----------|--------------|---------------|-----------|--------------|
| Year          | Agate Flat | Colestine | Oregon Gulch | Agate Flat    | Colestine | Oregon Gulch |
| 2003          | 1.006      | 1.015     | 1.056        | 1.059         | 1.048     | 1.079        |
| 2004          | 1.083      | 1.057     | 1.004        | 1.090         | 1.055     | 1.065        |
| 2005          | 0.983      | 0.995     | 0.939        | 0.979         | 0.993     | 0.979        |
| 2006          | 0.964      | 0.997     | 0.942        | 1.023         | 1.061     | 1.026        |
| 2007          | 1.048      | 0.986     | 0.967        | 1.260         | 1.017     | 1.048        |
| 2008          | 0.987      | 0.946     | 0.970        | 1.031         | 1.016     | 1.012        |
| 2009          | 1.007      | 0.911     | 0.968        | 1.315         | 0.893     | 1.018        |
| 2010          | 0.927      | 0.899     | 0.906        | 1.044         | 1.005     | 0.921        |
| 2011          | 0.993      | 0.997     | 0.971        | 1.027         | 1.039     | 1.024        |
| $\lambda_{s}$ | 0.980      | 0.959     | 0.951        | 1.090         | 1.005     | 1.004        |

**Table 3.** Current and long-term ( $\lambda_s$ ) population growth rates for the control and fenced treatments at each site. Growth rates >1 represent a population increase.

#### CLIMATE-DRIVEN POPULATION MODELS

Of the eleven vital rate functions, nine were associated with a climate driver (P < 0.1), and the two that were not (probability of flowering and seedling establishment) were allowed to vary randomly ( $P \ge 0.1$ ) (Table 4) in our models. Total precipitation, average minimum temperature, and total reference evapotranspiration were the three climate drivers chosen, and most were during the growing season (roughly spring and early summer), except for annual growth and the probability of entering dormancy. Four chosen climate drivers were during the dry season

(roughly summer and early fall), and two were from weather previous to the year of the annual monitoring interval (t-1). Plant growth had the same driver (dry dormant season precipitation in year t) as the probability of entering dormancy, but with opposite effects. Survival and the probability of leaving dormancy as well as the plant size after dormancy were linked by the same or similar climate driver (wet growing season minimum temperature in year t and t-1), but survival was positively correlated with climate. All dormant season functions were negatively correlated with climate. Reference evapotranspiration in year t-1 was positively correlated with the probability of producing viable fruit, but negatively correlated with the number of fruit produced and a season later (wet vs. dry).

**Table 4.** Correlations (with P-values and r coefficients) between chosen climate drivers and vital rate function coefficients (n=9). Bolded coefficients varied by year and treatment, while others were held constant (x = plant leaf widths). Climate drivers are of current year's weather (t) and previous year's weather (t-1) relative to the annual monitoring interval. Precip = precipitation, Min Temp = minimum temperature, rET = reference evapotranspiration.

| Climate driver                    | Р     | r      | Intercept                         | Slope                                     |
|-----------------------------------|-------|--------|-----------------------------------|---|
| Dry Dormant season Precip (t)     | 0.010 | 0.800  | growth                            | growth*x                                  |
| Dry Growing season Precip (t-1)   | 0.042 | 0.685  | growth variance                   | growth variance*x                         |
| Wet Growing season Min Temp (t)   | 0.021 | 0.746  | P(survival)                       | P(survival)*x, P(survival)*x <sup>2</sup> |
| Dry Dormant season Precip (t)     | 0.031 | -0.715 | P(enter dormancy)                 | P(enter dormancy)*x                       |
| Wet Growing season Min Temp (t)   | 0.010 | -0.798 | P(leave dormancy)                 |   |
| Wet Growing season Min Temp (t-1) | 0.019 | -0.753 | mean leaf width after dormancy    |   |
| Wet Growing season Min Temp (t)   | 0.000 | -0.938 | std dev leaf width after dormancy |   |
| Random                            | ~     | ~      | P(flowering)                      | P(flowering)*x                            |
| Wet Growing season rET (t)        | 0.016 | 0.766  | P(viable capsules)                | P(capsules)*x                             |
| Dry Growing season rET (t)        | 0.017 | -0.763 | # of viable capsules              | # of capsules*x                           |
| Random                            | ~     | ~      | P(seedling establishment)         |   |

