

INVESTIGATIONS INTO THE SPATIAL GROWTH  
PATTERNS OF KINCAID'S LUPINE

by

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A THESIS

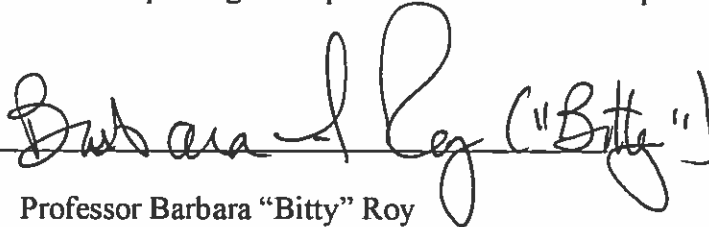
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## An Abstract of the Thesis of

Paul Metzler for the degree of Bachelor of Arts  
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Title: Investigations into the spatial growth patterns of Kincaid's Lupine

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Professor Barbara "Bitty" Roy

Large patches (20–40 meters wide) of threatened Kincaid's Lupine, *Lupinus oreganus* Heller, (host plant to endangered Fender's Blue butterfly *Icaricia icarioides fenderi* Macy) exhibit a striking ring-like pattern of density suggestive of central die-back, in which there is a less dense outer zone, a denser middle zone, and a less dense center zone. To determine what might be causing this pattern, different zones were tested for seed germination, and the plants were analyzed for pathogen and herbivore damage, carbon and nitrogen content, and reproduction. Total germination and growth of the germinants did not significantly vary across zones or with plant density. Herbivory types and nitrogen levels were greater in the less dense outer zones of the patches. Flowering stems per leaf were more abundant in the denser middle zone. Kincaid's lupine displays spatially explicit stoichiometric and herbivory patterns similar to those seen in other lupines, in which leaves in the less dense outer zone (margin) are more nutrient rich and receive greater herbivore damage. However, the less dense center zone had less damage from herbivory, implying that herbivory is not strictly density dependent, but instead depends on zone.

## **Acknowledgements**

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## Introduction

On a few fragments of the once common Willamette Valley upland prairie habitat, a threatened purple wildflower provides sustenance and shelter to a small endangered blue butterfly that depends on it for survival. The endangered status of Fender's Blue butterfly (*Icaricia icarioides fenderi* Macy) means that the fate of this butterfly and its host plant are linked to the management practices of private and governmental agencies, including the US Army Corps of Engineers (USACE). The host, Kincaid's lupine (*Lupinus oregonus* Heller) is surveyed by USACE botanists who recently discovered patches displaying conspicuous rings of dense growth (Figure 1). This study was undertaken to try to determine why these patterns occur. Understanding the spatial growth patterns of this important plant could improve management techniques, and might lead to a better understanding of the role of Kincaid's lupine in Willamette Valley upland prairie ecosystems.

Less than 1% of the widespread upland prairie habitat present at the time of European settlement in Oregon (USA) still exists today (Alverson 1993). Prior to European settlement, land management practices by the Kalapuya Indians over at least the past 2,400 years decidedly changed the course of the evolutionary history of many plants and animals that inhabit the Willamette Valley (Lepofsky and Lertzman 2008, Christy and Alverson 2011, Storm et al. 2006). Willamette Valley grasslands were managed by the Kalapuya Indians who burned the prairies for, among many other things, the production of Camas (*Camassia sp.*), berries, and improved hunting grounds (Boyd 1999, Storm et al. 2006). When native management was removed, Douglas fir trees, non-native blackberries, and other woody species encroached on the prairie

habitat (Christy and Alverson 2011). In addition other prairies were converted to farmland, developed, or suffered from fragmentation (Clark and Wilson 2001). As a result of the change in management, this habitat now hosts a disproportionately high number of endangered and threatened species which still rely on human actions, such as those by lawmakers, land management agencies, and scientists (U.S. Fish and Wildlife Service 2010).

Fender's blue butterfly has been especially adversely affected by habitat loss, and the rare insect was thought to be extinct for 60 years before its rediscovery in the 1980's (Schultz 2003). The butterflies lay their eggs on the leaves of Kincaid's lupine where the larva feed until they drop down and enter diapause in the leaf litter (Schultz 2003). In the spring, they move above ground, feed, form a chrysalis on the leaves, emerge as a butterfly, and then mate and lay eggs for about ten days before they die (Wilson et al. 2003). Fender's blue butterfly can also consume spur lupine (*Lupinus arbutus*) or sickle-keeled lupine (*Lupinus albicaulis*) but only when Kincaid's lupine is nearby (Wilson et al. 2003).

Kincaid's lupine is a perennial herbaceous legume with palmately dissected leaves creating a branched crown and many inflorescences per plant made up of a spike of purple flowers. It grows 20-30cm high, and spreads through rhizomes creating genets (a group of genetically identical "individuals" or ramets) as large as 27 meters across and as much as several centuries old (Wilson et al. 2003, Severns et al. 2011). Fruits are heavily damaged by herbivores, primarily by short nosed weevils and silvery blue butterfly larvae, resulting in low seed set, 0.5 to 1 seeds per pod and 0.4-8.9 seeds per inflorescence, which could explain the low abundance of seedlings other researchers



have found in the field (Wilson et al. 2003). In a study by Schultz (2001) 10,400 seeds were planted at two different sites in autumn of 1995 and only 25 survived until autumn of next year, however, others have found as high as 24 percent germination after one year (Guerrant et al. 2007). Kincaid's lupine cultivates nitrogen fixing bacteria on root nodules and has an unknown relationship with vesicular-arbuscular mycorrhizae, though usually these mycorrhizae have a beneficial relationship with lupines (Wilson et al. 2003). Although not present at the sites visited in this study, it is known that Kincaid's lupine has a relationship with golden paintbrush (*Castilleja indivisa*) in which the paintbrush appropriates the toxic alkaloids that Kincaid's Lupine uses to avoid herbivory (Adler 2000).

The USACE surveys and manages the Kincaid's lupine populations referred to in this paper. Management techniques include controlled burns, mowing, removal and replacement of invasive grasses, propagating and establishing native plants, planting Kincaid's lupine beds for the production of seed, and growing Kincaid's lupine plugs in the greenhouse for out planting. A survey of the 2013 lupine population revealed a unique spatial growth pattern in three Kincaid's lupine patches (Figure 1-3). These three patches form a ring of dense growth with the area inside and outside of the ring growing at a lower density. Data from 2009 and 2013 shows that the Green Oaks North patch seems to be increasing in diameter but then dying back in the center (Figure 1 and 4). Unfortunately there is no spatially explicit data for the other two sites in this study, therefore we cannot assume that the other sites are undergoing the same growth pattern.

