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CLONAL DIVERSITY OF QUAKING ASPEN (*POPULUS TREMULOIDES*): HOW MULTIPLE  
CLONES MAY ADD TO THE RESILIENCE AND PERSISTENCE OF THIS FOREST TYPE

by

Richard S. Gardner

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

MASTER OF SCIENCE

in

Forestry

Approved:

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Dr. Karen E. Mock  
Major Professor

---

Dr. James N. Long  
Committee Member

---

Dr. Zhao Ma  
Committee Member

---

Dr. Douglas J. Shinneman  
Committee Member

---

Mark R. McLellan  
Vice President for Research and  
Dean of the School of Graduate Studies

UTAH STATE UNIVERSITY  
Logan, Utah

2013

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## ABSTRACT

Clonal Diversity of Quaking Aspen (*Populus tremuloides*): How Multiple Clones May  
Add to the Resilience and Persistence of This Forest Type

by

Richard S. Gardner, Master of Science

Utah State University, 2013

Major Professor: Dr. Karen E. Mock  
Department: Wildland Resources

Conservation and restoration of quaking aspen in the western United States requires an understanding of how and when aspen clones became established, how clones adapt to environmental challenges, and how individual clones interact within stands. I used molecular tools to identify individual clones in a natural population of aspen in southern Utah and detected high and low levels of clonal diversity within stands. Stands with high clonal diversity were located in areas with a more frequent fire history, indicating that fires may have prepared sites for seed germination and establishment over time. Conversely, areas of low clonal diversity corresponded to areas with less frequent fire. The same molecular tools were then used to investigate clonal interactions/succession over relatively recent time. For this portion of the study I sampled small, medium, and large aspen ramets (stems) at 25 subplots within spatially separated one-hectare plots, and mapped the clonal identities. I found that approximately 25% of the clones appeared to be spreading into adjacent clones, while 75% of the clones

had a stationary pattern. In the final portion of the study, I again used molecular tools to identify aspen clones and investigated tradeoffs between growth and defense chemistry in mature, naturally-occurring trees. Growth was estimated using a ten-year basal area increment, and the percent dry weight of salicortin, tremulacin, and condensed tannins was measured in the same trees. Overall I discovered evidence for a tradeoff between growth and salicortin/tremulacin, and a marginally significant but positive relationship between growth and condensed tannins.

(90 pages)

## PUBLIC ABSTRACT

Clonal Diversity of Quaking Aspen (*Populus tremuloides*): How Multiple Clones may  
add to the Resilience and Persistence of this Forest Type

by

Richard S. Gardner

Aspen forests and woodlands are widespread across the western United States and are a primary component of many ecosystems in the west. Aspen is a clonal species, with reproduction occurring both by root sprouting (suckering) and seeding. Traditionally, western aspen forests were thought to consist almost entirely of large clones established several thousands of years ago, with seeding events being rare and ecologically negligible. Although clones in the western US can grow to be many acres, recent studies have demonstrated a far greater proportion of small clones than had been previously thought to exist. In this study I wanted to answer some important questions about how local conditions may lead to the recent establishment of these small clones, how clones interact with one another in genetically diverse stands, and how individual clones cope with environmental pressures over time.

In the first portion of my study, I used genetic tools to identify clones across Cedar Mountain, Utah (~10 miles southeast of Cedar City) and found areas of high and low clonal diversity (a greater number of individual clones in a stand would lead to higher clonal diversity). Areas of high clonal diversity occurred in areas where fires have been more frequent over recent time, suggesting that fire may play a role in preparing landscapes for aspen seedlings to germinate and become established.

In another portion of the Cedar Mountain study I showed at how adjacent aspen clones interact with one another. In particular I determined how frequently clones seemed to be spreading into adjacent clones (versus having stable boundaries). I found that approximately a quarter of the clones seemed to be spreading into surrounding clones, and three-quarters of the clones displayed more stationary behavior. These findings suggest that the process of clonal displacement and replacement within stands may be quite slow but does occur.

The third portion of my thesis addressed ecological tradeoffs that might occur in aspen. Aspen leaves are consumed by many species of insects, and must cope with this pressure over their lifetimes. Alternative ways of coping with herbivory include resisting attacks by producing defense chemicals to reduce attacks or growing new tissues vigorously following herbivory. Previous greenhouse studies have shown that aspen experiences tradeoffs between resistance (defense chemistry) and resilience (growth following attack) in experimental settings, and I wanted to determine if these tradeoffs were also present in mature, naturally-occurring aspen forests. I did detect a tradeoff between a particularly effective group of defense chemicals and growth, and the tradeoff varied among individual aspen clones. I also found that individual clones differed with respect to the concentration of all defense chemicals and also with respect to growth.

The findings of this study may help influence management decisions when objectives are to promote aspen stand resilience and persistence over time. Forest managers can create conditions favorable to seedling establishment and the promote establishment of new clones which will likely increase the chances for some clones to tolerate changing conditions over time. The establishment and maintenance of clonal

diversity should also provide forest resilience, both in terms of ecosystem function and adaptation to changing conditions.



## DEDICATION

I dedicate this work to my great friend Thomas Taylor whose life was lost during the time that this work occurred. Through encouragement and example, Tom motivated me to pursue an education in forestry and to not waver when faced with adversity. Tom was one of the finest humans I've ever known.

Richard S. Gardner

## ACKNOWLEDGMENTS

First and foremost I would like to thank my academic advisor Karen Mock who provided me with a tremendous amount of support and guidance, both as an undergraduate and graduate student. Karen is an instinctual mentor who seems to place more merit on the success of her graduate students than on her own, a quality unmatched by any other with whom I have worked with. I also would like to thank James Long, Zhao Ma, and Douglas Shinneman for their perspectives, input, and for serving as members of my graduate committee. This work also benefited greatly from the previous work and input of Rick Lindroth, R. Justin DeRose, and Paul Rogers. Susan Durham, Josh Leffler, and Richard Cutler provided substantial statistical advising. The collection of field data and samples would not have been possible without assistance from R. Justin DeRose, Seth Ex, Cody Mittank Jerrell Mock, Jon Mock, and John Rentschler, and Kelly Sivy.

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Richard Scott Gardner

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## CHAPTER 1

### INTRODUCTION

Quaking aspen (*Populus tremuloides*) is one of the most widespread tree species in North America (Little, 1971), and over much of its range is ecologically, economically, and socially important (DeByle and Winokur, 1985). Isolated incidents of declining health and mortality in aspen have been observed in the interior western United States (Worrall et al., 2008, 2010), and climate change is projected to have further negative effects on aspen coverage in the west (Rehfeldt et al., 2009). Conservation and restoration efforts have already been occurring where aspen has particularly high ecological and economical value. Much of the emphasis of aspen restoration has focused on stimulating the growth of new stems (DeByle and Winokur, 1985), relief from ungulate browsing (Kay and Bartos, 2000) and reducing succession to conifers (Bartos, 2001). Although these restoration methods are likely to promote positive short-term results, they largely ignore adaptive potential and resilience in the face of future environmental hardships.

Our current understanding of aspen regeneration ecology in the western United States is changing (Long and Mock, 2012) and this recent awareness may lead to improved conservation efforts in aspen dominated ecosystems. Historically, it has been assumed that western aspen stands consist of one or few clones, and conditions necessary for sexual regeneration are generally not present. Recent discoveries have challenged these assumptions, and aspen in the west have been found to be clonally diverse

(DeWoody et al., 2008, 2009; Mock et al., 2008) and undergoing sexual reproduction recent time (Elliott and Baker, 2004; Kay, 1993; Romme et al., 1997).

The discovery of more clonally diverse stands in the Mountain West raises questions about clonal establishment and the ecological implications of clonal diversity in aspen stands. Stands with a greater number of clones are expected to add to the phenotypic diversity and potential resilience to changing conditions. Identifying site conditions which lead to successful seedling establishment could inform management actions to create favorable conditions for seedling establishment and persistence.

In this thesis I characterized patterns of aspen clonal richness in southern Utah, and determined whether locations with significantly higher levels of clonal diversity also had a history of greater fire frequency. I then investigated how multiple clones interact in clonally diverse stands, asking whether some clones seem to be replacing others over relatively recent time. Finally, I investigated whether naturally-occurring aspen display a tradeoff between growth and defense chemistry, two contrasting strategies for dealing with herbivory.

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## CHAPTER 2

LINKING PATTERN AND PROCESS: ASPEN (*Populus tremuloides* Michx.)CLONAL DIVERSITY AND LANDSCAPE HISTORY <sup>1</sup>

## ABSTRACT

Recent studies suggest that clonal diversity in Western aspen (*Populus tremuloides*) is far greater than once presumed, raising questions about when and how clones establish and what clonal diversity may mean ecologically. In this study we found areas of significantly high and low levels of clonal diversity in aspen stands of southeastern Utah, a location near the geographic fringe of the species distribution; areas where stands are assumed to be dominated by large clones. Recent fire was more common in areas where we discovered greater clonal richness, and relatively few fires were reported in areas with lower clonal richness. In study plots where multiple clones existed we detected evidence of clonal boundary stability, although approximately one quarter of the clones showed evidence of encroaching into neighboring clones. Clonal boundary stability and clonal encroachment represent potentially contrasting and complex successional dynamics. Clonal boundary stability would prevent competitive loss of clonal richness in a stand, but may result in a loss of aspen coverage as clones succumb to stressful conditions. While clonal encroachment may result in a reduction of diversity over time, this phenomenon may allow more competitive clones to persist throughout a range of environmental challenges.

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<sup>1</sup> This chapter is co-authored by Richard S. Gardner and Karen E. Mock

## 1. Introduction

Recent studies have shown that aspen stands in the Intermountain West have substantially higher clonal diversity than previously assumed (DeWoody et al., 2008, 2009; Mock et al., 2008), a finding that could fundamentally change our understanding of aspen ecology (Long and Mock, 2012). Even in landscapes dominated by large clones (e.g., Fishlake National Forest in Central Utah, home of the enormous Pando Clone) patches of high clonal diversity exist (DeWoody et al., 2008; Mock et al., 2008). Clonal richness has inherent ecological and evolutionary value; stands with a greater number of clones are likely to contain a variety of phenotypes (Kanaga et al., 2008; Osier and Lindroth, 2006; Stevens et al., 2007), providing increased ecological amplitude and increased potential for adaptation to future environmental challenges. The spatial clustering of small clones (Mock et al., 2008) suggests that conditions sufficient for genet establishment and persistence vary across landscapes and may be occurring over a relatively recent time scale. Understanding the landscape processes underlying these patterns of genet richness in western landscapes could inform management practices directed toward aspen restoration and resiliency.

Widespread and successful genet establishment events have occurred in the past century in western landscapes (Elliott and Baker, 2004; Romme et al., 1997, 2005). As a dramatic example, the Yellowstone fires of 1988 led to successful establishment in some locations of thousands of seedlings per hectare (Kay, 1993), and although high levels of genet mortality occurred following the Yellowstone fires, some individuals remained vigorous and were greater than 2 meters tall 5 years after the fires (Romme et al., 2005). Aspen recruitment events appear to follow a pattern described in other clonal species

(Eriksson, 1992, 1993; Silvertown, 2008), with high levels of genet establishment, each with few ramets, followed by a decline in the number of genets over time, with an increase in ramets per genet.

We hypothesize that the configuration of genet (clonal) diversity within stands is the result of “windows of opportunity,” where a viable seed source, site preparation, and subsequent years of adequate moisture occurred simultaneously (Jelinski and Cheliak, 1992; Eriksson, 1993). While clonal age cannot yet be determined with precision and clonal size does not correlate with clonal age at a coarse temporal scale (Ally et al., 2010), seeding events necessarily result in small clones which expand at varying rates over time. Thus, patches of high clonal richness within a matrix of larger clones may be the result of seeding events in the past century. Alternatively, such a pattern may result from the long term persistence of clones that expand relatively slowly and unevenly, perhaps due to ecological conditions limiting the expansion of clones.