Overall, the fenced plots had higher vital rates than the control plots, which corresponded to the patterns in long-term population growth rates between treatments. Annual population growth rates from the climate-driven IPMs ranged from 0.90-1.05 in the control plots and 0.99-1.07 for the fenced plots, although the two highest growth rates occurred during the first two years of the study. Average long-term climate-driven growth rates for the 16 GCMs were 0.99 ( $\pm$  0.002 SE) for control plots and 1.05 ( $\pm$  0.005 SE) for fenced plots (Figure 14). Projections of both treatments to 2099 show a decreasing population size for control plots and an increasing population size for fenced plots (Figure 15).

Long-term forecasts suggest that projected changes in climate drivers of population growth in this species The projections of climate-driven population sizes closely followed those of randomly selected years, with a slight up-turn in the climate-driven population about midway through the simulated years for both treatments. The wide variation in climate-driven population projections comes from the different climate projections of the 16 GCMs. 50% extinction probabilities were much higher in the control plots after ten (8.3%) and fifty (80.4%) years compared to fenced

plots (0.6% and 2.6%, respectively) under climate-driven scenarios (Table 5). In contrast, the random-year-selected vital rates had no probability of 50% population extinction after ten years for both treatments, but a higher risk in 50 years for control plots (91.2%).

 Table 5. 10 and 50 year 50% population extinction probabilities for control and fenced treatments under both climate-driven and random-year (uniformly-distributed, current-year IPMs) demographic models.

|          | climate | -driven | random-year    |      |  |
|----------|---------|---------|----------------|------|--|
|          | control | fenced  | control fenced |      |  |
| 10 years | 8.3%    | 0.6%    | 0.0%           | 0.0% |  |
| 50 years | 80.4%   | 2.6%    | 91.2%          | 0.0% |  |



**Figure 14.** Long-term population growth rates from the climate driven vital rates, averaged across the 16 CMIP3 GCMs for control and fenced treatments. Error bars represent 95% confidence intervals. The dashed line represents zero growth with positive and negative growth above and below the line, respectively.



**Figure 15.** Population size (number of individuals) projections for (a.) control plots and (b.) fenced plots under climate-driven (red) and random-year (blue) demographic models (log<sub>e</sub> scale). Solid lines represent median projections across 16 GCMs (red) or 1,000 projections (blue). Dashed lines represent 95% quantiles of the projections.

# DISCUSSION

#### Herbivory

Calochortus greenei was negatively affected by herbivory from large and small mammals at both the individual and population level. When excluding large herbivores (deer and occasionally cattle) only, this effect was immediate in terms of reduced damage to leaves, while leaf width, flowering frequency and flower number increased in response to protection from herbivory after two or three years of fencing. We found this pattern to be consistent across the three study areas of our research at the Cascade-Siskiyou National Monument, even though the associated vegetation varied among sites from exotic annual grass dominance to native perennial grasslands.

The effects of small and large mammal herbivores on plant size, flowering frequency and flower number appear to be similar, although they may affect flowering and fruiting structures differently. In some years, the small mammal grazers appeared to have stronger impacts on fruiting structures than the large mammals. This likely fluctuates with small mammal population sizes and the variety of food available to them. It is unknown whether small mammals may remove and transport the large C. greenei capsules away from plants, which may provide some seed dispersal benefits to C. greenei. Insects do appear to be agents of herbivory, as over 50% of plants in all-mammal exclosures still had signs of leaf damage. We found all types of herbivory could vary greatly between years, potentially due to fluctuations in the abundance of insects, small and large mammal populations, and their predators.

Population viability analyses indicated a slight increase in annual population growth when large mammals were excluded, suggesting that C. greenei populations may be limited by herbivores.