# Kincaid's lupine at Fern Ridge: 2013 Green Oaks North (North Main Patch)

2013 Census based on rectangular grids with 1 meter cells

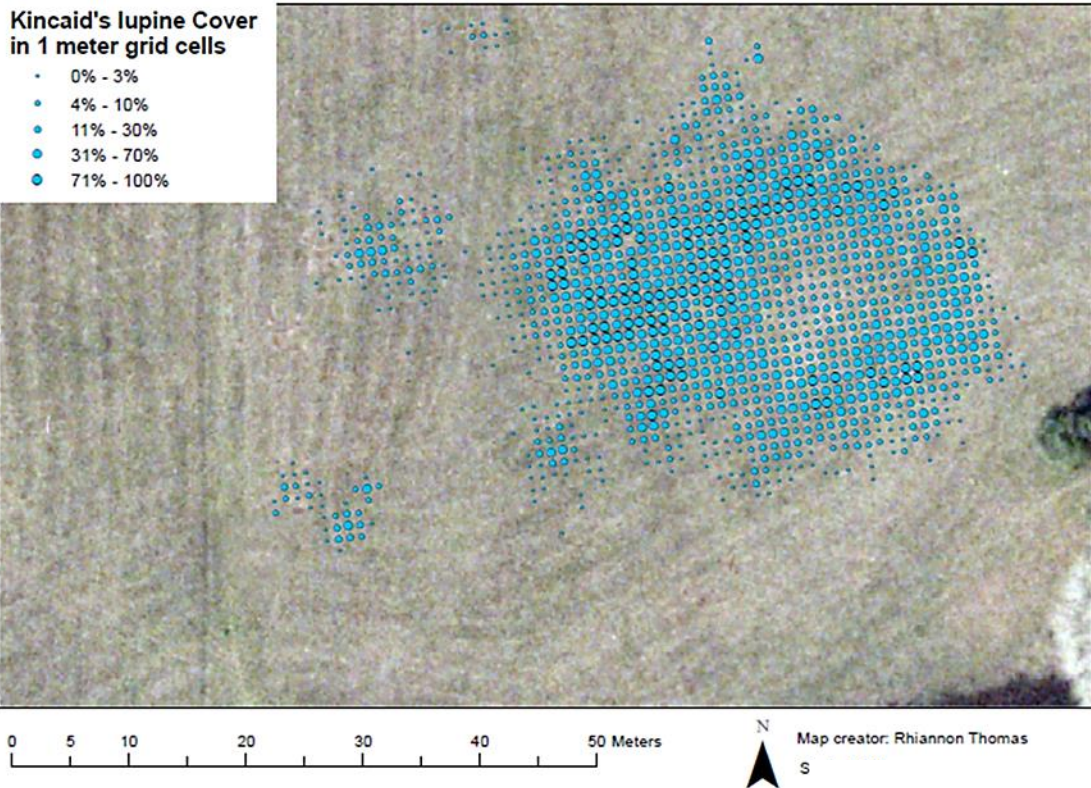


Figure 1.

# Kincaid's lupine at Fern Ridge: Green Oaks South

2013 Census based on rectangular grids with 1 meter cells

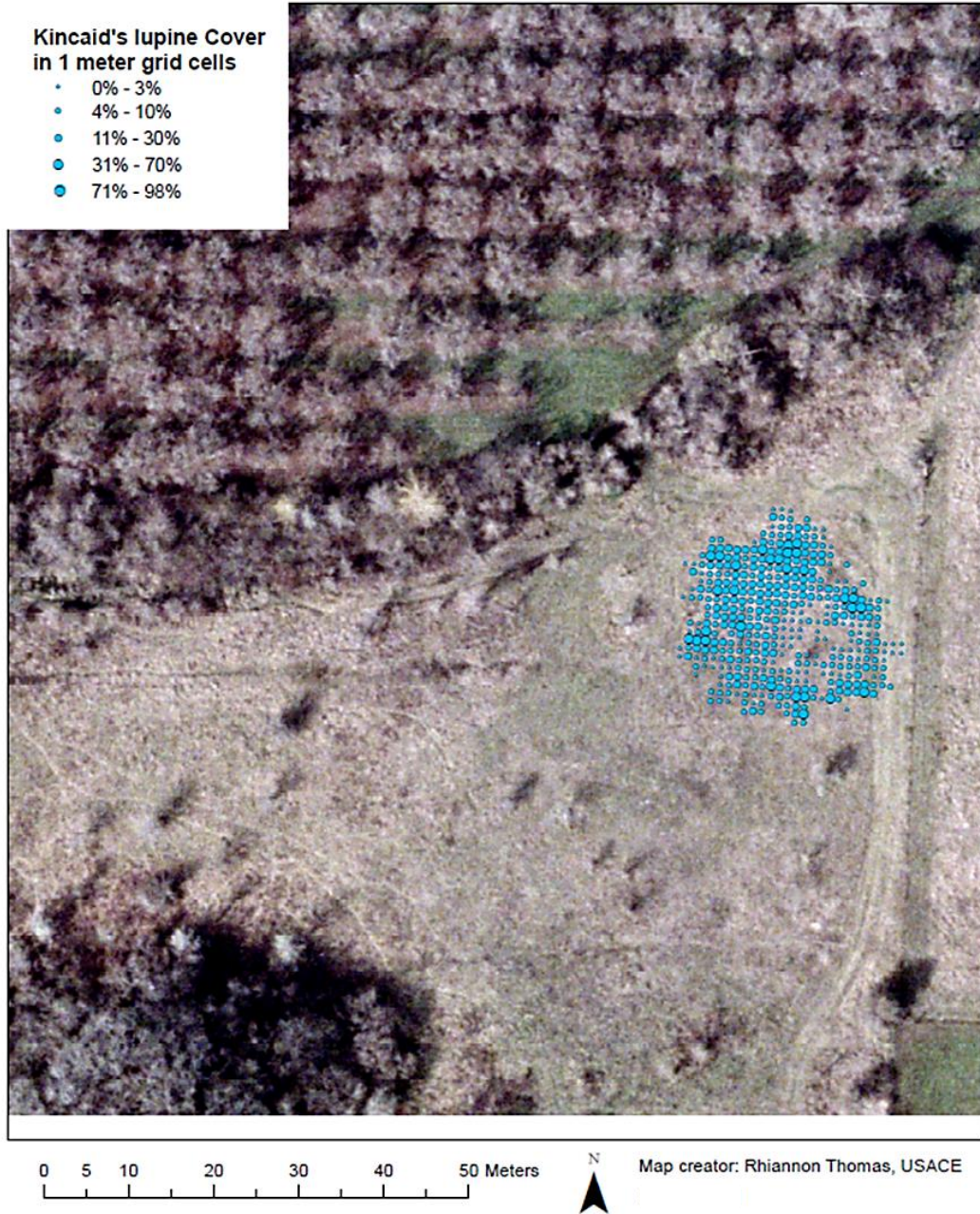


Figure 2.