The first objective of this study was to determine whether patterns of clonal diversity correspond spatially with areas prone to fires, since fires often create conditions favorable for aspen seed germination (McDonough, 1979). Such a correlation would suggest that areas of high clonal richness are indicative of past seeding events following fires. Our second objective was to characterize clonal interactions within stands. Once clones become established, they are expected to compete for resources, resulting in the loss of some clones and the expansion of others. Thus, in the absence of stand-replacing disturbance, we expect clonal succession (more adapted clones replacing less adapted ones) to reduce clonal diversity over time and the rate of this succession would reflect the rate of loss of genetic diversity. These dynamics may be particularly important in

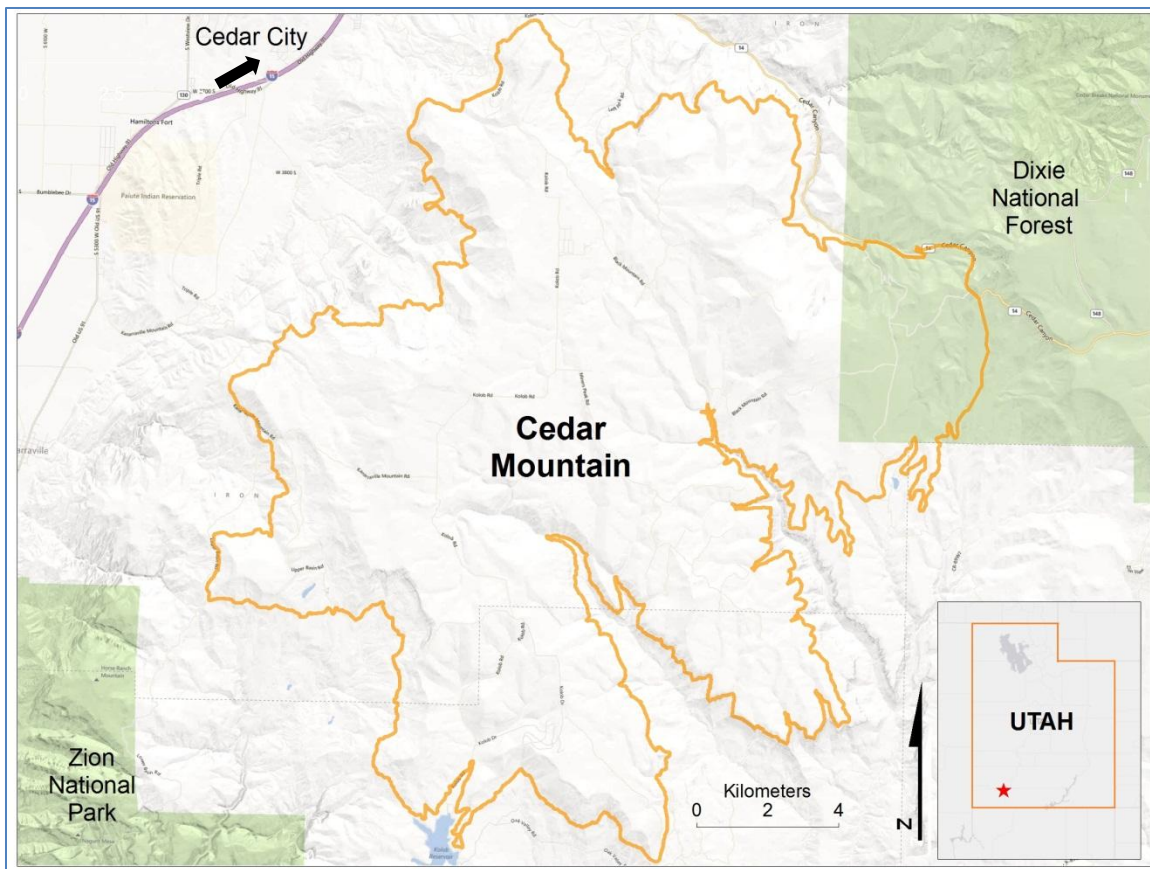
western landscapes, where clones can become very large. If clonal succession is rapid, we expect to see a change in the vertical composition of clonal diversity – i.e. the understory aspen may be a different clone than the overstory aspen as one clone advances and occupies the area of an adjacent clone. Alternatively, if clonal composition is rather stable over time, we expect understory clones to be the same clone as those in overstory positions.

## **2. Methods**

### *2.1. Study site description*

This study took place on Cedar Mountain, Utah (Fig. 2.1), located approximately 10 kilometers south-southeast of Cedar City, Utah, USA, in southern Iron and northern Washington counties (37° 38' 15'' N, 113° 01' 39'' W). The forest type across the plateau is mostly pure aspen, with minor components of mixed aspen and conifer (Douglas-fir (*Pseudotsuga menziesii*) at lower elevations, and true fir (*Abies concolor*) and Engelman spruce (*Picea engelmannii*) at upper elevations). With the exception of the extreme eastern portion of the plateau (Webster Flat, which is managed by the US Forest Service), Cedar Mountain is privately owned, and the plateau has a history of seasonal livestock grazing of sheep and cattle. Although livestock grazing exists throughout the study area, Webster Flat likely has policies different from the rest of the plateau, which may have an effect on vegetation dynamics. Fire occurrence on Cedar Mountain is relatively infrequent, but the eastern side has experienced the highest number of fire events over the last 20 years, based on fire history records obtained from the Wildland Fire Occurance Database (Brady, 2012). One substantial fire (The C Trail fire) occurred

on the northwest edge of Cedar Mountain in June 2002, burning approximately 320 acres and lasting for approximately 3 months.



**Fig. 2.1.** The Cedar Mountain study area.

## 2.2. Field methods

### 2.2.1. Clonal richness

Sample points were generated by projecting a 900 meter hexagonal grid over the Cedar Mountain study area. We preserved only those points that fell within aspen coverage according to Southwest Regional Gap Analysis data (Lowry et al., 2007), resulting in 134 points. Of the 134 points, 90 were randomly selected to represent our

area of inference, and after access was granted by landowners and ground-truthing each point for aspen coverage, a final 83 points remained.

One-hectare plots (100m x 100m) were centered at each of the 83 sample points across the Cedar Mountain Plateau. At each plot, 9 evenly spaced (50 meters apart) subsample locations were established in a 3x3 matrix. At each subsample location tissue samples (either leaf or cambium) were obtained from overstory and understory ramets within 10 meters of the center of each subsample location. Tissue samples were preserved in labeled paper coin envelopes and submerged in silica gel desiccant, and GPS coordinates were collected at each sample site using hand held GPS units (UTM coordinate system and NAD83 map datum).

### *2.2.2. Clonal Boundary Interactions*

From the 83 plots described above, 17 were sampled to investigate clonal boundary interactions. The 17 plots were selected on the basis of 1) having multiple size classes of ramets, 2) having at least two genotypes detected in the genetic analysis (see subsection 2.3. *Clonal ID*), and 3) attempting to distribute sample locations across the entire plateau at Cedar Mountain. Within each of the 17 plots, a square grid of 25 subplots (20 meters apart, each with a five meter radius) was established. The 20 meter spacing was influenced by the results of the Mock et al. (2008) study, where two size classes of ramets (overstory and understory) were sampled at 50 meter spacing; in this study we were interested in detecting clonal distributions at finer horizontal and vertical scales. At each subplot, leaf tissue was collected from ramets in each of three size classes (when present): an overstory ramet (>12.5cm dbh), a mid-story ramet (2.5-12.5cm dbh),



and an understory ramet (<2.5cm dbh). Leaf tissue samples were preserved in paper coin envelopes containing silica gel desiccant and taken to the lab for genotyping.

### *2.3. Analytical methods*

#### *2.3.1. Clonal identity methods*

DNA was extracted from leaf and cambium samples using a QIAGEN DNEasy 96 Plant Kit following the manufacturer's protocols. Six highly variable microsatellite loci were amplified in each sample following Mock et al. (2008): GCPM970, PMGC433, PTR14, PMGC2571, WPMS15, WPMS14. Microsatellite alleles were scored using the program Genemapper v4.0 (Applied Biosystems, Inc.). Individual samples differing by just one allele were considered the same genet. A probability of identity (PI) analysis was conducted on all diploid samples using the GenAIEx v6 (Peakall and Smouse, 2006) add-in for Microsoft Excel version 2010 to assess the power of these six loci to identify individuals. A PI analysis reports the probability of two randomly selected genets from a population having the same multi-locus genotype. This statistic takes into account the allele frequencies in the population and the number of loci investigated per individual. Low PI values (0.01 – 0.0001) indicate a high likelihood of accurate detection of individuals (Waits et al., 2001).

#### *2.3.2. Clonal Richness and Fire Occurrence*

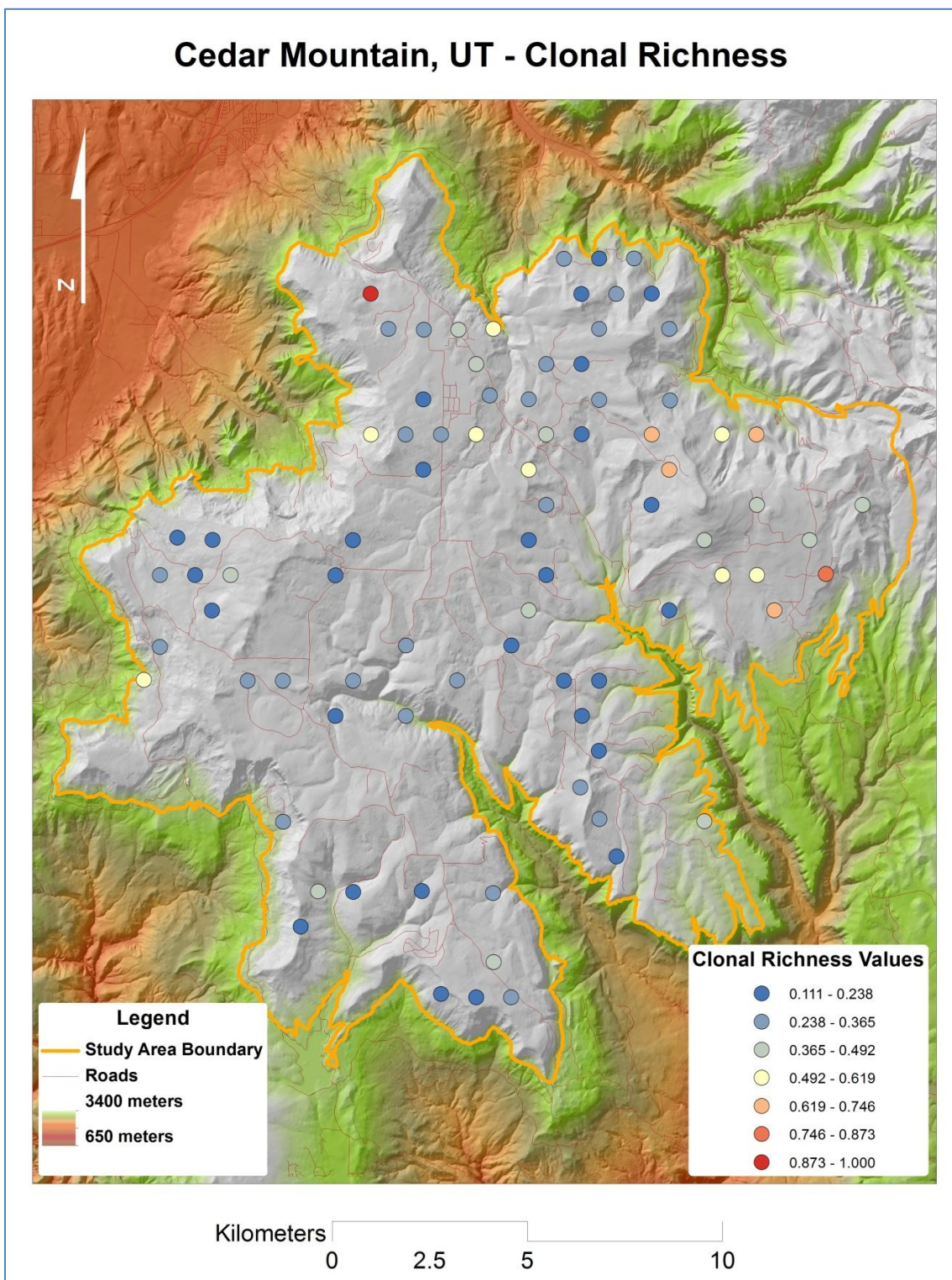
Clonal richness values were calculated for each of the 83 plots by dividing the number of individual clones by the total number of samples collected at each plot (maximum of 9 samples). Clonal richness values were then mapped (Fig. 2.2) using

ESRI ArcGIS Desktop v10. We used the ArcGIS Desktop Hot Spot Analysis, with the Getis-Ord  $G_i^*$  spatial statistic (Getis and Ord, 2010), to determine whether any significant ‘hot spots’ existed within the study area. The Moran’s Spatial Autocorrelation tool in ArcGIS Desktop v10 was used to determine the most appropriate distance to compare a given point to its neighbors in the Hot Spot Analysis (Fig 2.4). The Spatial Autocorrelation tool plots z-scores between given richness values over a range of distances; the highest z-score reported over a range of distances (2,400 meters for this analysis) can then be used for comparisons in the Hot Spot Analysis.