Population vital rates also improved in fenced plots. Growth, survival, flowering, and fruiting were all higher on average for fenced plot vital rate functions than for control plots functions. In an average year, the probability of entering dormancy was lower in fenced plots, and the average leaf width the year after dormancy was greater than in control plots. The average of these vital rates during the first two years were nearly identical for both treatments, suggesting a lag in the effect of fencing on population processes. A strong pattern in all population differences was not detectable until the fourth year of the study. This corresponds the results from the plot level analyses for similar vital rates.

In contrast to C. greenei, plant community dynamics did not significantly differ in and outside the large-mammal exclosures over the ten years of this study. Across a variety of habitats on the Cascade-Siskiyou National Monument, spanning between mostly native perennial bunchgrass communities to exotic annual grasslands, protecting plant communities from grazers did not change the ratio of native non-native species, or result in significant shrub expansion. This suggests these dry grassland habitats were relatively stable in their composition over the last ten years, whether they are dominated by natives or non-natives. In these habitats, exclusion of grazers alone does not appear to be an effective tool to restore native habitats.

## **Climate-driven Population Dynamics**

Climate change is forecasted to improve population growth for C. greenei in the Cascade Siskiyou National Monument. Strong correlations of climate with plant vital rates in fenced and unfenced plots suggests these populations are not fluctuating randomly through time. Along with biotic factors such as herbivory, climate likely drives the population dynamics of C. greenei, directly and/or indirectly. Climate may not drive vital rates to the same extent as herbivory, but including it in population models generally matches population size trends better than a randomly-iterated environment (Quintana-Ascencio et al. in prep.).

Climate strongly covaried (r > 0.68) with nine out of eleven population vital rates (Table 4). For ecample, dry dormant season precipitation correlated positively with growth and negatively with the probability of entering dormancy the next summer. Increased rainfall in this period may be a chance for plants to acquire more water and nutrients before temperatures drop for the winter, promoting growth and increasing the likelihood they will re-emerge. Wet growing season minimum temperatures correlated positively with survival and negatively with emerging from dormancy and with plant size after dormancy that same year. Warmer springs may lower the risk of vegetative frost damage. Plants may survive better in warmer weather, but at the cost of reduced resources (e.g., through inter- and intraspecific competition) for dormant plants, which are mostly buffered from aboveground temperature changes, but may still be affected by competition for nutrients or water. In contrast to fruit set, the probability of flowering did not have a strong climate driver. Plants may flower regardless of environmental conditions, but may abort fruit development if resources are limited. Fruiting increased with wet growing season evaporative demand, but evaporative demand during the following season (dry growing) was negatively related to fruit production. Drier, hotter conditions in the spring may increase the likelihood of successful pollination by relieving stress on pollinators, and more humid, cooler weather in the early summer might relieve stress on C. greenei plants, allowing greater resource

allocation to developing capsules. There are many possible climate drivers of recruitment, particularly since it is a function of capsule and seed numbers, but this process was challenging for us to detect given the difficulty of finding seedlings in the year they emerge in the field. Further field experiments specifically targeting seedling emergence may be required to identify a clear climate driver for this process.

Climate change simulations projected higher population growth rates than expected if climates remain the same as during the period of this study. This was the case regardless of fencing treatment, although populations were expected to grow more in fenced plots (Figure 15), suggesting the impact of changes in precipitation, temperature, and evaporative demand will improve population growth in the latter half of this century. Climate change, at least in these selected drivers, may benefit C. greenei in the long term. These findings should be considered cautiously as our climate-driven population model only included single drivers of each vital rate. Realistically, multiple factors determine plant growth, survival, and fecundity, including but not limited to inter- and intraspecific competition, pollination, insect grazing and disease, as well as direct and indirect climate interactions on these factors. We were limited, as most demographic studies are, by the number of observed years, which constrains the power of finding reliable drivers statistically.