# Kincaid's lupine at Fern Ridge: Spires West

2013 Census based on rectangular grids with 1 meter cells

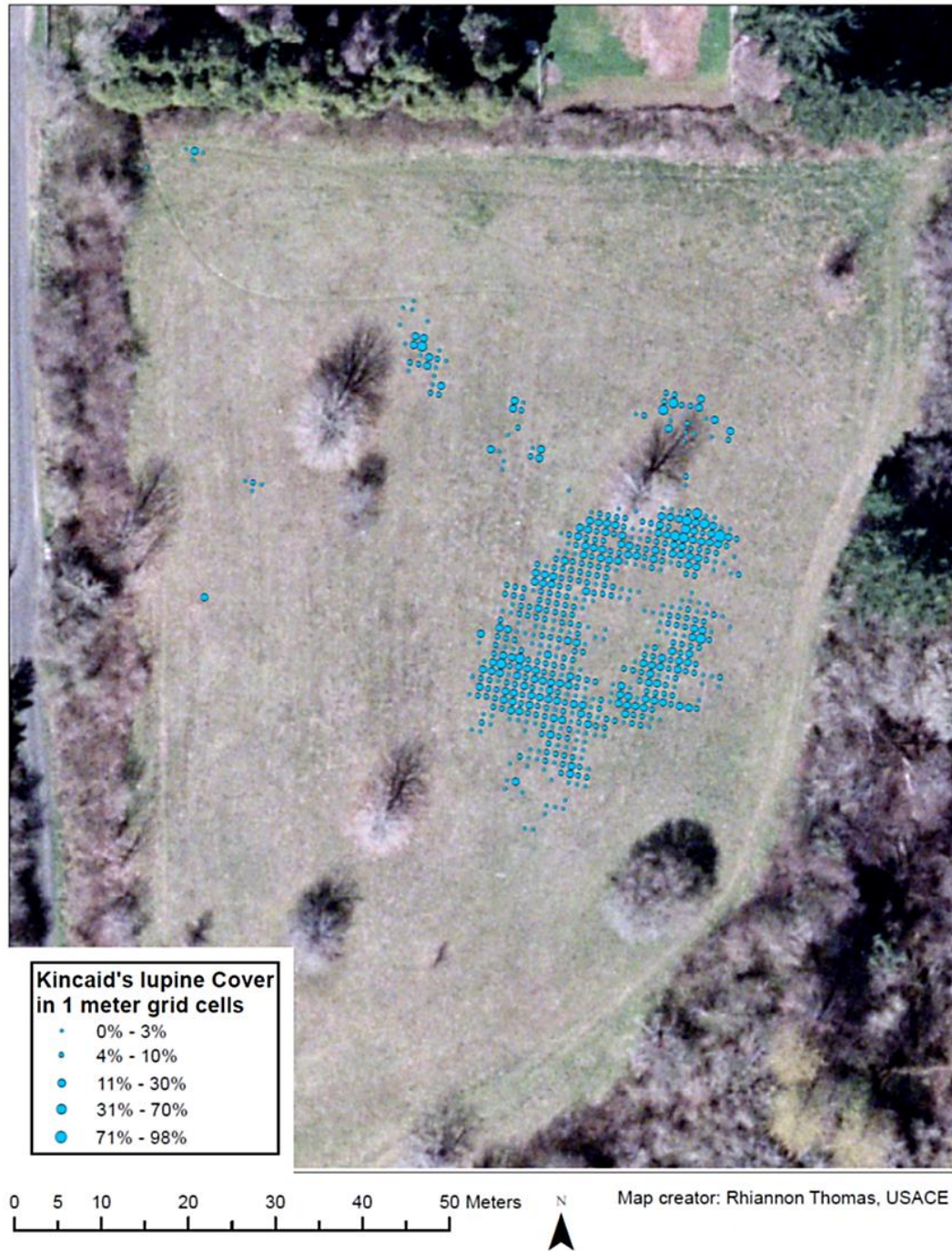
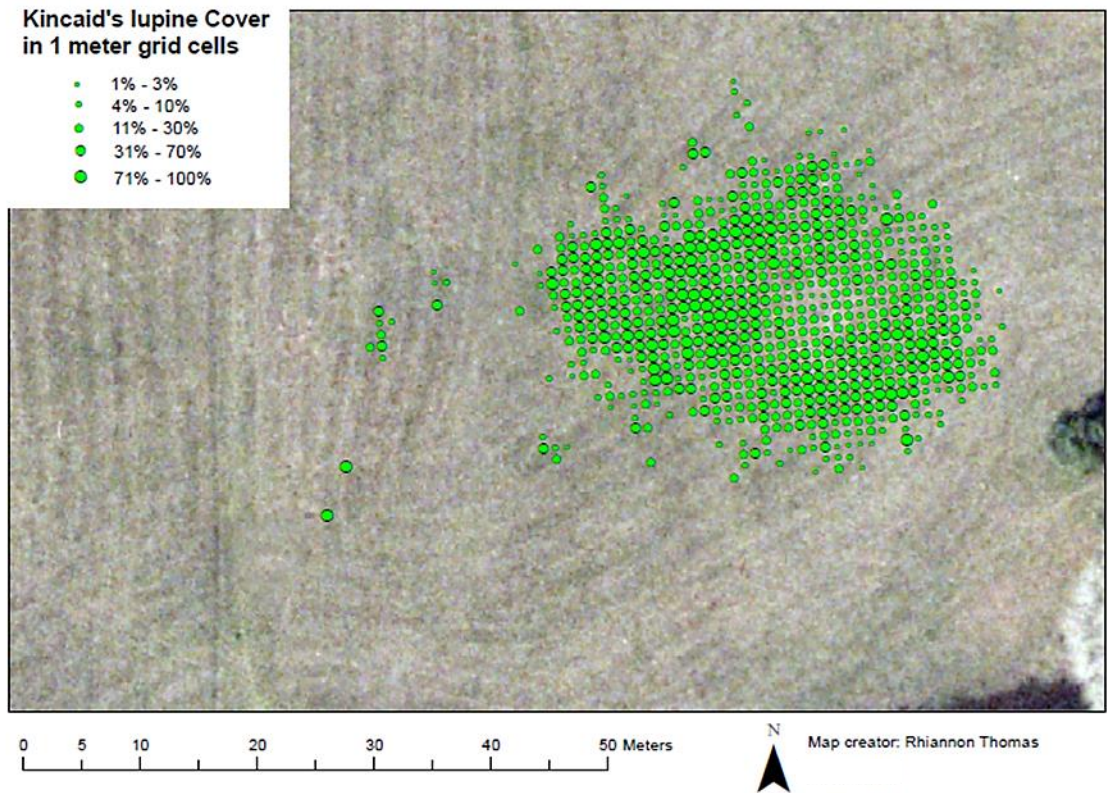


Figure 3.

## Kincaid's lupine at Fern Ridge: 2009 Green Oaks North (North Main Patch)

2009 Census based on rectangular grids with 1 meter cells



**Figure 4.**

This central die-back phenomenon could have any number of explanations and nature is teeming with examples of circular patterns in plant growth. One example of a circular pattern in plants comes from trees in eastern Oregon that are parasitized by reportedly the world's largest organism, one individual of *Armillaria ostoyae*, or honey mushroom (Stamets 2005). The mysterious fairy circles in the Namib Desert, circles of grass with bare land in their center, have recently been attributed to termite colonies (Juergens 2013). Others have attributed abiotic factors to circular patterns such as resource limitation, ramet division of labor, and clonal spreading characteristics

inherent in the plant (Sheffer 2007, Wang et al. 2011, Wong et al. 2011). The central die-back phenomenon has also been observed in colonizing plants in the lava fields of Mount Fuji, although only in monoclonal patches (Adachi et al. 1996). Lupine spreads clonally, but genetically distinct individuals are relatively evenly distributed within a patch (Severns et al. 2011).

I chose to examine fitness, damage, and nutrient content across spatial zones of different densities because of current research findings on Kincaid's lupine and other lupine in the Pacific Northwest. Fitness, measured by germination, germinate growth, and abundance of flowering stems was analyzed because of evidence that Kincaid's lupine may be severely affected by seed herbivory (Wilson et al. 2003). Reproductive behavior such as the distribution of flowering stems, germination inhibition, or seedling survival could be explanatory variables for understanding this central die-back phenomenon.

Percent leaves damaged and damage types were analyzed to characterize what organisms, such as insects, fungi, etc., are acting on these patches, and if their abundance changed depending on location within the patch or with density. Then, leaf nutrients were analyzed and compared to density and location within the patch. Specifically, I wanted to know whether Kincaid's lupine herbivory and nutrient content was inversely dependent on density as seen in *Lupinus lepidus*, a colonizing lupine species studied on the pumice fields created after the eruption of Mount St. Helens (Marleau et al. 2011, Apple et al. 2009, Fagan et al. 2004, 2005, Adamski et al. 2009). It is also possible that nutrient limitation is causing the central die-back phenomenon, as

some native Willamette Valley nitrogen fixing plants are phosphorous limited (Thorpe et al. 2013).

The ultimate goal of these investigations is to inform restoration efforts. The USACE spends a lot of time and resources growing lupine seeds, and if this study helps us understand what a seed needs to be successful, that could help streamline efforts. Surveying damage types and comparing them to their location within a patch will give us clues as to what is acting on and damaging these patches. A change in stoichiometry (the spatial distribution of nutrients) facilitated by lupine could cause succession to take place, as was the case with *Lupinus lepidus* mentioned above. This succession could in turn lead to the destruction of the lupine patch if woody species were to invade after the stoichiometric change. As this thesis is exploratory in nature, I cannot fully explore every possible cause of this phenomenon, but the above hypotheses should answer some interesting questions and lead to a great many more.