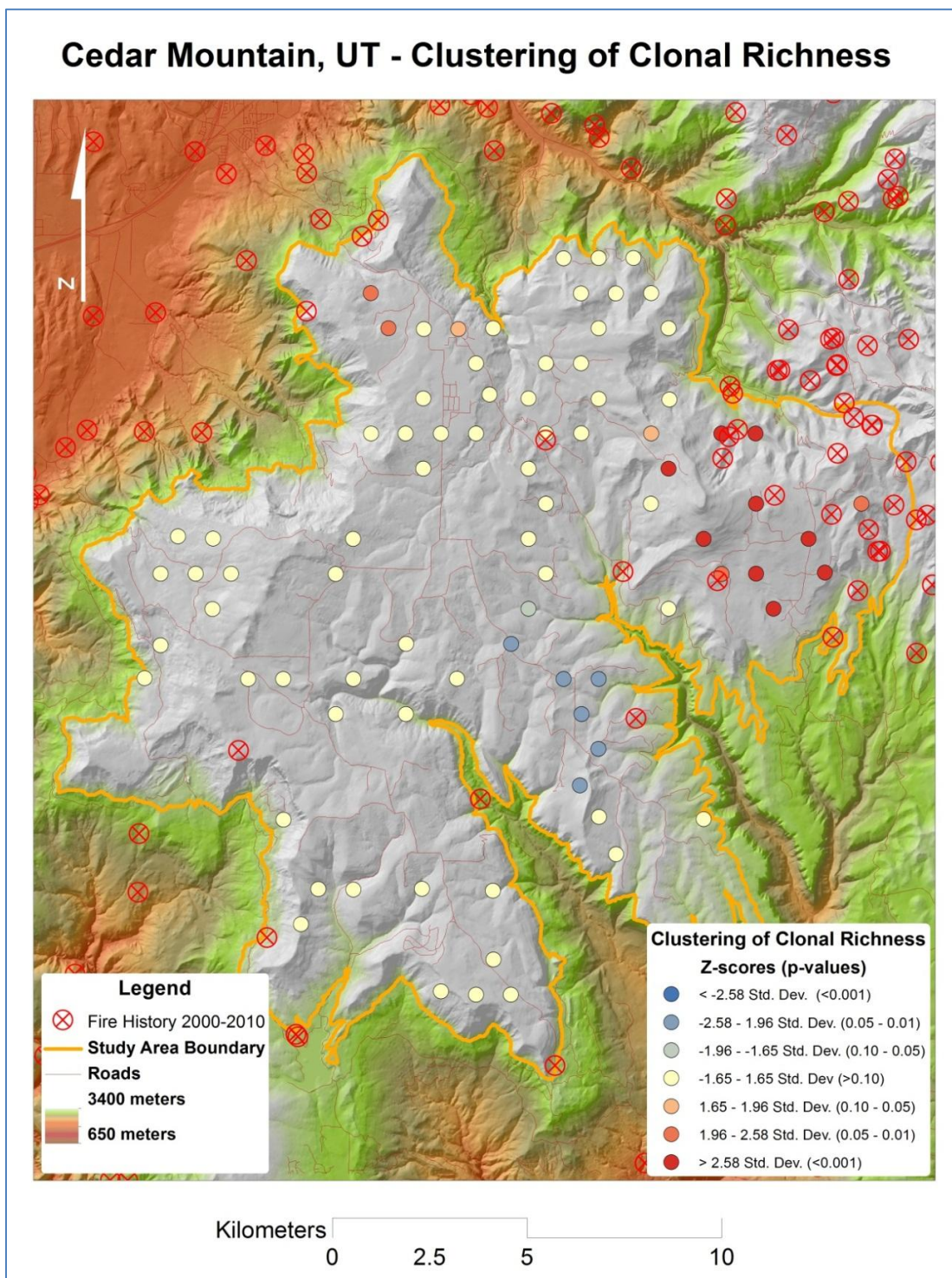
The significance values from the Hot Spot Analysis were plotted against fire frequency data obtained from the Wildland Fire Occurrence Database (Brady, 2012). The Wildland Fire Occurrence Database consists of point-specific data where fires occurred over a 20-year period. We made the assumption that fire frequency over the last 20 years could be used as an indicator of fire frequency over longer periods of time. Point fire data were used in this study (over perimeter fire data) due to the lack of perimeter data available for private lands.

### *2.3.3. Clonal Boundary Interactions*

Genotypes were mapped to their respective sample locations using ArcGIS Desktop v10; understory, mid-canopy, and overstory samples were assigned unique symbology within each subplot (Fig. 2.4, Fig. 2.5, and Appendix A). To summarize the patterns found in the clonal boundary plot maps, we created a set of rules based on the resolution of the sampling design and the genetic spatial patterns detected in the 17 plots



**Fig. 2.2.** Map of clonal richness for each of the 83 plots, showing uneven levels of aspen clonal richness across the study area, but generally low clonal diversity overall.



**Fig. 2.3.** Results of the Hot Spot Analysis of genetic richness values at Cedar Mountain, showing clustering of high levels of aspen clonal richness to the east and north, and clusters of low levels to the south and center. Fire frequency is higher in areas with clustering of plots with higher clonal diversity.

we studied for clonal interactions. These rules conservatively characterized the patterns of our data, as we excluded roughly half of the clones detected because they did not meet our criteria for having enough data. For clones in a plot to be considered ‘spreading’ into neighboring clones they met the following criteria: 1) they must be present in at least four subplots (in any size class), 2) they must have at least eight potential subplots to where detection of understory ‘spreading’ is possible (i.e. a different clone was present in the overstory in either the largest or mid-size class), and 3) they are found as ‘spreading’ in at least two sub-plots. A table summarizing the clonal distribution and evidence of clonal encroachment is summarized in Appendix B.

### **3. Results**

#### *3.1.1 Clonal Identification*

A total of 80 clones were detected across the study area. The PI analysis indicated that six microsatellite loci were sufficient to identify individual genets in this population with reasonable accuracy;  $P_{(ID)} = 6.5E-05$ .

#### *3.1.2 Clonal Richness*

There was pronounced heterogeneity across Cedar Mountain with respect to clonal richness (Fig. 2.2), with a clustering of significantly higher diversity in the eastern part of the plateau and a clustering of significantly lower diversity in the central and southern portions (Fig. 2.3). Table 1 lists the genetic richness and z-scores for all 83 plots on Cedar Mountain. The eastern portion of the plateau also had more frequent fires.

**Table 2.1**

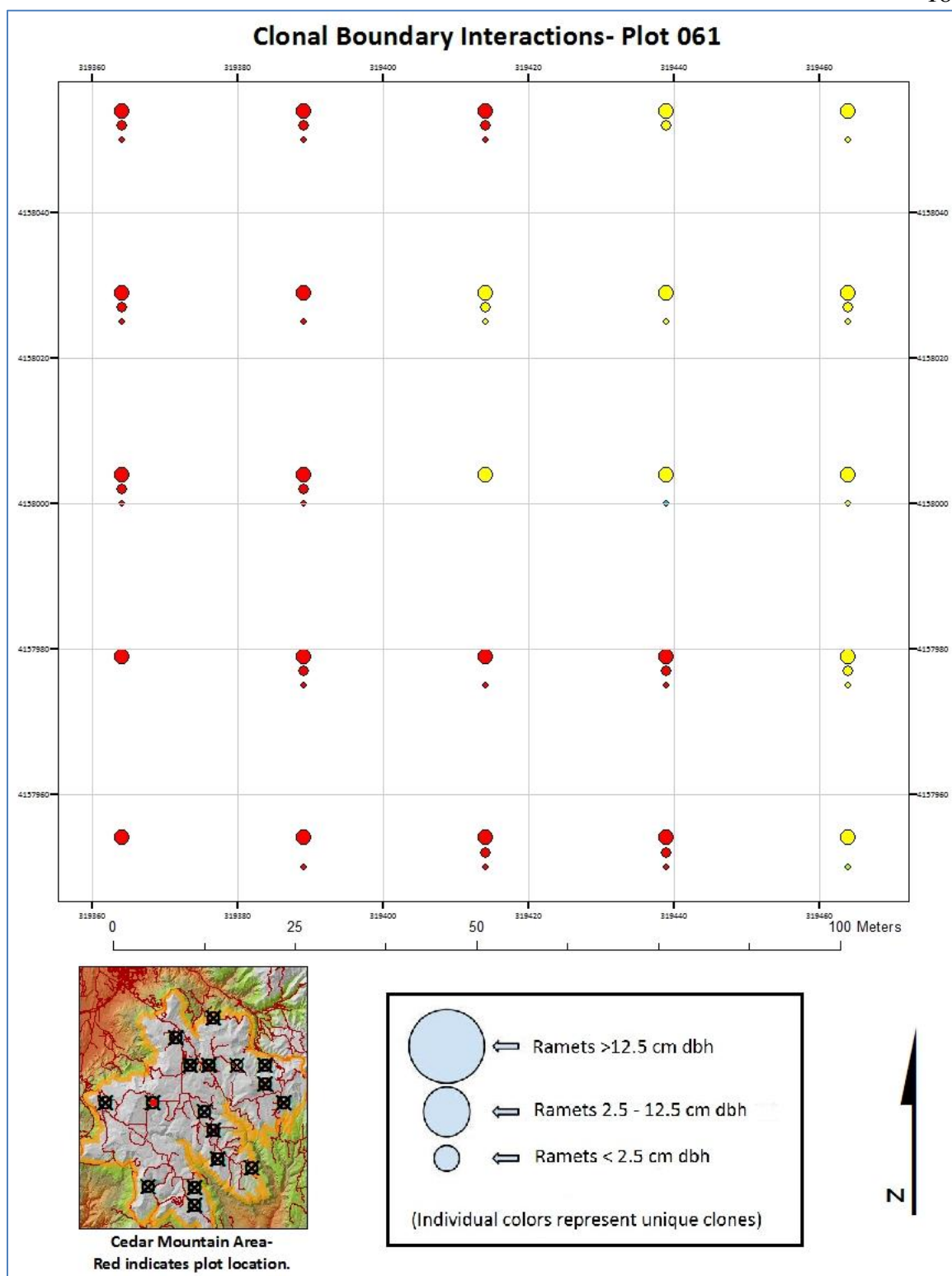
Clonal richness and z-scores for all 83 plots. Clonal richness scores are derived from the number of clones detected, divided by the number of ramets sampled per plot (maximum of nine). Z-score signs represent clustering of high (positive) and low (negative) clonal richness and *p*-values indicate significance levels for clustering.

Plot #	Clonal Richness			Plot #	Clonal Richness		
	Value	z-score	<i>p</i> -value		Value	z-score	<i>p</i> -value
001	0.222	-0.857	0.391	072	0.111	-0.789	0.430
002	0.111	-0.550	0.582	073	0.111	-0.455	0.649
003	0.333	-0.550	0.582	076	0.429	2.748	0.006
007	0.429	-0.866	0.386	078	0.429	3.323	0.001
008	0.111	-0.837	0.403	082	0.250	-0.153	0.878
011	0.400	-1.146	0.252	083	0.222	1.565	0.117
012	0.222	-1.146	0.252	084	0.444	4.491	0.001
013	0.167	-0.445	0.657	086	0.400	2.143	0.032
015	0.286	-0.693	0.488	088	0.111	0.164	0.870
016	0.111	-1.003	0.316	090	0.556	-0.519	0.604
018	0.250	-0.837	0.403	091	0.714	2.584	0.010
021	0.250	-1.381	0.167	093	0.556	-0.320	0.749
022	0.400	-0.643	0.521	094	0.333	0.262	0.793
023	0.333	-2.138	0.033	095	0.286	0.952	0.341
027	0.111	-2.138	0.033	096	0.556	0.778	0.437
028	0.125	-0.880	0.379	097	0.429	0.613	0.540
029	0.250	-0.688	0.491	098	0.143	1.414	0.157
030	0.125	-2.107	0.035	100	0.625	1.953	0.051
031	0.500	-0.007	0.994	101	0.600	3.186	0.001
033	0.250	-0.435	0.663	102	0.714	3.599	0.001
034	0.250	-1.067	0.286	105	0.111	0.697	0.486
036	0.333	-1.042	0.297	106	0.333	0.402	0.688
038	0.286	-0.525	0.599	107	0.286	0.605	0.545
039	0.222	-1.975	0.048	108	0.333	0.806	0.420
040	0.111	-2.012	0.044	110	0.333	1.549	0.121
041	0.286	-0.724	0.469	114	0.375	0.674	0.500
046	0.333	-1.231	0.218	115	0.333	0.605	0.545
048	0.125	-1.975	0.048	116	0.222	-0.226	0.821
049	0.222	-1.178	0.239	118	0.333	2.205	0.027
051	0.444	-1.851	0.064	119	0.333	1.624	0.104
053	0.111	0.746	0.456	120	0.444	1.859	0.063
056	0.625	2.722	0.006	121	0.500	0.095	0.924
057	0.333	-1.013	0.311	123	0.333	-1.071	0.284
058	0.125	-1.476	0.140	125	0.250	-0.512	0.609

059	0.375	-1.646	0.100	126	1.000	2.395	0.017
061	0.222	-0.565	0.572	128	0.111	-0.832	0.405
063	0.111	-0.911	0.362	129	0.286	-1.191	0.234
065	0.500	2.393	0.017	130	0.222	-1.268	0.205
066	0.571	2.722	0.006	131	0.250	-0.797	0.426
068	0.750	3.286	0.001	132	0.222	-1.356	0.175
069	0.111	-1.476	0.140	133	0.286	-1.228	0.220
070	0.111	-1.506	0.132				