It is important to consider is the variation in General Circulation Model outputs along with our results. We used the 16 CMIP3 GCMs due to availability of bias-corrected and downscaled data appropriate for our sites. However, not all GCMs are similarly developed, and thus not all GCMs are appropriate or should be considered equally for a given region. Some GCMs model physical properties that others do not, and weighting models according to how well they match observed climate patterns is a recommended approach (Mote and Salathé 2010). This might tighten our confidence around the climate-driven population size projections, where we see some GCMs project increases and some decreases in population size of C. greenei. Use of a smaller set of appropriate GCMs may be preferable, at least until new GCMs are developed with greater accuracy. Still, the lack of an analog to what we expect from future climate is problem that cannot be easily solved.

# Life History

Calochortus greenei has a complex life history, including dormancy that can last multiple years. This bet-hedging strategy corresponds to the species' low mortality, but any fitness advantage seems to be offset by delayed fecundity and low annual seedling recruitment. Flowering likely occurs at least two to three years after recruitment with fruiting occurring after roughly four to five years. Unfortunately, accurate seedling observations were very low (<20), causing a likely underestimation of seedling recruitment and mortality. Miller et al. (2004) found evidence for dormancy longer than a year in two sympatric Calochortus species in British Columbia (C. *Iyalli* and C. *macrocarpus*), but only C. *macrocarpus* had a low mortality, low reproduction trade-off as in C. greenei (Miller et al. 2007).

Our demographic study was challenged by the difficulty of identifying seedlings and quantifying dormancy. Locating wild seedlings was difficult and actual observed seedlings were rare due to their small size (<1 mm wide), which meant they were often over-looked or mistaken for

graminoid species. In addition, dormant plants could re-emerge with leaf widths <10 mm and be mistaken for one-year old plants. A side-effect of waiting for plants to re-emerge from dormancy to record their survival is that both survival and dormancy are intimately linked, and thus both must be estimated instead of observed during the most recent monitoring year. Currently, this methodology is adequate for an understanding of the annual variation in vital rates, but at the cost of accuracy. With more years of data, the general belowground dormancy proportion would become more accurate as more plants are included and tracked, possibly increasing the estimate of seedling recruitment as well. Also, if plants were completely grazed prior to monitoring we may have missed them and assumed they were dormant, which may have artificially elevated dormancy estimates and masked the effect of herbivory.

# IMPLICATIONS FOR CONSERVATION AND MANAGEMENT

- Herbivores negatively affected plant size and population viability of C. greenei. Fencing improved conditions for the species, and may be warranted to enhance some patches or populations of plants. Even protecting plants from just large herbivores provided a substantial benefit to the plants.
- Removing herbivores from plots generally did not result in improvements in native plant abundance, even after 10 years. Grassland vegetation on the Cascade-Siskiyou National Monument that has been degraded due to long-term grazing by livestock is unlikely to improve without additional restoration practices, such as removal of non-native plants and seeding with native vegetation.
- Climate change may improve conditions of C. greenei, at least at sites similar to those examined in this study. This conclusion is preliminary but suggests that some aspects of climate change could benefit the species.
- Further research to better measure seed germination and seedling establishment would improve C. greenei population modeling. Such studies could include a combination of greenhouse and field experiments in which the fate of individual seeds is tracked at multiple points in the wet and dry growing season for multiple years. Experiments across study areas might not be essential, since C. greenei plant performance appears generally similar across the plant communities of the Cascade-Siskiyou National Monument.

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# APPENDIX 1: MAPS









Sketch map of controls, large-mammal and all-mammal exclosure locations in Agate Flat.



Sketch map of Oregon Gulch walk in plots 11-14 and plots 15 & 16 on Rosebud Mountain. Double squares represent fenced plots.



Sketch map of plots 17-20 on Keane Ridge. Follow flagging up and over ridge to reach plots. Double squares represent fenced plots.



Sketch map of control, large-mammal and all-mammal exclosure locations in Colestine study area.

# **APPENDIX 2: PLOT DIRECTIONS**

#### Agate Flat Study Area (Plots 1-10):

Access with assistance from BLM only.