## Methods

### *Site and Surveys*

The three sites in figures 1-4 are native patches, meaning that these plants were discovered, not planted intentionally like many other patches. They are also the three largest patches on USACE property. These three patches were the only sites exhibiting the central die-back pattern from the USACE survey, and likely from all 13 units of critical habitat designated by the US Fish and Wildlife Service, since most other Kincaid's lupine patches are much smaller than the sites in this paper (U.S. Fish and Wildlife Service 2006). The patches can be thought of as having three zones, inner, edge, and outer, in which the edge zone is the dense ring of growth and the inner and outer zones are defined as being within the center of the ring or outside of it. For the germination experiment, zones were determined visually, as they are obvious when in the field and can sometimes even be observed from readily available satellite imagery (Figure 5).

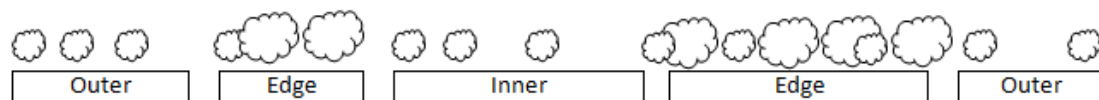


**Figure 5.** Green Oaks North. Image from Google Earth showing the same pattern and shape seen in the USACE lupine survey, Figure 1. (Not to scale)

Zones for the leaf damage data and the nutrient experiment were based on the previous year's (2013) percent cover data. I assumed that the 2013 data was



representative of the 2014 patch because during the four year period between 2009 and 2013, the patches grew 1-4 meters in diameter. Assuming a growth rate of less than 1 meter from 2013 to 2014, this would be smaller than the resolution of the 2013 survey. The entire USACE lupine population was surveyed by observing percent lupine cover over each square meter that made up the population. A transect from the southwest to northeast corner of each patch created a cross section of these patches so that the density of plants in each zone could be quantified meter by meter (Figure 6). Since the patches are different sizes, the transect sizes varied. Transects for Green Oaks North, Green Oaks South, and West Spires were 33 meters, 24 meters, and 40 meters long respectively. Using the length and the bearing of these transects, I was able to overlay each transect on the 2013 density data so that each lupine I observed in my 2014 survey had a corresponding measure of density.



**Figure 6.** Transect Cross Section

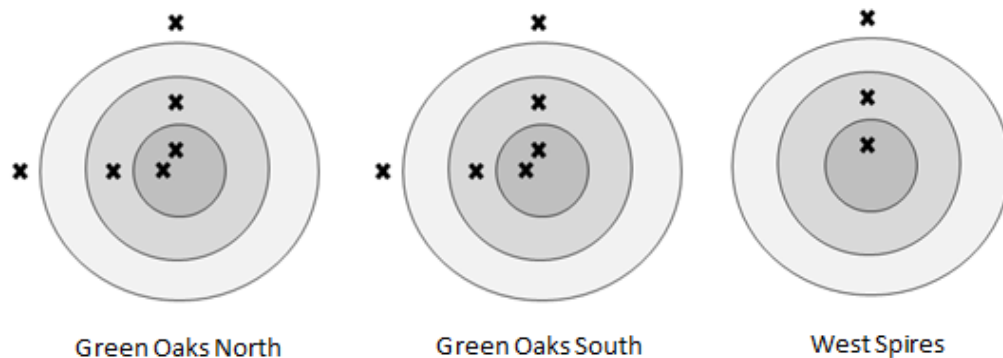
Hypothetical schematic (not to scale) of zones within a transect of a lupine patch, depicted as a cross section. Note the lack of uniformity in zone size. Edge zones generally have larger and more densely clustered plants and correspond to the dense ring seen in the aerial images, (Figures 1-5).

The leaf damage and nutrient data were tested for association with lupine density, and also tested against patch zones, which would allow me to differentiate between density effects and other processes. To examine the zone patterns, the edge was defined as containing the highest one third of the density plots, and that was calculated independently for each site to account for site differences. For Green Oaks North, that is all data greater than 29% cover, for Green Oaks South, all points greater than 14% cover, and for West Spires, all data greater than 2% cover. The inner and

outer zones were determined by their relationship to the edge zone, either within the ring of dense growth or outside of it.

### *Germination Experiment*

Seed germination was measured in small plots that were placed in the inner, edge, or just outside of the outer zones. Plots were placed along a straight line north from the middle of the patch and along a straight line going west from the middle of the patch. In total six germination plots were placed at each site and two within each zone in each site, with the exception of West Spires (Figure 7). At West Spires three of the six plots were planted with seeds from a different source, which did not germinate as readily, so they were dropped from the analysis. Analyzed seeds were from a 2012 harvest from the USACE nursery. Each germination plot consists of 20 different seeds planted as described below.



**Figure 7.** Germination Plots

Schematic of germination plots (x) over a schematic of patch zones at three sites. Note that West Spires is missing the west pointing leg of the germination plots.

Germination plots were 20.3 cm by 25.4 cm and had twenty seeds evenly planted within them, the even pattern was produced by using a section of wire mesh as a template. Mechanical scarification has been shown to be an effective method for

encouraging germination of Kincaid's lupine so the three hundred seeds that were needed to fill all plots were scarified by hand with a metal file (Wilson et al. 2003). The seeds were glued with the radicle facing downward a third of the way up a wooden toothpick. Seeds were planted about 2.5 cm apart and just under the surface of the soil to allow the light necessary for germination (Nava et al. 2010). Planting occurred on March 14, 2014. Seeds were planted in the spring because higher germination of Kincaid's lupine has been recorded in spring rather than fall (Schultz 2001). Plots were checked six times for germination, but only on the last four times was the number of true leaves and herbivory recorded for each seed as well. Census dates were April 1, April 4, April 11, April 27, May 12, and July 14, 2014.

#### *Lupine Pathogen Survey*

Each plant along the transects described was examined for damage types, percent of total leaves damaged, and number of flowering stems. Percent of total leaves damaged was defined as the total number of leaves containing any of the damage types (described below) divided by the total number of leaves. Damage types were recorded in three categories and separated by those caused by herbivory, fungi, or senescence. Senescence is when a portion of the plant dies back, the cause of which is unknown. Since each plant surveyed was a random size (the plant closest to the meter mark on the transect tape was chosen) number of damage types and flowering stems were divided by the number of leaves before analysis. Types of herbivory included leaf roll, rasping, edge bite, center hole, window, slug damage, small yellow insect, thrip, slug, small black bug, spider web, spider egg sack, and ant. Fungal pathogens were recorded as either, stem splotch, black spot (about 3mm), dark ring light center, brown leaf spot,

many small black spots, or yellow with black splotches. Senescence types were, red leaf, dead/dying leaves, dead/dying leaf tips, leaf curl/wilt, purple leaves, and chlorotic (lacking chlorophyll). Pictures were taken of certain categories that were not obvious so they could be referenced if something similar was found (Appendix 1). Each type was recorded as present or absent, and the total number of types was summed. The reason for looking at damage types rather than just percent damaged was to get a sense of the diversity of pests (herbivores and pathogens) acting on the lupines. Damage types were then divided by number of leaves to account for differences in size of plants surveyed. The number of flowering stems was also recorded on each plant for a measure of fitness. The survey was completed twice, (between April 26<sup>th</sup> and May 1<sup>st</sup> and between June 19<sup>th</sup> to June 27<sup>th</sup>) because different species are active at different times. Damage tends to accumulate over time, thus damage increases over the season.

### *Nutrient Study*

To determine whether nutrient levels vary across space in lupine patches, I collected leaves from lupines each meter along the transects. Taking the leaves in late June decreased the chance of harming the young threatened plants but also increased the amount of damage the leaves were exposed. The leaves were immediately dried then ground into a fine powder so percent carbon (C) and percent nitrogen (N) could be quantified through combustion of organic materials using a C and N analyzer (Costech Analytical Technologies Inc., Valencia, CA). N/C ratios were compared to density and zone. A higher N/C ratio was considered a more nutritious leaf overall, as a high level of N is often correlated with higher levels of other nutrients and herbivores tend to prefer leaves with higher levels of N. Leaves were taken during the second pathogen

survey so they could be easily compared to pathogen data and related parameters without affecting the result of the pathogen survey.