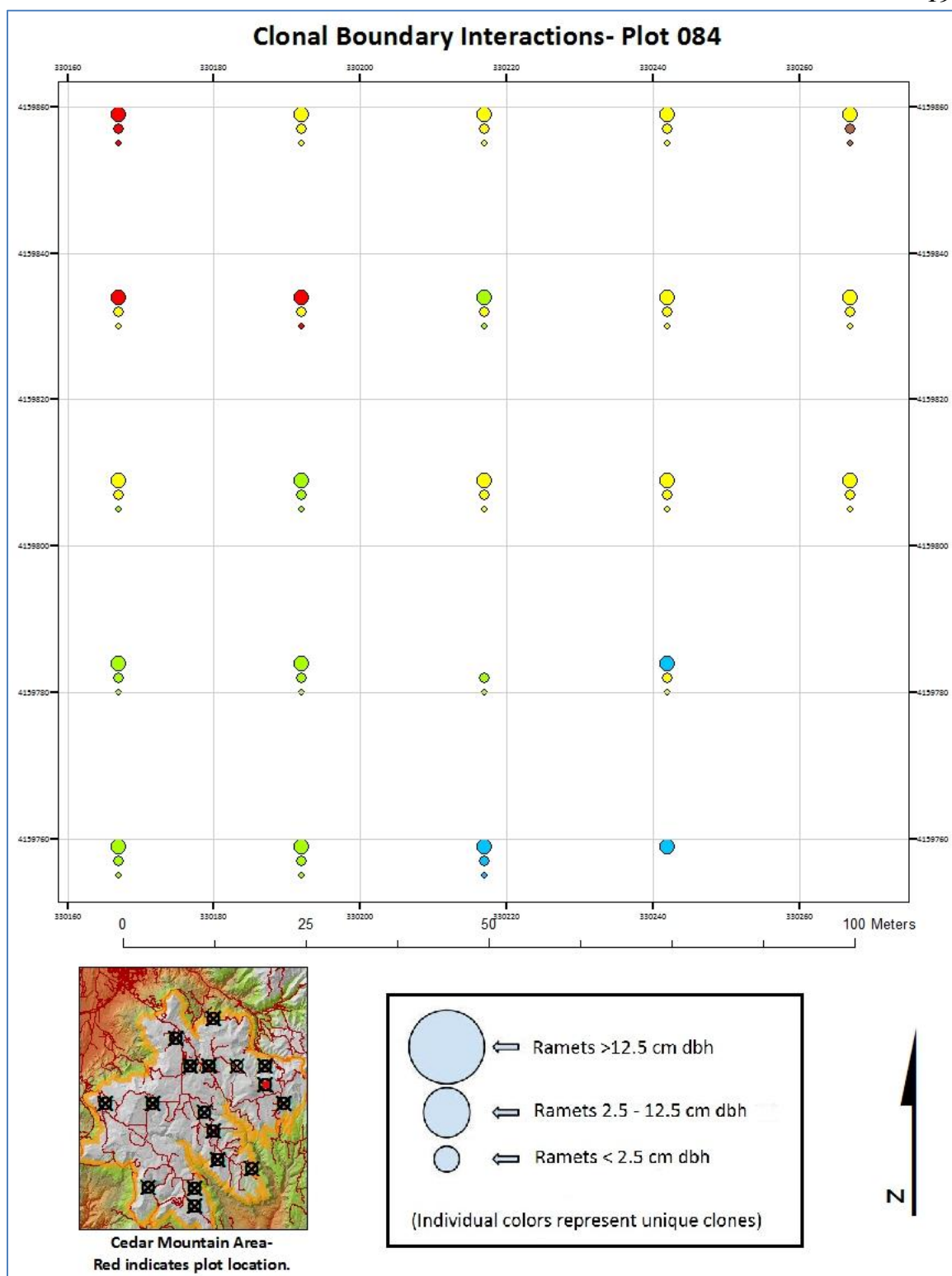
### 3.1.3 Clonal Boundary Interactions

Eighty individual clones were detected in the 17 plots investigated for clonal boundary interactions. Forty-two clones met our criteria required for assessment of clonal encroachment (i.e. present in at least four subplots), and 10 of the 42 clones (23.8%) displayed a pattern of clonal encroachment. Clones meeting the criteria for clonal encroachment occurred in nine of the 17 plots and one plot contained two clones classified as encroaching. Figures 4 and 5 illustrate examples of clonal boundary stability (Plot 61, Fig. 2.4) and clonal encroachment (Plot 84, Fig. 2.5). Clone-specific maps of all 17 plots are presented in Appendix A.



**Fig. 2.4.** Example of a subplot showing clonal boundary stability. At nearly all subplots, the same genotype is represented in all three size classes.





**Fig. 2.5.** Example of a subplot suggesting clonal replacement. The yellow clone exists in the understory of four adjacent clones.

#### 4. Discussion

Current understanding of western aspen ecology, and subsequently its appropriate management, is evolving (Long and Mock, 2012). Discoveries such as aspen invasion of conifer stands (Elliott and Baker, 2004), high levels of clonal diversity within stands (Mock et al., 2008; DeWoody et al., 2009), and evidence of recent sexual reproduction (Kay, 1993; Romme et al., 1997, 2005) are challenging and expanding current management paradigms. With these paradigm shifts in mind, we sought to describe and interpret patterns of clonal diversity in a large study area (Cedar Mountain) in southern Utah.

Our first research objective was to describe patterns of clonal diversity across the 27,500 hectare study area, and to look for potential spatial relationships between diversity and fire history. We hypothesized that if areas of high clonal diversity and small clonal size of western aspen (observed in previous studies by Dewoody and Mock (2008, 2009)) were indicative of recent episodes of aspen seedling recruitment, then such areas would be spatially correlated with more frequent fire occurrence, because fire positively influences seedling establishment and recruitment. Although there are likely other factors affecting clonal establishment over time (i.e. presence of conifers and grazing regimes), fire is likely and capable of creating the conditions necessary for seedling establishment, is tractable over time, and relatively frequent in our study area. With the lack of fire severity information over time, this study simply considers spatial relationships between fire and clonal diversity and is not intended to be corollary or causal.

We detected clusters of both high and low levels of clonal diversity across Cedar Mountain, and high levels of clonal diversity corresponded well to areas with recent fires. We suggest that when a site burns periodically, there are more frequent “windows of opportunity” for seedling establishment, as described by Eriksson (1993), and Jelinski and Cheliak (1992). The complex post-fire structure of living and dead remnant trees (of multiple species) may also enhance opportunities for new clones to establish by providing shelter from herbivores and soil desiccation.

Our second research objective was to determine whether we could detect evidence of rapid clonal succession at clonal boundaries in the Cedar Mountain study area. We hypothesized that if some clones were replacing others relatively quickly, we would detect clone-specific differences in age classes at sub-plots within one hectare plots across Cedar Mountain. According to this scenario, we would expect to see expanding clones represented as small ramets under larger remnant (older) ramets of static clones. Alternatively, if clonal succession is occurring relatively slowly, we would expect that boundaries between clones would be represented across all ramet size classes (i.e. clones would occupy a vertical distribution at all subplots). These scenarios are generally consistent with the “guerrilla” vs. “phalanx” patterns described by Cheplik (1997) and Namroud et al. (2005), with the added consideration of ramet age classes.

Overall, 23.8% clones showed evidence of encroachment into neighboring clones. These encroaching clones were dispersed across the study area and (with the exception of one plot with two encroaching clones) were observed as independent occurrences in eight of the study plots. Stable clonal boundaries can be seen as both a benefit and potentially detrimental in the context of succession and stand resilience. Clonal boundary stability

may be due to local adaptation and competitive evenness among genets. Clonal succession, by contrast, is expected to lead to loss of genetic diversity over time in the absence of sexual reproduction, and loss of genetic diversity can reduce stand resilience in the face of changing environmental conditions. Our findings suggest that clonal boundaries are generally stable on Cedar Mountain, but that a small but important proportion of clones may be expanding rapidly into adjacent clones. These results do not suggest that genetic diversity is rapidly declining on Cedar Mountain due to clonal succession. However, studies at finer scales assessing clonal boundary changes over time may provide more definitive evidence of the rate of these clonal dynamics over time.

## **5. Conclusions and Implications**

We found stands with higher clonal richness and small clonal size (more typical of aspen in eastern landscapes, as described by Kemperman and Barnes (1976)) in areas that burned more frequently. In contrast, locations with low clonal diversity and larger clonal size were located in areas without frequent fires. This finding is consistent with the interpretation that clusters of high clonal richness are signatures of seedling recruitment (e.g. Mock et al., 2008). Our findings also suggest that most clones have stable boundaries, although 24% of the clones appeared to be encroaching into adjacent clones.

Understanding the landscape and biological processes influencing aspen clonal diversity and interactions could provide valuable information for land managers. Formulating management actions which favor clone establishment over time could result in higher clonal richness of aspen stands. Presuming that clonal diversity is important to

resilience of aspen forests in light of changing environmental conditions, we suggest that an effort be made to identify and protect seeding events following fires.

Management actions such as prescribed fire can help create and maintain “windows of opportunity” for successful seedling establishment. Aspen generally produce copious amounts of viable seed (McDonough, 1979) that are wind dispersed. Preparing bare mineral soil conditions in areas that are likely to support seedling establishment (Kay, 1993) could increase the chances of seedlings successfully establishing, especially if protected from herbivory.

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## CHAPTER 3

GROWTH-DEFENSE TRADEOFFS IN POPULATIONS OF ASPEN (*Populus tremuloides* Michx.) IN THE INTERIOR WESTERN UNITED STATES <sup>1</sup>

**Abstract-** Ecological tradeoffs in aspen have been studied for decades, but a majority of these studies have been performed in either common gardens or greenhouses, and have involved young genets. In this study we assessed 18 mature aspen genets in wild populations of northern Utah to determine whether tradeoffs between resistance and tolerance were ameliorated under field conditions. The eighteen genets were blocked by cytotype, which allowed us to partition the effects of diploid vs. triploid clones. We found evidence of a tradeoff between radial stem growth and foliar dry weight of current year foliar phenolic glycosides, and a (marginally) significant positive relationship between radial stem growth and the foliar dry weight of condensed tannins. We also found evidence of diploid aspen having lower basal area increment (BAI) than triploids as they aged. In addition to investigating ecological tradeoffs, our results support previous greenhouse and common garden studies where individual aspen clones displayed significantly different levels of growth, foliar condensed tannins, and foliar phenolic glycosides. There was also evidence that triploid aspen produce significantly more phenolic glycosides than diploids, a finding that may be regionally important as high levels of triploidy have recently been reported in western aspen.

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<sup>1</sup> This chapter is co-authored by Richard S. Gardner, R. Justin DeRose, Richard L. Lindroth, and Karen E. Mock

## INTRODUCTION

A major driving force in the persistence of plants is balancing the allocation of available resources between growth and the production of chemical or physical defenses (Herms and Mattson, 1992). Regrowth following herbivory (tolerance) and production of defensive compounds or structures (resistance) are two common strategies used by plants for coping with herbivory. While each of these strategies can be effective, one may come at a direct carbon cost to the other, resulting in negative correlations (tradeoffs) between growth and defense (Kozlowski and Pallardy, 1996). Attempts to explain the tendency of particular species, populations, or individuals to invest in growth vs. defense have invoked evolutionary histories of both plants and herbivores (Rhoades, 1979; Crawley, 1983; Rausher, 1992) as well as plant resource availability (Bryant et al., 1983; Coley et al., 1985) with the most likely explanations involving elements of both (Hamilton et al., 2001).

Plants resist herbivory by producing physical and chemical barriers. The more obvious physical defenses such as trichomes, spines, and thorns reduce the amount of herbivory by increasing the handling time of plant tissues or obstructing access altogether. Less obvious are the chemical defenses (plant secondary metabolites or PSMs), which can reduce palatability and digestibility, and/or limit herbivore fitness and health (Rhoades, 1985; Rausher, 1992). The production and sequestration of PSMs have been directly linked to herbivore resistance (Bryant et al., 1987; Kozlowski, 1992), but the production of these compounds draws from the same carbon pool required for physical growth and primary metabolism of woody plants. Therefore, when carbon is

limiting, a broad tradeoff exists between growth and defense, and potentially among various defense compounds (Kozlowski and Pallardy, 1996; Koricheva et al., 2004).

The cost of plant defenses has been an active area of ecological research for decades. Previous investigations assessing resistance and tolerance tradeoffs in various plant species, manifest through correlations between growth and defense compounds, have had mixed results, with inconsistencies likely due to species differences (Han and Lincoln, 1994; Adler et al., 1995; Leimu and Koricheva, 2006) and methodological differences among studies (Hwang and Lindroth, 1997; Stevens et al., 2007). Nearly all of these studies have been conducted in common garden settings, which have allowed researchers to partition genetic and environmental influences on the production of defense compounds and growth, and have provided valuable insights into growth-defense tradeoffs (Han and Lincoln, 1994; Adler et al., 1995; Hwang and Lindroth, 1997; Stevens et al., 2007). However, the controlled setting of a common garden, particularly greenhouse studies, may limit inferences to natural settings, where environments are more variable over time and multiple interacting stressors may alter ecological relationships.

Ecological tradeoffs between growth and defense have both theoretical and practical importance in understanding how plants and animals coexist over time, yet empirical knowledge about tradeoffs in natural populations is limited (Leimu and Koricheva, 2006). A more thorough understanding of growth-defense tradeoffs could be particularly important on landscapes where dominant and/or foundation species are predicted to experience significant changes in distribution. One such species is quaking aspen (*Populus tremuloides*), a member of the Salicaceae family. Quaking aspen is an

ecologically and economically valuable forest species which covers large areas of the North American continent and is predicted to undergo large scale distributional shifts and a reduction in distribution in the coming decades (Rehfeldt et al., 2009).