#### Oregon Gulch Study Area (Plots 11-20):

#### Plots 11-14 "Walk in Sites"

From Ashland, take the Hwy 66 exit from I-5 and head east. Just before milepost 21, turn right on Mill Creek Road (40 3E 12 Road). After 1.4 mi, turn left on Lincoln Creek Road (40 3E 12.1 road on map, not labeled on sign), then after 4.3 mi (total mileage from pavement) veer right towards Agate Flat. At 4.5 miles veer left, and at 4.9 miles there is a barbed wire fence across the road, which no longer has a gate, so park and start walking here. After about a 1-1.5 miles (20-25 min) walking, you'll reach an area where slash piles were burned on BOTH sides of the road and there is somewhat of a landing on the left side (look for dead ponderosa pines). Look for an old road blocked off by soil berms, and walk in for 10-15 minutes on this road. Plots 11 and 12 will be visible from the road, on the left side. To reach plots 13 and 14, turn right (before you reach 11 and 12) at the red and yellow/black striped flagging on the right side of the road. Head up hill along a drainage, following flagging.

#### Plots 15-16 "Rosebud Mountain"

From Ashland, take the Highway 66 exit from I-5 and head east. Just before milepost 21, turn right on Mill Creek Road (40 3E 12 Road). After 1.4 mi, turn left on Lincoln Creek Road (40 3E 12.1 road on map, not labeled on sign). After 4.3 mi (total) veer left towards Rosebud (gate), and at 4.6 miles stay left. At 4.9 miles, stay straight/right, and at 5.0 miles take the left fork. At 5.4 miles take the left fork again. Park where possible (road disintegrates) and walk less than an 1/4<sup>th</sup> of a mile past second big slash pile on right, and hike up the hill to right, to bald openings. Fenced plot is just uphill 60 degrees and 75' from the unfenced plot.

#### Plots 17-20 "Keane Ridge"

From Ashland, take the Highway 66 exit from I-5, and head east. Just before milepost 21, turn right on Mill Creek Road (40 3E 12 Road). After 1.4 miles, stay straight, don't veer left as for Agate Flat. Pass through gate at 1.7 miles. Then at 1.9 miles (total miles from pavement), stay left/straight (private road goes right). After 2.2 miles, keep going straight/left, and at 2.5 miles veer left. At 2.9 miles, stay right, then just between 3.0 and 3.1 miles, take a left turn and go through a yellow locked gate (push down on gate to get it to open). At just over 4.1 miles, pass a heli pond on the right side, and at 4.4 miles, stay right. At 4.5 miles veer right, and at just over 5.1 miles, turn right on the 40-3E-25 road. At 5.5 miles veer left. Keep driving a little further, park at 2nd open area, hike up and over hill on right side, following flagging through oak galleries to the sites.

#### Colestine Study Area (Plots 21-30):

#### Plots 21-24 "Colestine Freeway"

From Ashland take I-5 south, and pull off the freeway between mileposts 2 and 3, just past two CHP turnarounds through the center median. Pull off just past metal guardrail. Be sure to leave the BLM research permit on the dash. Hike up the cut bank, and curve up right, through a barbed wire fence. Look for flagging and fencing to find plots. Call Oregon State Patrol Dispatch (541-776-6111) so you don't get tagged and towed.

#### Plots 25-28 "Colestine Overpass"

On I-5, take Exit 1 from the northbound direction (only access is from I-5 north) and park in chain-up pullout at the east end of overpass at top of off ramp. Walk over the overpass, and cross over the guardrail on west side of road, then over a flagged barbed wire fence. Look for exclosure fencing, flagging and fat rattlesnakes.

#### Plots 29-30 "Colestine Corral"

On I-5, take Exit 1 from the northbound direction (can only access from I-5 north), and continue north on the Old Siskiyou Highway past the parking spot for plots 25-28, to the corral turnoff (about 0.5 miles). Take the first right turn, looking for the corral about 100 yards down the road. Park by the corral, and walk east-northeast up an old, washed out, rutted road, then follow flagging to the plots. The fenced plot is slightly uphill from unfenced plot.