### *Statistical Analysis*

The statistical model for total germination included site, zone, and the interaction between site and zone. Total germination was categorical (a seed either germinated or did not) and was compared to the categorical values, site (“Green Oaks North”, “Green Oaks South”, “West Spires”) and zone (“outer”, “edge”, “inner”). A likelihood-ratio chi squared test (L-R  $X^2$ ) was used to analyze results.

Growth of seedlings was assessed by counting the number of true leaves, a continuous value, and was analyzed with a repeated measures MANOVA (multivariate analysis of variance) because growth was measured more than once. Repeated measures accounted for the internal correlation of the data between surveys (the number of true leaves on the same seed recorded on different dates are not independent). Percent leaf damage, damage types per leaf, and number of flowering stems per leaf, were also analyzed with these methods. The statistical model included site, zone, and the interaction between site and zone.

Percent of leaves damaged, number of damage types per leaf, and nutrient ratios were compared against percent cover from a 2013 lupine density survey as described above. An ANCOVA (analysis of covariance) was used, the covariate being percent cover. An ANCOVA was also used to look at flowering stems with damage types as a covariate; this examined the effect of pest damage on fitness. The statistical model included site, percent cover, and the interaction between site and percent cover.

For nutrient data analysis a MANOVA and ANCOVA were performed as described above except there was no need for a repeated measures analysis because nutrients were sampled only once. A Tukeys HSD test was used to see which specific zones and sites were significant. The statistical analysis software JMP Pro 10 was used for all analysis.

## Results

### *Seedling Germination and Growth*

To test for possible germination or seedling inhibition, total germination and growth of lupine leaves was assessed over time across the three different zones. A likelihood-ratio chi squared test showed that total germination was not significant across outer, edge, or inner zones, (L-R  $X^2 = 3.30$ ,  $P = 0.19$ ; Table 1, Figure 8).

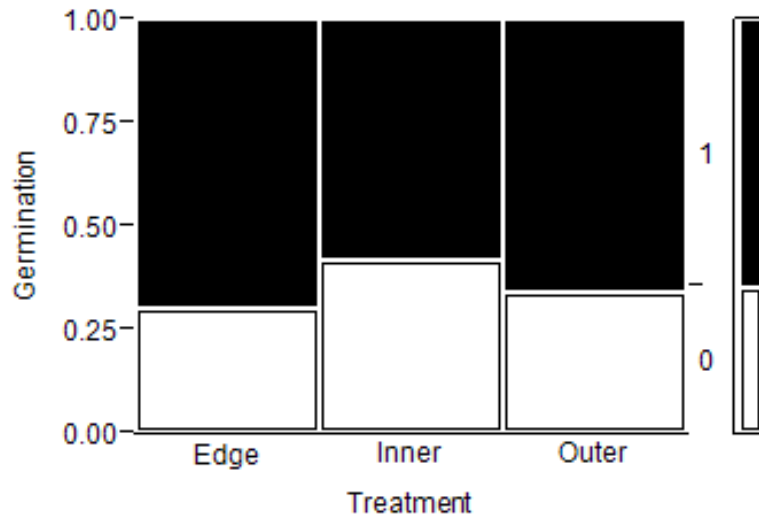
However, there was significant site effect and a site by zone interaction (L-R  $X^2 = 20.08$ ,  $P < .0001$ ; L-R  $X^2 = 26.48$ ,  $P < .0001$ ; Table 1). South Green Oaks showed significantly higher germination than West Spires and Green Oaks South (Figure 9).

The significant interaction of site by zone means that there was no consistent zone effect across the sites (Figure 10).

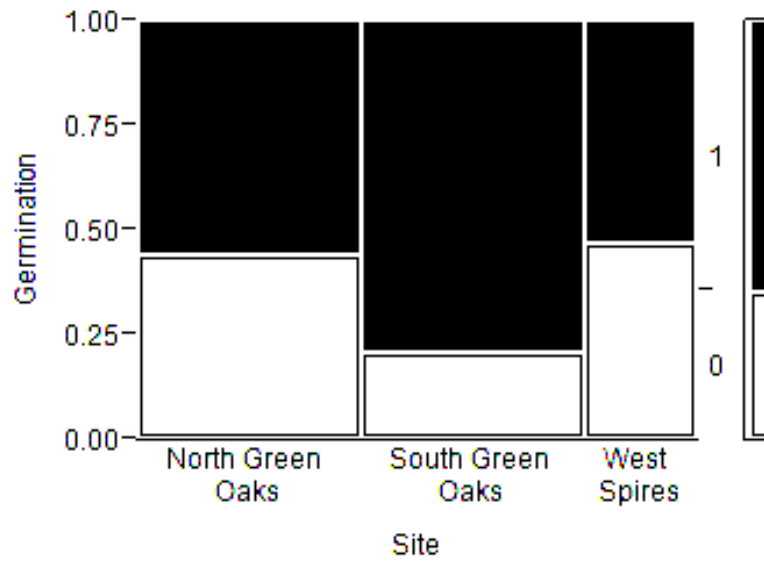
**Table 1.** Germination effect likelihood ratio test

Source	Nparm d.f.	L-R ChiSquare	P-value
Site	2	20.08	<b>&lt;&lt;0.001</b>
Zone	2	3.30	0.190
site*zone	4	26.48	<b>&lt;&lt;0.001</b>

\*indicates an interaction term, Nparm=non-parametric, d.f.=degrees of freedom. Bold probabilities are significant.

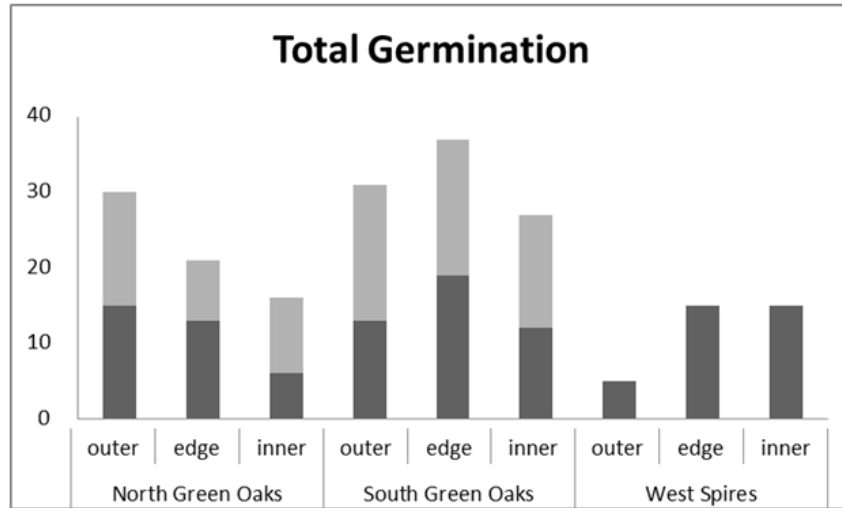


**Figure 8.** Frequency distributions for each zone where 1 represents germination and 0 represents no germination.



**Figure 9.** Percent germination for each site where 1 represents germination and 0 represents no germination.





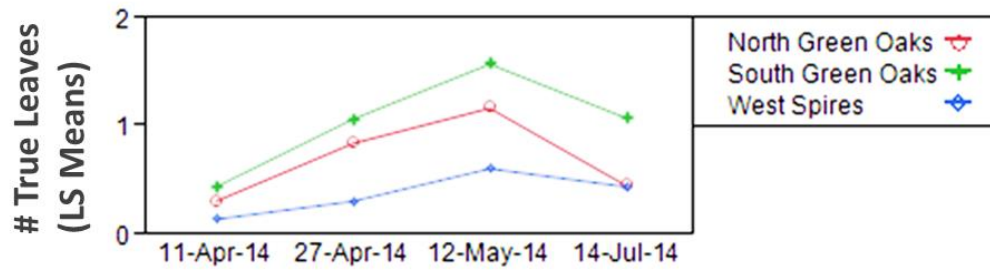
**Figure 10.** Total germination across zones within each site. Note that West Spires has half the sample size. The two shades of grey represent the two germination plots in each zone.