Aspen in the Western U.S. is a tractable species for assessing ecological tradeoffs in natural environments because of its tendency to form large clones and the existence of pronounced heritable phenotypic differences among clones (Stevens et al., 2007; Donaldson and Lindroth, 2007; Kanaga et al., 2008). Clonal trait variation may even influence soil chemistry, nutrient cycling, and microbial communities (Madritch et al., 2009), and has been shown to alter broader community traits (Whitham et al., 2003). Mock et al. (2008) note that larger clones in the Interior West tend to be triploid, a discovery that may have considerable ecological impacts because polyploid plants often show more robust vegetative growth, potentially altering growth-defense dynamics. These characteristics provide an opportunity to study extended patterns of tolerance and resistance tradeoffs between clones, as well as between cytotypes (diploid vs. triploid).

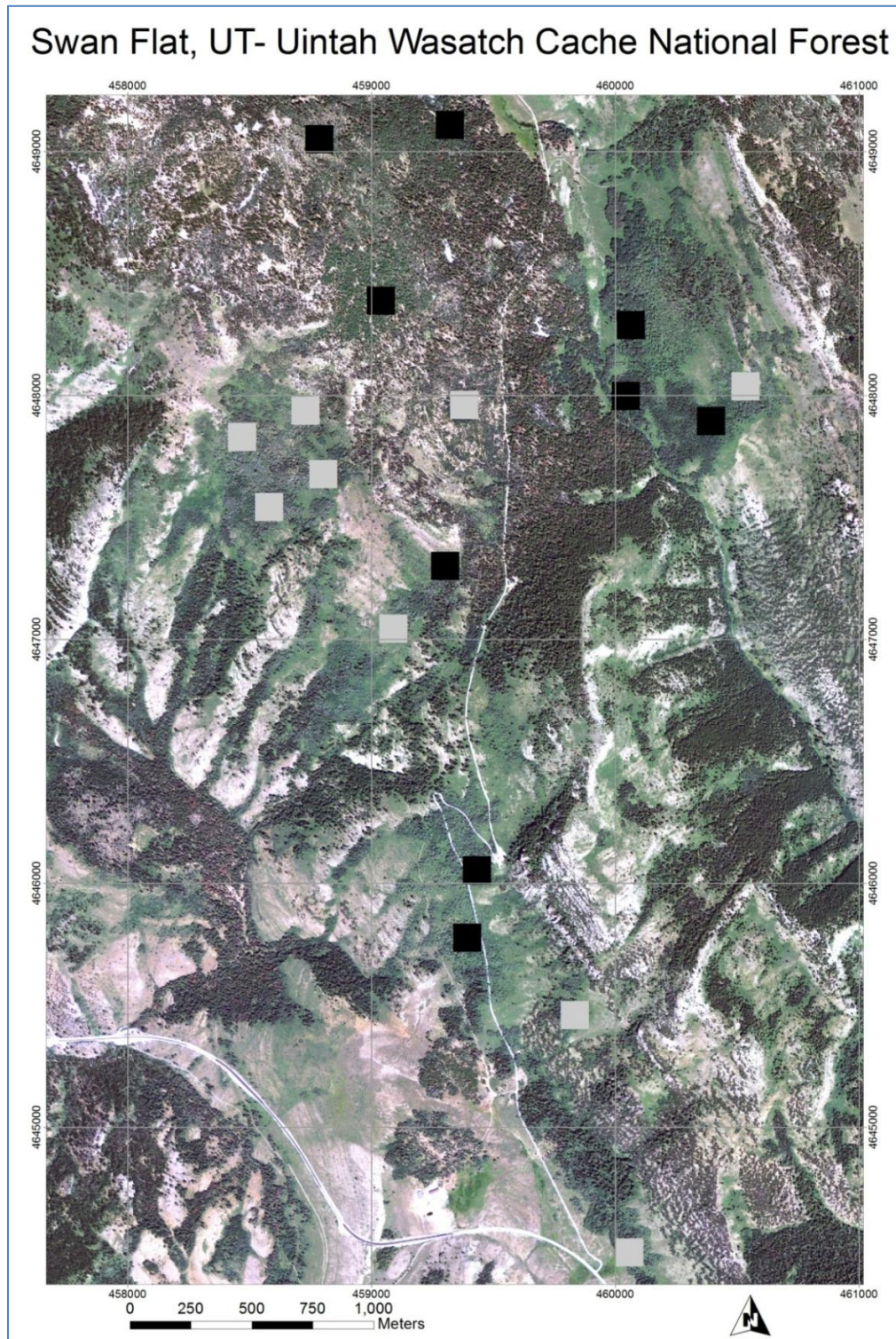
Defense chemicals of the Salicaceae family have been studied for over 100 years (Boeckler et al., 2011). Pharmaceutical benefits were the original motivation for exploring these secondary compounds, but more recently the focus has shifted toward their ecological importance. Phenolic glycosides (PGs) and condensed tannins (CTs) have been identified as being particularly effective at resisting insect herbivory (Tahvanainen et al., 1985) and are the only secondary metabolites found in any appreciable amounts in plants of the Salicaceae family (Palo, 1984). Some studies have shown that specialist consumers can increase herbivory of plants with higher concentrations of phenolics via detoxification or sequestration, but phenolics are still

regarded as being highly effective at deterring generalist herbivores (Rhoades, 1985; Boeckler et al., 2011). Common garden studies on aspen have shown that PGs are particularly effective at deterring herbivory from both insects and mammals (Boeckler et al., 2011). Similar studies have also shown that CTs are less effective at deterring herbivory, but may function to deter fungal (Bailey et al., 2005) pathogens or even prevent UV damage (Close and McArthur, 2002). These compounds can comprise a significant proportion of leaf dry weight, often up in the range of 30%.

In native aspen populations we sought to determine 1) whether there was evidence of a tradeoff between growth and chemical defense and 2) whether levels of growth and defensive chemistry varied between genotypes or cytotypes. This assessment of tradeoffs in naturally occurring aspen populations provides an important complement to common garden studies, as the complexity of field conditions may dampen or exacerbate the ecological responses and tradeoffs observed in more controlled settings.

## METHODS AND MATERIALS

*Study Site.* Our study was conducted at Swan Flat in northern Utah, located within the Uintah/Wasatch/Cache National Forest (41°58'05"N 111°29'21"W). This site consists of both pure aspen and mixed aspen-conifer (*Abies*, *Pinus*, and *Pseudotsuga*). The average elevation of the site is 2,400 meters and aspen exists on all aspects. This site was chosen because it is typical of the semi-arid Intermountain West aspen and because of the availability of previous aspen genotypic data from Mock et al. (2008).



**Fig. 3.1** Map of the Swan Flat study site in northern Utah. Gray squares represent plots in diploid clones ( $n=9$ ), while black represent plots in triploid clones ( $n=9$ ). Within each of these plots, 10 ramets were originally sampled ( $n=180$ ).

*Study Design.* In order to detect clonal influences on tradeoffs, we established plots that contained ramets from just one clone. Previous genetic work by Mock et al. (2008) had delineated clonal boundaries of aspen at Swan Flat at a 50m grid scale, revealing areas of small and large clones. We established 18 50m x 50m plots (2,500 m<sup>2</sup>) in spring 2008 within areas where large clones were known to be present. Of the 18 plots established, nine were within diploid clones and the other nine plots were within triploid clones. Within each of the 18 plots, 10 ramets were selected randomly, but with an effort to sample throughout the entire 2,500 m<sup>2</sup> area within each of the 18 plots (n=180). Ramets measuring 10-15 cm in diameter, measured 1.4m above the soil surface, were selected to account for ontological shifts in phytochemistry (Donaldson et al., 2006) and growth. Each ramet was permanently marked with a nail and tag for future measurements and sampling.

*Clonal Confirmation Sampling and Analysis.* We used microsatellite genotyping to assure that all ramets within each plot were members of the same clone. Leaf tissue was collected from each ramet at the time of plot establishment and DNA was extracted using Qiagen's DNeasy 96 Plant Kit<sup>®</sup>. For each of the 180 samples, microsatellite analyses were performed on five highly variable nuclear microsatellite loci (PMGC2571, GCPM970, WPMS14, PMGC576, and WPMS20) following protocols for extraction and amplification described in Mock et al. (2008). The microsatellite data were scored using the program GeneMapper v4.0. Cytotype (diploid vs. triploid) was established by observing three alleles at one or more microsatellite loci and cytotpe was confirmed by the flow-cytometry method described in Mock et al. (2012). Three of the 180 ramets

were not of the expected clone (based on microsatellite genotyping) and were excluded from the remainder of the study.

*Phytochemistry Sampling and Analysis.* Leaf samples were collected in summer 2008 from a random subset of the original 180 ramets (n=98, between 5 and 7 ramets per clone) to determine concentrations of defense compounds. Phytochemical sampling required a representative sample of about 20 leaves per ramet, which were collected throughout each canopy, to account for any variation in phytochemistry within the canopies. All leaves from a particular ramet were placed into one paper envelope and stored in silica gel desiccant. Once dry, leaves were ground using a Wiley Mill with a size 40 mesh. The PGs salicortin and tremulacin were quantified using thin-layer chromatography methods described by Lindroth et al. (1993), using purified aspen PGs as standards. CTs were analyzed using the acid butanol method (Porter et al., 1985), using purified CTs from aspen as a standard.

*Dendrochronology Sampling and Analysis.* In fall 2008, increment cores were collected from 95 of the 98 ramets which had been sampled for phytochemistry and clonal ID; three of the ramets were too rotted to obtain cores. Tree cores were obtained using an increment borer at 1.4 m above the soil surface. Each core was prepared and analyzed using standard dendrochronological methods (Stokes and Smiley, 1968). Increment cores were glued to mounting blocks with vessels oriented upward and sanded with progressively finer grades of sandpaper until annual rings were apparent. Age of ramets and ring widths were measured using a binocular scope; conservative age estimates were made for six samples in which the borer missed the pith and had no arc for estimating ages. Hidden and missing rings were revealed using cross-dating



techniques (Holmes, 1983) and confirmed by shadowing direct light on the sample for detection of latewood vessels (DeRose and Gardner, 2010).

Although the increment cores obtained from each ramet provided us with a record of annual radial growth going back sometimes 100 years or more, the last ten years (1999 to 2008) of basal area increment was used as the growth metric, to facilitate closer correspondence with the sampling season of phytochemistry. Analyzing the cumulative growth over the last ten years also smoothed out annual variability of growth.

## STATISTICAL ANALYSES

*Clonal Confirmation Sampling and Analysis.* A probability of identity (PI) analysis was performed on the scored microsatellite data using the GenA1Ex v6.1 add-in for Microsoft Excel (Peakall and Smouse, 2006). A PI analysis reports the likelihood of two unrelated individuals, selected randomly from the same population, of having the same multilocus genotype. Because PI analysis requires population allele frequency estimation based on individual genotypes, and individual allelic composition cannot be determined for triploid individuals using microsatellite analysis, only diploid individuals were used in calculating the PI.

*Resistance / Tolerance Tradeoffs.* A random coefficient model was used to assess the effects of the predictor variables on growth (n=97). This model was appropriate given the nested design structure (ramets within clones) and fixed effect predictor variables, measured on both categorical and continuous scales. Ramets were blocked by clone as a random variable, since clones sampled represented a random sample from the population at Swan Flat, UT. The independent variables cytotype (categorical: diploid

and triploid), % dry wt. of PGs (continuous), % dry wt. of CTs (continuous), and age of ramet (continuous) were used to predict the last 10 years of growth. All predictor variables for growth were centered with means equal to zero to standardize intercepts in the model. The variables age and CTs were transformed using natural log to linearize their relationships with average growth and to reduce leverage of individual observations. The CORR procedure in SAS v9.2 (SAS Institute, 2004) was used to test for multicollinearity between predictor variables. Two-way interactions among predictor variables were explored by testing for improved fitting of the model. The only interaction which improved the model fit and remained in the final model was cytotype by age, meaning that cytotype explained more of the variability in growth as ramets aged. There was no support of random slopes by clone for any continuous scale predictor; hence we report results for a fixed slope, random intercept model grouped by cytotype. The growth variable was calculated as the area of the last ten years of radial growth, centered with means equal to zero to standardized intercepts, and was transformed using natural log to meet assumptions of normality of residuals and homogeneity of variance. It was necessary to remove one outlier because its growth was an order of magnitude less (likely due to a chronic/non-lethal fungal pathogen, observed by very slight incremental growth within dark discoloration of the core) than the average and proved to be a leverage point. Data computations were generated using the GLIMMIX procedure in SAS/STAT software, Version 9.2 in the SAS System for Windows (SAS Institute, 2004).

*Growth and Chemical Defense Differences by Cytotype.* We used the TTEST procedure in SAS v9.2 (SAS Institute, 2004) to determine differences by cytotype in growth, PGs, and CTs. Since annual growth was negatively correlated with age ( $r = -$

0.68), it was necessary to perform a data transformation to account for this relationship; multiplying growth by the respective age of each sample nearly eliminated the influence of age on growth ( $r = 0.05$ ) while maintaining the original relationships between predictor and response variables. The natural log of age was used to meet assumptions for normality of residuals for the t-test.