A repeated measures analysis suggests that the number of true leaves depends on site ( $F_{6,580} = 3.44$ ,  $P = 0.002$ , Table 2, Figure 11). However, zones within the patch had no effect and there was no site by zone interaction ( $F_{6,580} = 0.55$ ,  $p = 0.76$ , Table 2, Figure 12). Not surprisingly, the number of true leaves increased over time, and the sites differed over time as well (significant time and site by time interaction).

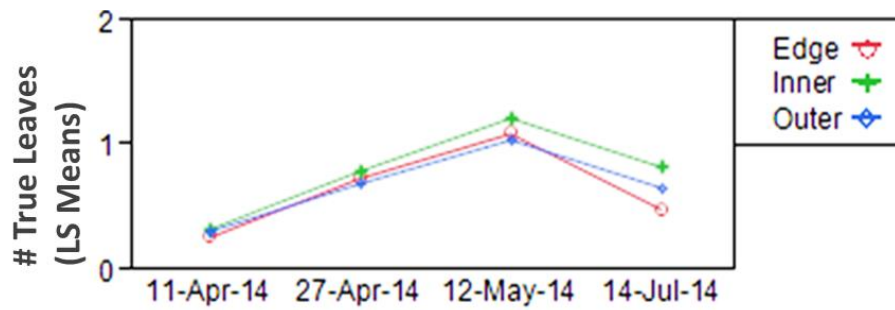
**Table 2.** Number of true leaves ANOVA

	Test	Value	Approx. F	Num DF	Den DF	P-value
All between interactions	F Test	0.14	5.05	8	291	<< <b>0.001</b>
Site	F Test	0.06	9.42	2	291	< <b>0.001</b>
Zone	F Test	<.01	0.54	2	291	0.582
Site*Zone	F Test	0.07	5.23	4	291	< <b>.001</b>
All within interactions	Pillai's Trace	0.13	1.69	24	873	0.019
Time	F Test	0.33	32.16	3	289	<< <b>0.001</b>
Time*site	Pillai's Trace	0.07	3.44	6	580	<b>0.002</b>
Time*zone	Pillai's Trace	0.01	0.55	6	580	0.765
Time*site*zone	Pillai's Trace	0.05	1.40	12	873	0.159

\*indicates an interaction term, Nparm=non-parametric, d.f.=degrees of freedom. Bold probabilities are significant.



**Figure 11.** Average number of true leaves per seedling over four dates



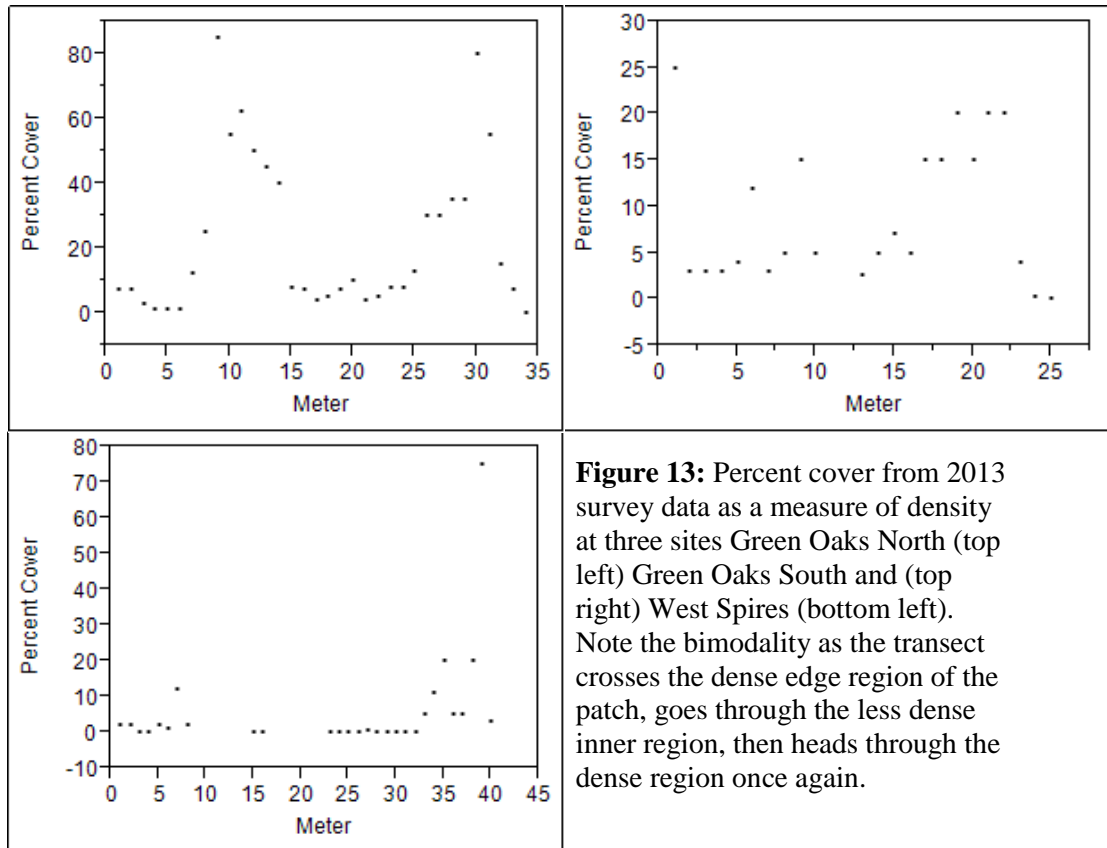
**Figure 12.** Zone effect across time.

Overall there was no consistent pattern of germination or seedling growth between inner, edge, and outer zones in the lupine patches. In fact, those variables depend more on site than they do on zone, so we cannot make any generalizations about how the three different zones of these lupine patches affect germination or first year seedling growth.

### *Lupine Pathogen Survey*

The first survey took place between April 26 and May 1, 2014, right before most of the flowering stems emerged, and the second survey took place between June 19 and

June 27, 2014, while the lupine was setting its seed. Percent leaf damage and damage types were tested for covariance with the preceding year's percent lupine cover (Figure 13). An analysis of repeated measures was also used to compare damage to zone.



**Figure 13:** Percent cover from 2013 survey data as a measure of density at three sites Green Oaks North (top left) Green Oaks South and (top right) West Spires (bottom left). Note the bimodality as the transect crosses the dense edge region of the patch, goes through the less dense inner region, then heads through the dense region once again.

An ANCOVA of percent leaves damaged with lupine cover as a covariate found no significant association between damage and percent cover ( $F_{1,77} = 0.82$ ,  $P = 0.37$ , Table 3). However, site and time did significantly explain leaf damage and there were no interactions (Table 3). Time was the only factor significantly explaining the number of damage types per leaf (Table 4). Predictably, both leaf damage and pathogen types increased over the summer. Leaf damage varied significantly depending on the site; South Green Oaks had less damage than the other sites (Figure 14).