*Mean Differences in Growth and Defense Between Genotypes.* Three individual single-factor analyses of variance (ANOVAs) were conducted to determine if there were genotypic differences in PGs, CTs. As described in the t-test methods, the age-transformed growth was used to account for the effect age had on growth. The ANOVAs were conducted in SAS v9.2 using the GLIMMIX procedure (SAS Institute, 2004). To meet assumptions for normality of residuals, natural log transformations were used on the variables CTs and growth.

## RESULTS

*Resistance / Tolerance Tradeoffs.* Results of the random coefficient model are reported in **Table 3.1**. Our results provide evidence for a tradeoff between growth and PGs (correlation estimate of  $-0.028$ ;  $p=0.015$ ) and an opposite and less significant relationship between CTs and growth (correlation estimate  $0.093$ ;  $p=0.096$ ). Model fit increased when ramets were blocked by clone (**Table 3.2**), indicating that clonal identity helps explain the variation in growth. Age was a significant predictor of growth, where older ramets grew less than younger ramets (correlation estimate  $0.378$ ;  $p<0.001$ ). The only significant interaction between predictor variables was age by cytotype, meaning that older diploids grew more slowly than triploids as age increased (correlation estimate

**Table 3.1** Results of the random coefficient model, investigating tradeoffs between defense chemistry (independent) and growth (dependent).

	Parameter Estimate	Standard Error	<i>T</i> - <i>statistic</i>	<i>df</i>	<i>P</i> - <i>value</i>
Intercept	3.133	0.063	49.53	16	<0.01
Diploid	-0.035	0.098	-0.35	16	0.726
Phenolic Glycosides	-0.028	0.011	-2.50	72	0.015
Condensed Tannins (ln)	0.093	0.055	1.69	72	0.096
Age of Ramets (ln)	-0.378	0.119	-3.85	72	<0.01
Age of Ramets by Diploid Interaction (ln)	-0.379	0.170	-2.23	72	0.029

-0.379;  $p=0.029$ ), but cytotype alone was not a good predictor of growth (correlation estimate -0.035;  $p=0.726$ ).

*Growth and Defense Differences by Cytotype.* Results for the T-tests are presented in **Table 3.3** and **Fig. 3.2**. These tests revealed no significant differences in growth between the means for diploid and triploid samples ( $t=1.25$ ;  $p = 0.447$ ). This result agrees with the result of the random coefficient model where cytotype was not a significant predictor of growth in the tradeoff model, but in the random coefficient model cytotype by age was significant. Similarly there was no significant difference between the mean concentrations of CTs ( $t= -1.36$ ;  $p = 0.177$ ) in diploid vs. triploid clones. There was, however, a significant difference between the means for concentrations of PGs by cytotype ( $t= -4.28$ ;  $P = <0.001$ ), where triploid clones had significantly higher concentrations of PGs than diploid clones.

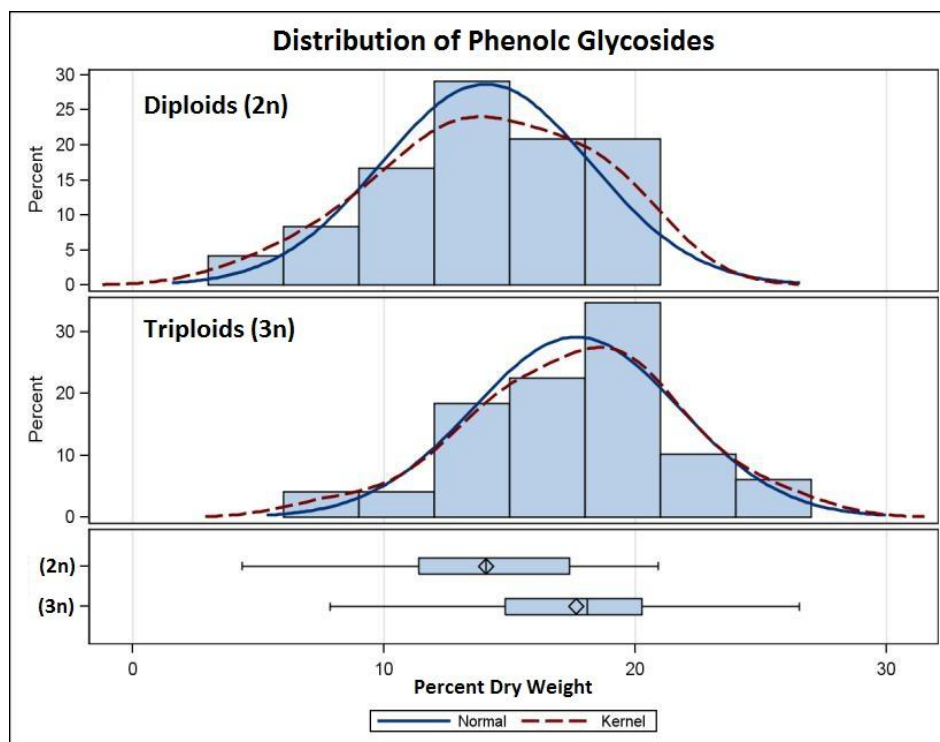
**Table 3.2** Model fit statistics for the random coefficient model (smaller values indicate a more appropriate model). Including genotype in the model improves the model for three of the fit statistics, suggesting genotype helps explain the variance in this growth model.

	BIC	CAIC	HQIC
With Genotype included as random effect	118.12	125.12	112.74
Without genotype included as random effect	129.69	136.69	119.07

**Table 3.3** Results of the T-tests between cytotypes (diploid vs. triploid).

	Degrees of Freedom	Equality of Variances	T-statistic	P-value
Growth of Ramets (ln)	92	Yes (P=0.447)	1.25	0.4474
Phenolic Glycosides	95	Yes (P=0.921)	-4.28	<0.001
Condensed Tannins (ln)	86	No (P=0.037)	-1.36	0.177

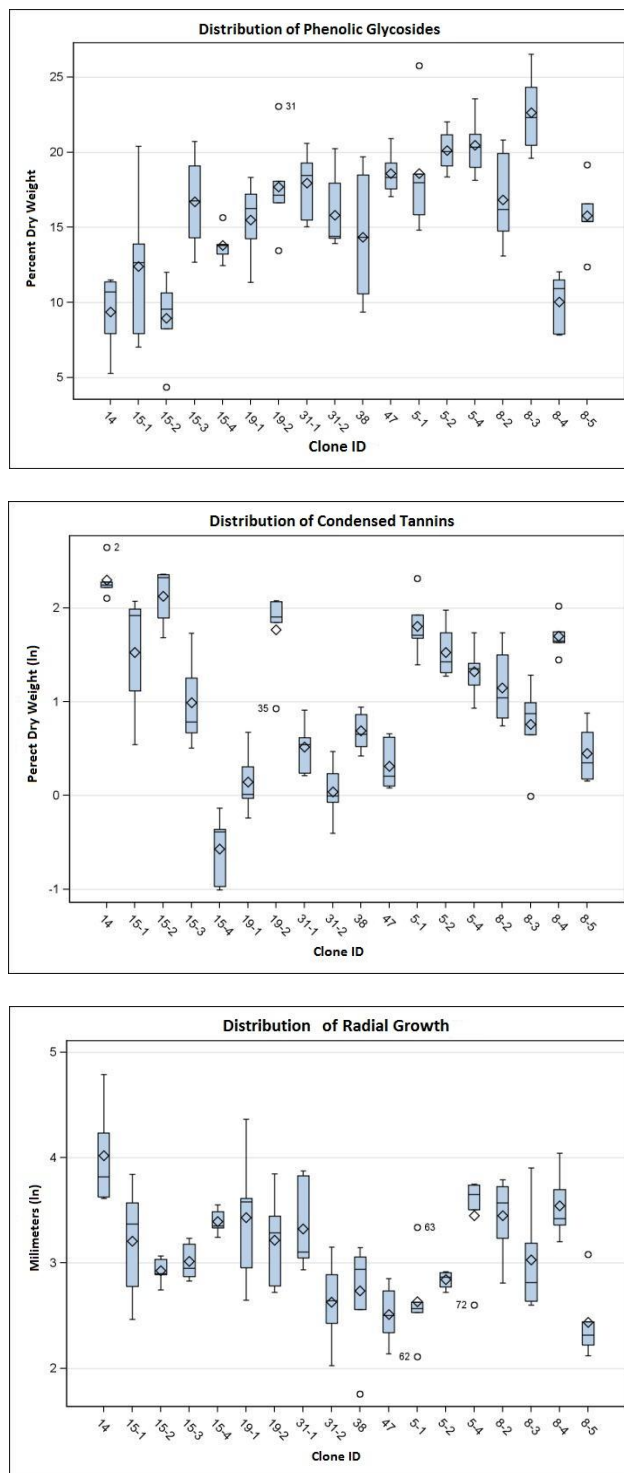
*Growth and Defense Differences Between Genotypes.* Results for the ANOVA procedures are in **Table 3.4 and Fig. 3.3**. Mean levels of growth ( $f=5.46$ ;  $p<0.001$ ), PGs ( $f=8.66$ ;  $p<0.001$ ), and CTs ( $f=25.46$ ;  $p<0.001$ ) were significantly different among the 18 aspen genotypes in this study.



**Fig. 3.2** Results of T-test of phenolic glycosides by cytotype. This T-test compared the means of the percent dry weight of foliar phenolic glycosides between diploid (2n) and triploid (3n) ramets.

**Table 3.4** Results of ANOVA tests between clones.

	Degrees of Freedom	F-statistic	P-value
Growth of Ramets (ln)	17	5.46	<0.001
Phenolic Glycosides	17	8.66	<0.001
Condensed Tannins (ln)	17	25.46	<0.001



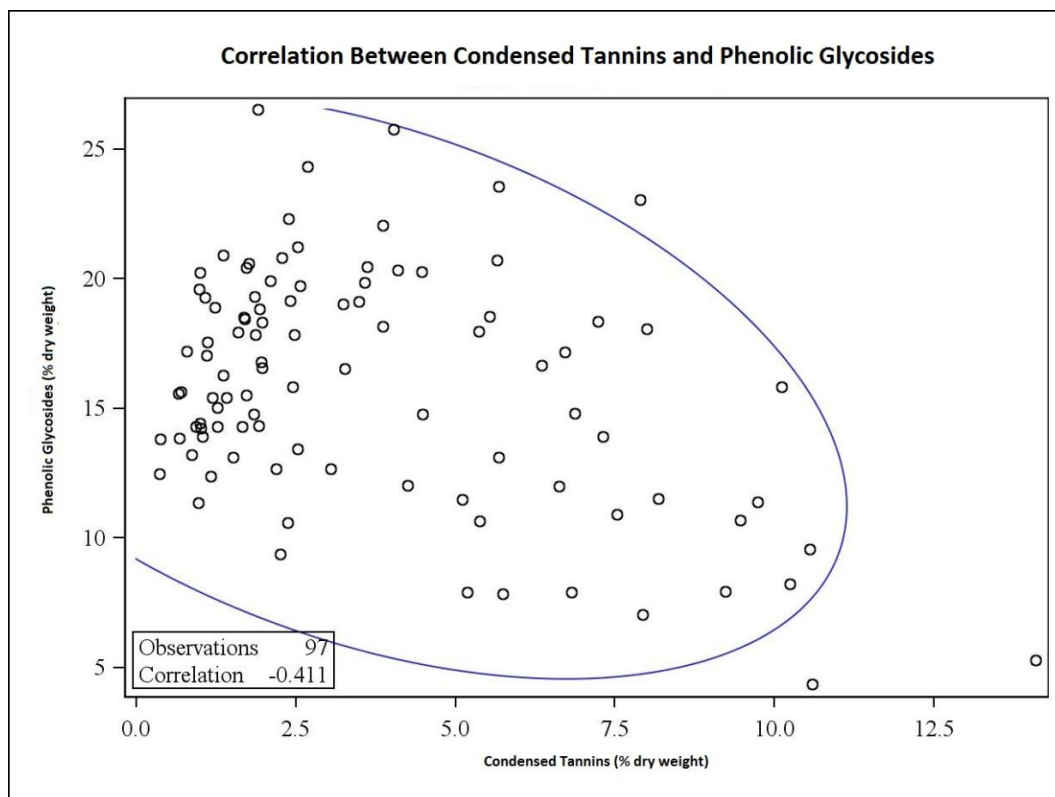
**Fig. 3.3** Results of ANOVAs for phenolic glycosides, condensed tannins and radial growth, showing the significant differences in defense chemicals and growth between clones.

## DISCUSSION

Our finding of a negative correlation between PGs and growth provides evidence of ecological tradeoffs between growth and defense in natural populations. This result is consistent with those of previous common garden studies, our results even more closely matched those where experimental aspen were subjected to limited nutrients (Hwang and Lindroth, 1997; Osier and Lindroth, 2006), and providing further evidence that these tradeoffs have ecological relevance in a natural setting. Additionally, although common garden studies have shown tradeoffs between growth and defense manifest as overall biomass differences in saplings, our results demonstrate that these tradeoffs are also operating in mature trees, are persistent over years, and can impact the secondary growth of mature stems. Counter to our hypothesis and previous work on ecological tradeoffs in aspen (Hwang and Lindroth, 1997), CTs displayed a marginally significant ( $p=0.096$ ) and positive correlation with growth, suggesting that the carbon required for CT production does not come at the expense of growth. This positive correlation between CTs and growth is, however, consistent with the negative tradeoff found between PGs and CTs (**Fig. 3.4**) which has been observed in other wild species (Koricheva et al., 2004) and also between constituent and inducible defense chemicals in aspen (Stevens and Lindroth, 2005). PGs and CTs can be both constitutive and induced after herbivory (Stevens and Lindroth, 2005); CTs are induced rapidly (on the scale of days) and PGs having a more intermediate induction (on the scale of months) (Stevens and Lindroth, 2005). Ramets in this study generally have low levels of CTs, but those with higher



levels of CTs generally have lower levels of PGs (**Fig. 3.4**), which could be evidence of a tradeoff between the current levels of these two defenses.



**Fig. 3.4** Negative correlation between phenolic glycosides and condensed tannins. In most clones with higher levels of CTs, there are lower levels of PGs.

A previously unexplored factor in patterns of ecological tradeoffs and morphological or physiological diversity in aspen is triploidy. Polyploid plants are expected to have distinct growth and physiological properties compared to their diploid counterparts (Levin, 1983). If physiological properties are enhanced in triploids, they may help explain persistence of aspen in western landscapes where seeding events are rare and episodic (Mock et al., 2012). Genetic assays suggest that larger clones tend to be triploid (Mock et al., 2008), a phenomenon suggesting increased growth and/or persistence in triploids. Enhanced growth properties of triploid aspen have been of

interest to the wood technology industry for over 50 years (Joranson, 1957; van Buijtenen, 1957), but the influence of triploidy on the ecology of aspen in western landscapes is still relatively unexplored. From a growth-defense perspective, we expected triploid clones to be less well defended than diploids, and we did observe this pattern in triploid aspen as they aged, but not in younger ramets. These findings may have implications for the persistence of triploid clones; i.e. if the tradeoff between growth and chemical defense is more pronounced in older ramets of triploid clones, maintenance of chemical defenses in these clones may require periodic disturbance to maintain younger ramet age classes.

Of the 18 clones in this study, triploids had significantly higher levels of PGs than diploids, a finding that seems counter to the growth-defense tradeoff hypothesis since these clones did not appear to have increased incremental growth compared to diploid clones. However, there were several aspects of growth which were not measured in our study, namely root growth, suckering rates, crown growth, or overall increases in biomass. The finding of elevated PGs in triploid clones may have a biological basis in triploidy *per se*, perhaps due to gene dosage effects or genetic regulatory mechanisms unique to triploids. Alternatively, the elevated PGs in triploids could be a result of indirect processes (e.g. disproportionate induction of PGs in triploids due to some other triploid-specific trait), or a result of local selection disproportionately impacting triploids. This is a finding that should be investigated at a broader geographic scale. Ploidy in western populations of aspen presents us with an entirely new perspective on factors contributing to the current and future state of western aspen forests, and management of

aspen would likely benefit from more empirical studies which include the effects of ploidy on aspen ecology.

The clonal habit is an essential trait in the ecology of aspen in the Interior West and is often underappreciated in management and restoration efforts of aspen dominated forests and woodlands (Long and Mock, 2012). Aspen clones display varied and deterministic phenotypic traits (Barnes, 1975; Hwang and Lindroth, 1997; Lindroth, 2001; Stevens and Lindroth, 2005; Stevens et al., 2007; Kanaga et al., 2008) and many of these traits can be directly linked to the resilience of this species, such as growth, drought resistance and frost resistance (Kanaga et al., 2008; Schreiber et al., 2011). Consistent with these previous studies, we detected pronounced differences in growth and chemical composition among clones as they have been observed in controlled common garden and greenhouse settings. Because both growth and defense chemistry vary by clone, the behavior of tradeoffs is likely to be a clone-specific phenomenon, as indicated by our improved model fit when ramets were blocked by clone. In western aspen, where clones can be many hectares in size, such clonal differences can be manifest at a very large scale within and among stands. It is important that studies of aspen growth and physiology in natural settings carefully account for clonal composition and distribution. Ecologically, our findings add to the growing evidence that clonal diversity is an important component of long-term resilience, and we recommend that the promotion and maintenance of clonal diversity in western aspen forests become a management goal.

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## CHAPTER 4

## SUMMARY AND CONCLUSIONS

Despite the fact that aspen is one of the most widely distributed tree species in North America (Little, 1971), it is sensitive to extreme climate events (Worrall et al., 2010) and in the western United States, is projected to undergo significant range shifts and dieback over the next 10 – 20 years (Rehfeldt et al., 2009). Recent advances in our understanding of aspen reproductive ecology (Long and Mock, 2012) could lead to new and effective methods of restoring and conserving aspen in the west. As our knowledge of western aspen increases, new questions are emerging about the establishment, persistence and interaction of clones over time. In this thesis I assess correspondence between aspen clonal diversity and fire frequency, I describe spatial interactions among clones within stands, and I assess evidence for tradeoffs in aspen clones between growth and chemical defense. These results could add substantially to the effective management of aspen forest and woodlands in the west as future climate challenges are encountered.

In the first portion of this study (Chapter 2) I found patterns of high and low clonal diversity on Cedar Mountain, UT (located about 15 miles southeast of Cedar City, UT) and showed that areas with evidence more frequent fires had higher clonal diversity. I hypothesize that this relationship is due to an increased frequency of seedling establishment following fires. These results suggest that management influencing fire regimes may also influence the frequency of seedling establishment and thus the clonal diversity of aspen stands. Increased clonal richness, in turn, is expected to be important in species persistence and resilience, as genetic recombination and diversity should enhance

evolutionary potential. In this portion of the study, where I encountered stands with multiple clones I determined whether boundaries between clones appeared to be static or whether particular clones were expanding over time. I found that approximately 25% of the clones seemed to be advancing into bordering clones. Such a process would eventually be expected to reduce clonal diversity over time, perhaps allowing the persistence of the better-adapted clones.

In the second portion of this thesis (Chapter 3) I looked for evidence of ecological tradeoffs in wild populations of western aspen between growth (a resilience strategy) and defense chemistry (resistance). Such tradeoffs have been detected in greenhouse and common garden studies (Stevens et al., 2007), but I wanted to determine whether these tradeoffs persist in naturally-occurring populations of mature aspen, or whether complex environmental conditions appear to diminish this relationship. I found that that the tradeoff remained intact between phenolic glycosides (a group of defense chemicals that have been shown to deter insect herbivory) and growth, but there was a positive and less significant relationship between condensed tannins and growth (discussed in detail in Chapter 3). I also detected high levels of variability in this tradeoff among aspen clones, which is further evidence of the ability of aspen to display varied phenotypic responses in similar stand conditions when clonal diversity is present.

The results of this thesis add to the growing body of knowledge surrounding aspen ecology and will hopefully influence management decisions in conserving and restoring aspen populations in the western United States. Managing for increased clonal diversity at the stand level will likely increase aspen resilience as climates change in the coming years.

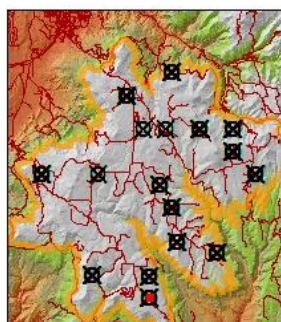
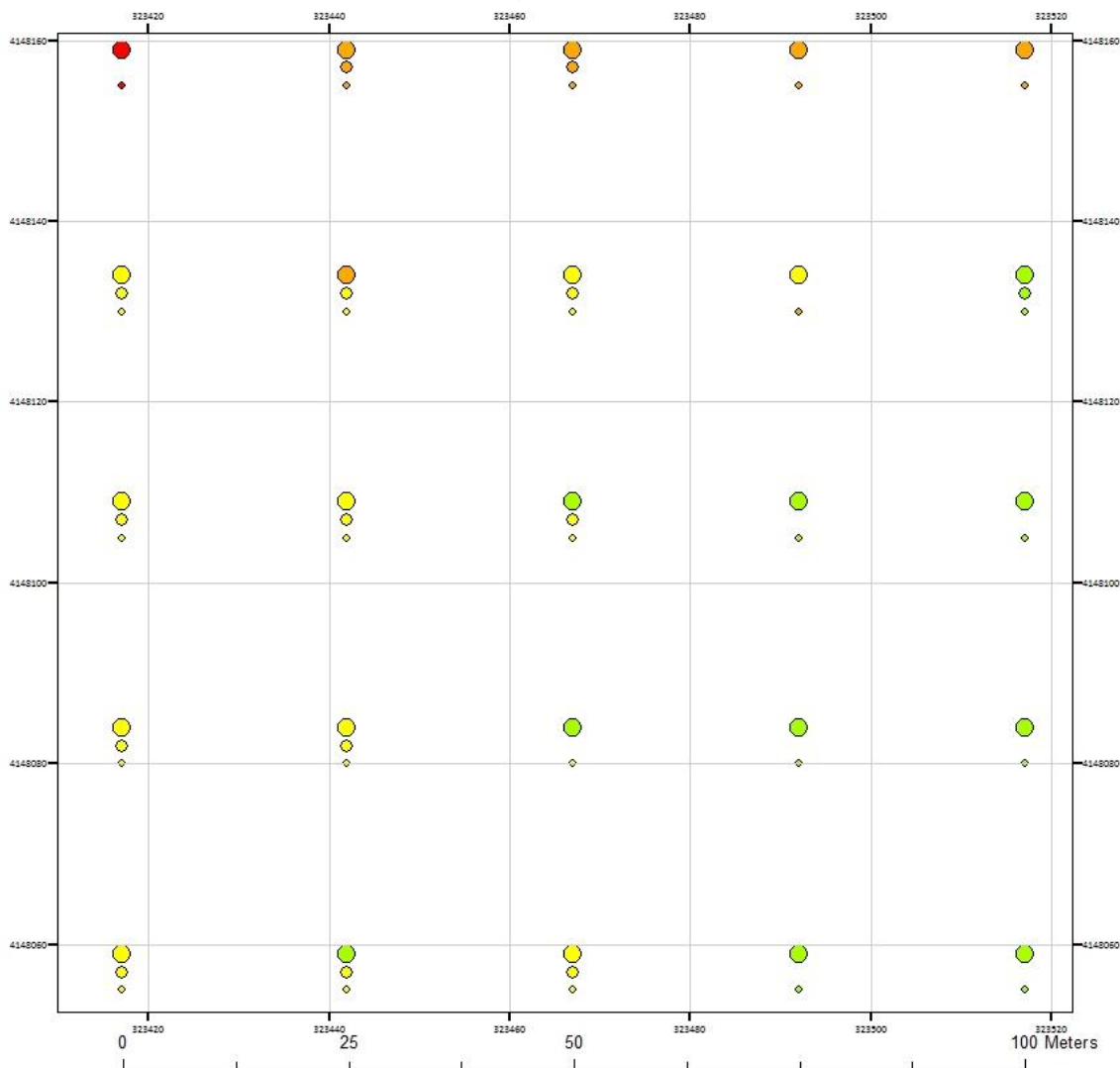
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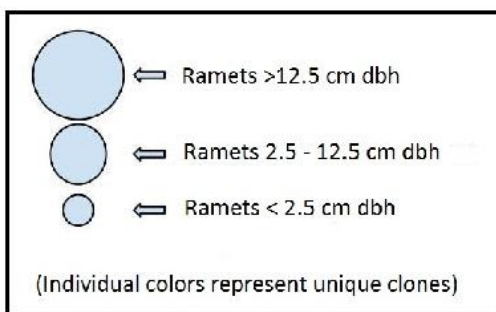
APPENDICES

**Appendix A** Individual maps of each of the 17 plots investigated for clonal replacement. Plots with nonaligned subplots are a result of navigating to subplots via compass and tape, rather than navigating to pre-determined GPS coordinates.