**Table 3.** ANCOVA on percent leaf damage. The covariate was percent lupine cover.

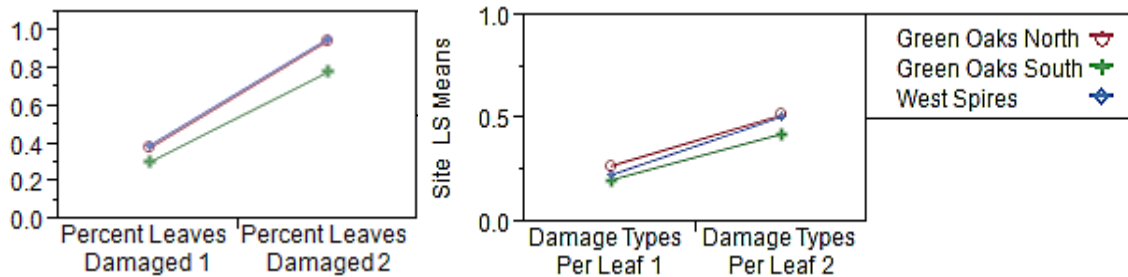
	Test	Value	Apprx. F	NumDF	DenDF	P-value
All between interactions	F Test	0.26	3.93	5	77	<b>0.003</b>
Site	F Test	0.19	7.49	2	77	<b>0.001</b>
Percent Cover	F Test	0.01	0.82	1	77	0.368
Site*Percent Cover	F Test	0.04	1.51	2	77	0.227
All within interactions	F Test	0.04	0.62	5	77	0.683
Time	F Test	2.23	171.97	1	77	<b>&lt;&lt;0.001</b>
Time*Site	F Test	0.02	0.73	2	77	0.484
Time*Percent Cover	F Test	<.01	0.10	1	77	0.751
Time*Site*Percent Cover	F Test	0.01	0.45	2	77	0.640

\*indicates an interaction term, Nparm=non-parametric, d.f.=degrees of freedom. Bold probabilities are significant.

**Table 4.** ANCOVA on number of damage types per leaf. The covariate was percent lupine cover.

	Test	Value	Apprx.F	Num DF	Den DF	P-value
All between interactions	F Test	0.04	0.65	5	77	0.660
Site	F Test	0.02	0.67	2	77	0.515
Percent Cover	F Test	<0.01	0.31	1	77	0.579
Site*Percent Cover	F Test	0.01	0.19	2	77	0.824
All within interactions	F Test	0.05	0.77	5	77	0.573
Time	F Test	0.48	36.99	1	77	<b>&lt;&lt;0.001</b>
Time*Site	F Test	0.01	0.26	2	77	0.771
Time*Percent Cover	F Test	<<0.01	0.00	1	77	0.997
Time*Site*Percent Cover	F Test	0.01	0.34	2	77	0.715

\*indicates an interaction term, Nparm=non-parametric, d.f.=degrees of freedom. Bold probabilities are significant.



**Figure 14.** Percent leaf damage and damage types per leaf over two surveys and three sites

The ANCOVAs included the percent lupine cover data, which made a distinction between the dense and not dense areas but did not distinguish between the inner and outer sections of the patch. The repeated measures analysis below is based on zone in order to make that distinction.

Damage types are significant across patch zones ( $F_{2,74} = 4.64$ ,  $p = 0.013$ , Table 5). Specifically, the outer zone was more susceptible to damage than the inner or edge zones (Figure 15). The interaction plot of groups of damage types shows that this significance is due to herbivory rather than fungal pathogens or senescence (Figure 15). The interaction in that figure (shown by crossed lines) is due to the three groups of damage types not having the same effect across zone, in fact, only herbivory has a significant relationship to zone. Time was also a significant factor as, predictably, damage types increased later in the summer (Figure 15).

**Table 5.** Damage types repeated measures analysis

	Test	Value	Apprx. F	NumDF	DenDF	P-value
All between interactions	F Test	0.20	1.83	8	74	0.085
Zone	F Test	0.13	4.64	2	74	<b>0.013</b>
Site	F Test	0.05	1.78	2	74	0.177
Site*Zone	F Test	0.06	1.02	4	74	0.403
All within interactions	F Test	0.14	1.32	8	74	0.246
Time	F Test	0.97	71.41	1	74	<b>&lt;&lt;0.001</b>
Time*Zone	F Test	0.03	1.12	2	74	0.331
Time*Site	F Test	0.05	1.78	2	74	0.176

Time*Site*Zone	F Test	0.11	1.95	4	74	0.111
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\*indicates an interaction term, Nparm=non-parametric, d.f.=degrees of freedom. Bold probabilities are significant.

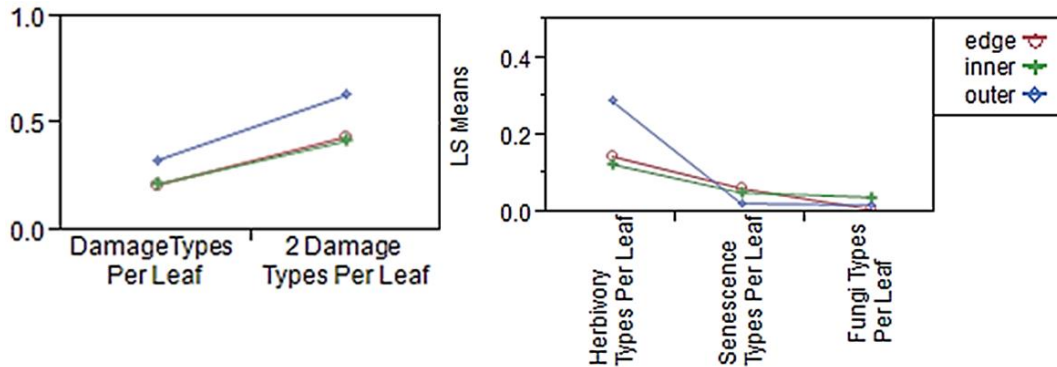


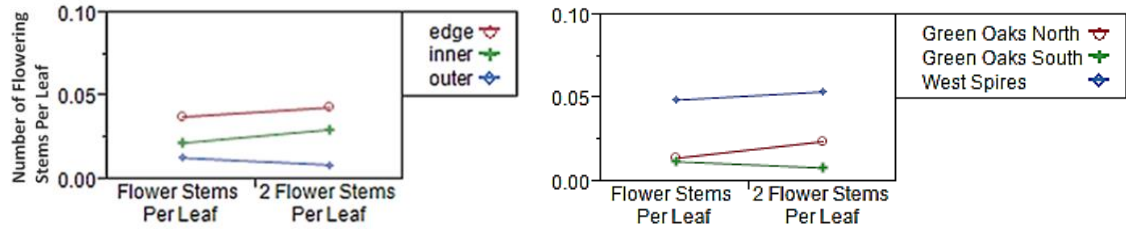
Figure 15. Lupine damage types per leaf by zone and groups of damage types

Table 6. Flowering stems repeated measures analysis

	Test	Value	Apprx. F	NumDF	DenDF	P-value
All between interactions	F Test	0.51	4.68	8	74	<b>&lt;0.001</b>
Site	F Test	0.25	9.43	2	74	<b>&lt;0.001</b>
Zone	F Test	0.14	5.34	2	74	<b>0.007</b>
Site*Zone	F Test	0.05	0.90	4	74	0.467
All within interactions	F Test	0.06	0.54	8	74	0.825
Time	F Test	0.01	0.46	1	74	0.498
Time*Site	F Test	0.02	0.67	2	74	0.515
Time*Zone	F Test	0.02	0.64	2	74	0.532
Time*Site*Zone	F Test	0.02	0.32	4	74	0.866

\*indicates an interaction term, Nparm=non-parametric, d.f.=degrees of freedom. Bold probabilities are significant.

To help answer the question of reproductive success posed in the germination study, I looked at flowering stems across zones. There was significant zone effect in that the most flowering stems were in the edge zone, followed by inner then outer ( $F_{2,74} = 5.34$ ,  $p = 0.007$ , Table 6, Figure 16). There was also significant site effect; West Spires had the greatest number of flowering stems followed by Green Oaks North then Green Oaks South ( $F_{2,74} = 5.34$ ,  $p = <.001$ , Table 6, Figure 16).



**Figure 16.** Flowering stems over two surveys separated by zone (left) and site (right)

Since damage types and fitness were both significant, I tested for an interaction between the two using a repeated measures ANCOVA with damage types per leaf as a covariant with flowering stems per leaf. There were no significant interactions, meaning that these variables have a simple relationship with fitness not being conflated with damage types (Table 7).

**Table 7:** ANCOVA on number of flowering stems per leaf. The covariate was damage types.

	Test	Value	Apprx. F	NumDF	DenDF	P-value
All between interactions	F Test	0.65	4.66	10	72	<b>&lt;&lt;0.001</b>
Site	F Test	0.09	3.27	2	72	0.044
Zone	F Test	0.32	11.47	2	72	<b>&lt;&lt;0.001</b>
Site*Zone	F Test	0.02	0.50	4	72	0.738
Damage Types (2)	F Test	0.02	1.62	1	72	0.207
Damage Types (1)	F Test	0.02	1.63	1	72	0.206
All within interactions	F Test	0.08	0.57	10	72	0.836
Time	F Test	0.01	0.71	1	72	0.403
Time*Site	F Test	0.01	0.36	2	72	0.696
Time*Zone	F Test	0.02	0.80	2	72	0.455
Time*Site*Zone	F Test	0.02	0.38	4	72	0.822
Time*Damage Types (2)	F Test	<0.01	0.15	1	72	0.700
Time*Damage Types (1)	F Test	0.02	1.36	1	72	0.247

\*indicates an interaction term, Nparm=non-parametric, d.f.=degrees of freedom. Bold probabilities are significant.

### Nutrient Analysis

To test for a possible stoichiometric explanation for the central die-back phenomenon, I analyzed N/C ratios in leaf tissue. A least squares analysis showed an interaction between site and percent cover (Table 8).

**Table 8.** An ANCOVA of N/C ratios. Percent cover is the covariate.

	NumDF	DenDF	Sum of Squares	F Ratio	P-value
Site	2	2	0.00001	0.68	0.508
Percent Cover	1	1	0.0002	17.26	<< <b>0.001</b>
Site*Percent Cover	2	2	0.0002	8.27	< <b>0.001</b>

\*indicates an interaction term, Nparm=non-parametric, d.f.=degrees of freedom. Bold probabilities are significant.

A MANOVA comparing N/C ratios to zone, determined that site, zone, and their interaction significantly explained the variation in the data (Table 9). The interaction term indicates that there is not a consistent effect of zone across sites. However, a Tukey's HSD showed that the outer zone had significantly higher nitrogen to carbon ratios than the edge zone and that Green Oaks South was significantly different than the other sites. This indicates that the outer zone has a more nutritious leaf (higher levels of N).

**Table 9.** MANOVA of N/C ratios compared to zone

	NumDF	DenDF	Sum of Squares	F Ratio	P-value
Site	2	2	0.00007	3.63	<b>0.029</b>
Zone	2	2	0.0002	7.73	<b>0.001</b>
Site*Zone	4	4	0.0003	9.16	<< <b>0.001</b>

\*indicates an interaction term, Nparm=non-parametric, d.f.=degrees of freedom. Bold probabilities are significant.

**Table 10.** Tukey's HSD

Effect	Level		Least Squares Mean
Zone	Outer	A	0.02187
Zone	Inner	A B	0.02068
Zone	Edge	B	0.02001
Site	Green Oaks South	A	0.02254



Site	West Spires	B	0.02014
Site	Green Oaks North	B	0.01989
Zone*Site	Outer Green Oaks South	A	0.02649
Zone*Site	Inner Green Oaks South	B	0.02213
Zone*Site	Edge West Spires	B	0.02105
Zone*Site	Inner Green Oaks North	B	0.02086
Zone*Site	Outer West Spires	B	0.02030
Zone*Site	Edge Green Oaks North	B	0.02000
Zone*Site	Inner West Spires	B	0.01906
Zone*Site	Edge Green Oaks South	B	0.01899
Zone*Site	Outer Green Oaks North	B	0.01883

Levels not connected by the same letter are significantly different

## Discussion

Understanding how a species spreads at a population level can have an enormous impact on how the species is managed. In this case, understanding the spatial dynamics of this threatened plant could improve population estimate surveys, methods of integrating new plants and seeds into restoration sites, or maintaining existing lupine sites. Trying to deconstruct this ecological phenomenon could lead to a greater understanding of a mechanism that could be critical in supporting this threatened plant or the endangered butterfly for which it is host.

One restoration practice is to broadcast seed into potential habitat, it would be helpful for land management agencies to know if germination is affected by nearby lupine. However, no difference in total germination or vigor of germinant was detected across density zones. Since the seedlings in the germination plots described above senesced along with their adult counterparts for the winter season, there is no way to know if the seedlings will reemerge in the spring. Zone was nearly a significant factor in the total germination analysis ( $p = 0.19$ ) with the inner zone having the least germination. If variance was reduced by putting more germination plots randomly within each zone (more than one or two as I have done in this study) that statistic may be significant in future studies. If true, that would be consistent with my prediction that germination may be less vigorous in the center of the patch, but that is just conjecture at this point.

Another measure of reproductive fitness, flowering stems per leaf, did vary across zones. Flowering stems were significantly more abundant in the dense edge zone than in either of the other zones. This raises other potential research questions, such as:

Do lupines intentionally produce reproductive parts where there is less chance of herbivory (where there is less nutritious dense leaves)? Are there less flowering stems per leaf in the outer zone because of herbivore damage? Do germinating lupine seeds need a dense patch of lupine (complete with protection from the sun and copious amounts of leaf litter) to successfully survive the fragile phase of germination?

Apple et al. (2009) found that less dense areas around the margin of a patch of *Lupinus lepidus* had more herbivory and higher percentages of nitrogen (N) and phosphorous (P). Herbivores that fed on the more nourishing leaves from the margin of these patches performed better than herbivores nourished on leaves from the dense middle of the patch (Fagan et al. 2004, Bishop et al. 2010). In the Kincaid's lupine patches analyzed above, both the number of herbivore types and nutrient content was higher in the outer zone. This is consistent with current research on lupine stoichiometric and herbivorous spatial dynamics (Marleau et al. 2011, Apple et al. 2009, Fagan et al. 2004, 2005, Adamski et al. 2009). However, their research has currently linked these trends to density dependency, which could be limiting. In my analysis, density did not co-vary with damage types or percent damaged leaves, but when the analysis was repeated, this time using the different zones, damage did depend on zones. This suggests that the outer and inner zones, though having similar density, were distinct from each other in some other way.

My analysis only measured levels of N not levels of P or other nutrients. At the Mount St. Helens lupine patches, herbivores preferred leaves with higher levels of P in a nitrogen and phosphorous co-limited primary succession system, and the availability of N was P-limited (Bishop et al. 2010). If P levels limit the growth of Kincaid's lupine

as well, testing for P across zones might be one of the best options for trying to pinpoint the cause of the central die-back.

There is still a huge body of explanatory factors that have not been explored. This study ignores below ground microorganisms that may be driving a negative plant soil feedback, for example. Potential relationships to other plants may also be driving this phenomenon as well. In fact, after nearly completing this thesis, I was browsing the historic imagery in Google Earth and found the images below (Google Earth, Figure 17).



June 2014



August 2012



August 2011

**Figure 17.** Satellite Images (not to scale)

Clearly in the last two photos, consistently two years in a row, maybe many more, there is something green in a field of senesced plants directly in the middle of the large Green Oaks North lupine patch. If the plant communities in the inner and outer zones are completely different, lupine may be facilitating succession. This would have enormous conservation implications because the incoming plants could be invasive and ultimately out compete the lupine.

Finally, what does this mean for the endangered Fender's Blue butterfly? Are larva larger and more numerous in the outer zone? If this ring-like pattern eventually grows to a size greater than the prairie that supports it, will the remaining leaves be less nutritious as a food source? The endangered and threatened statuses of these organisms provide scientists with a distinct legal and moral responsibility to learn more about them. This striking spatial arrangement certainly provides ample opportunity for research, most of which is outside of the scope of a short undergraduate thesis limited by only one season of field research. This project was exploratory in nature and attempted to address a broad range of hypotheses in order to lay out some questions for future research, and to bring up this fascinating growth pattern as something to consider in restoration efforts.

## Appendix 1





a. Slug and slug damage



b. Senescence of leaf tips



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