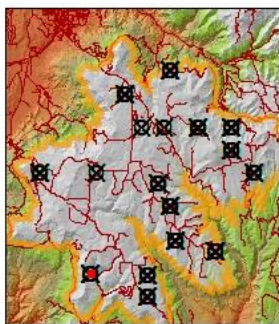
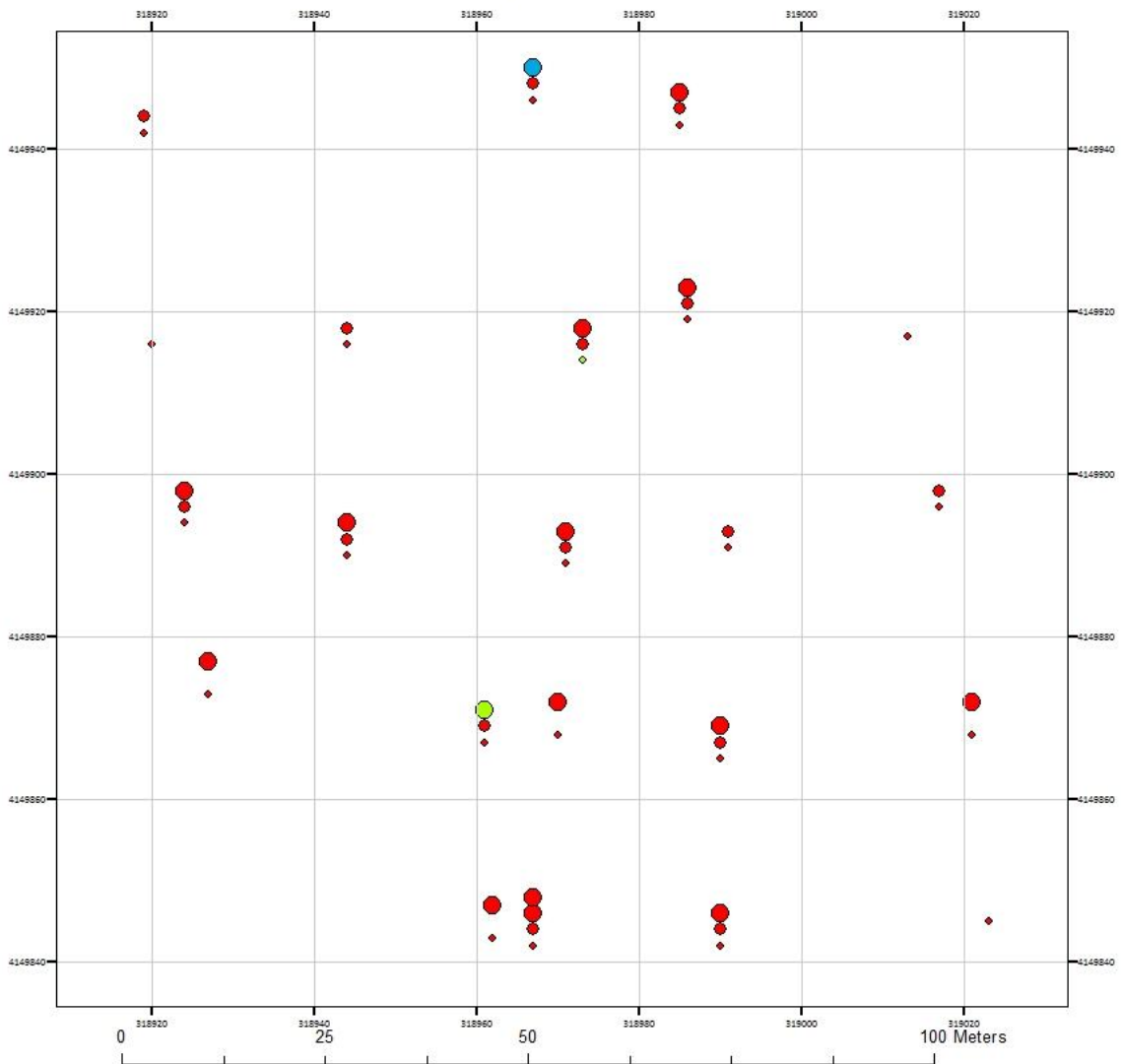
**Clonal Boundary Interactions- Plot 007**



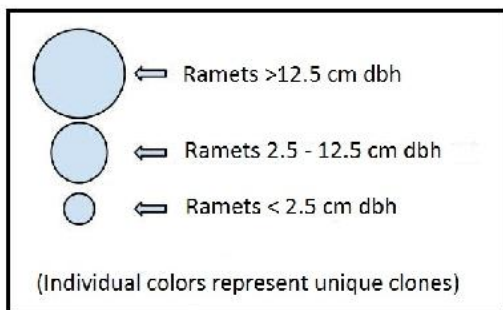
**Cedar Mountain Area-**  
Red indicates plot location.



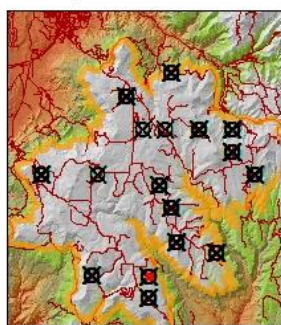
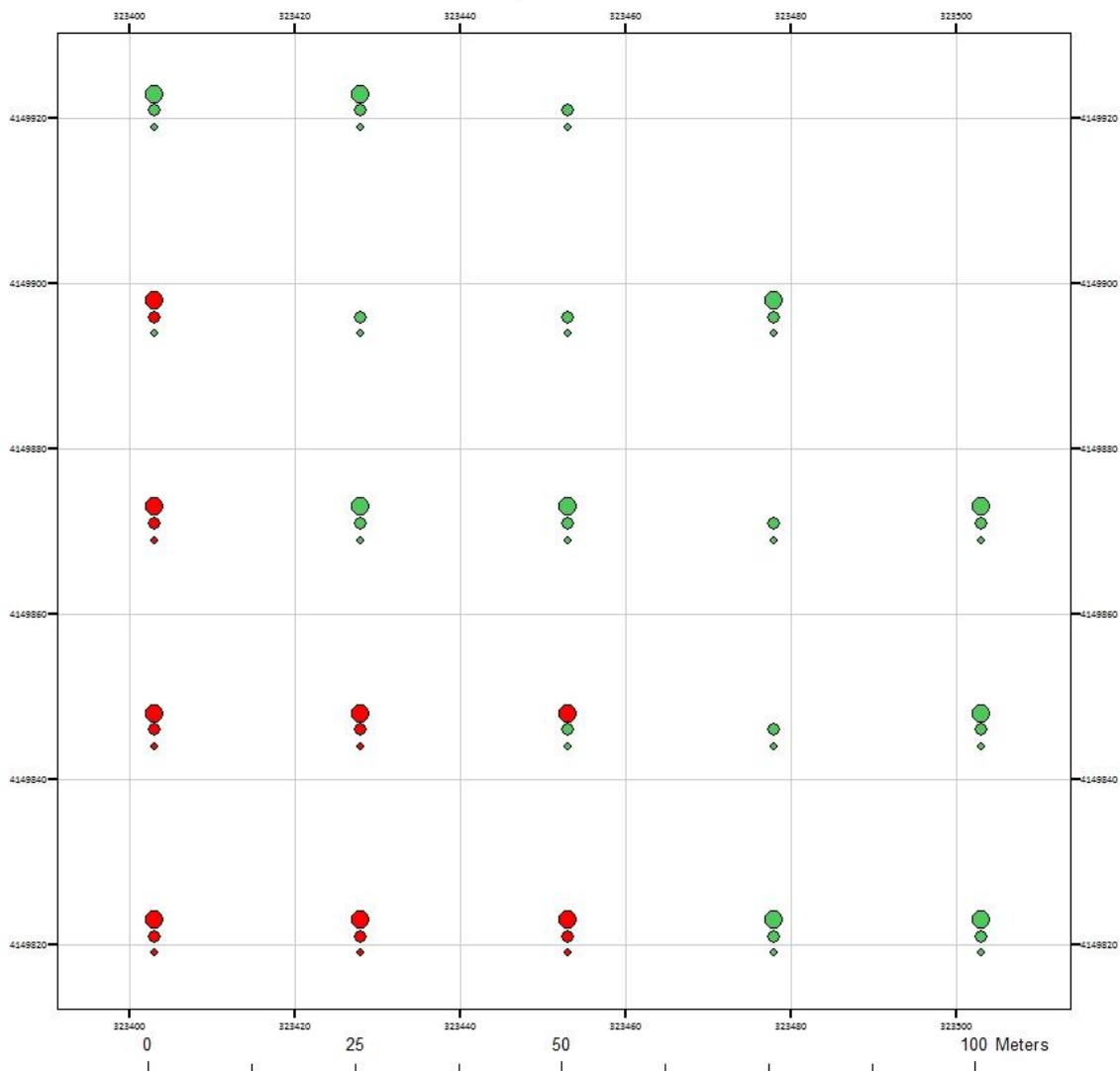
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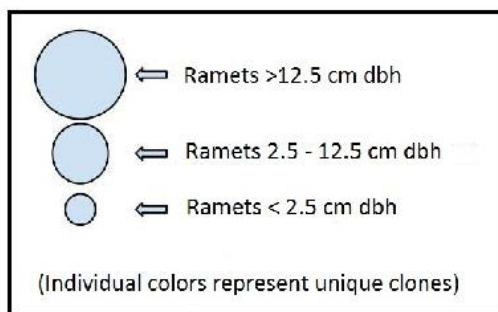
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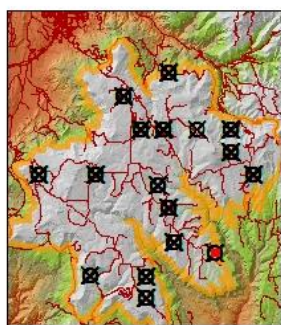
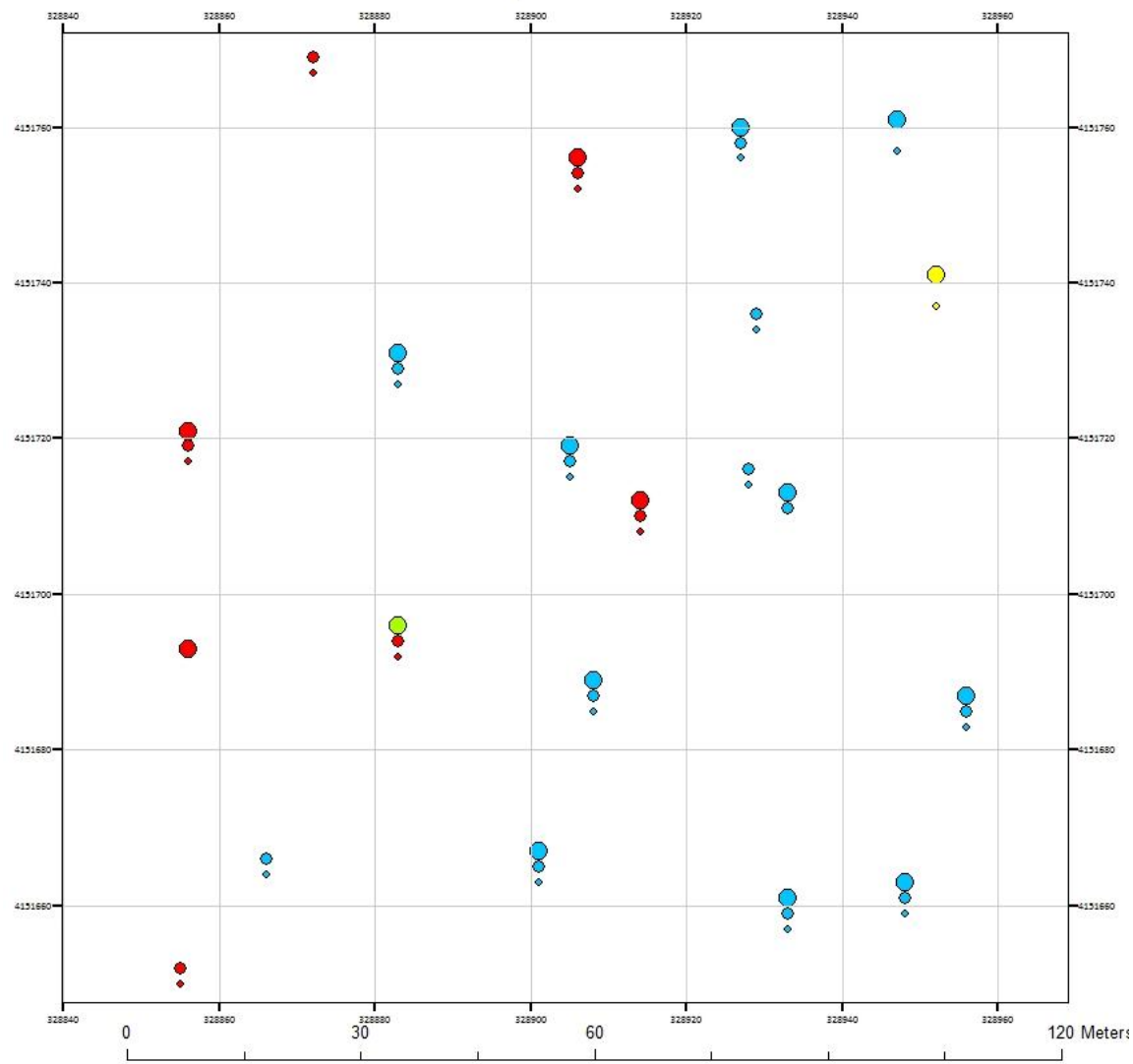


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Red indicates plot location.

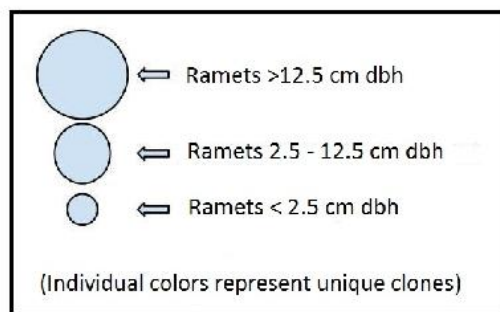




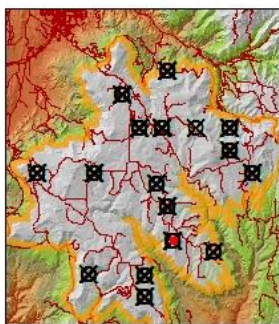
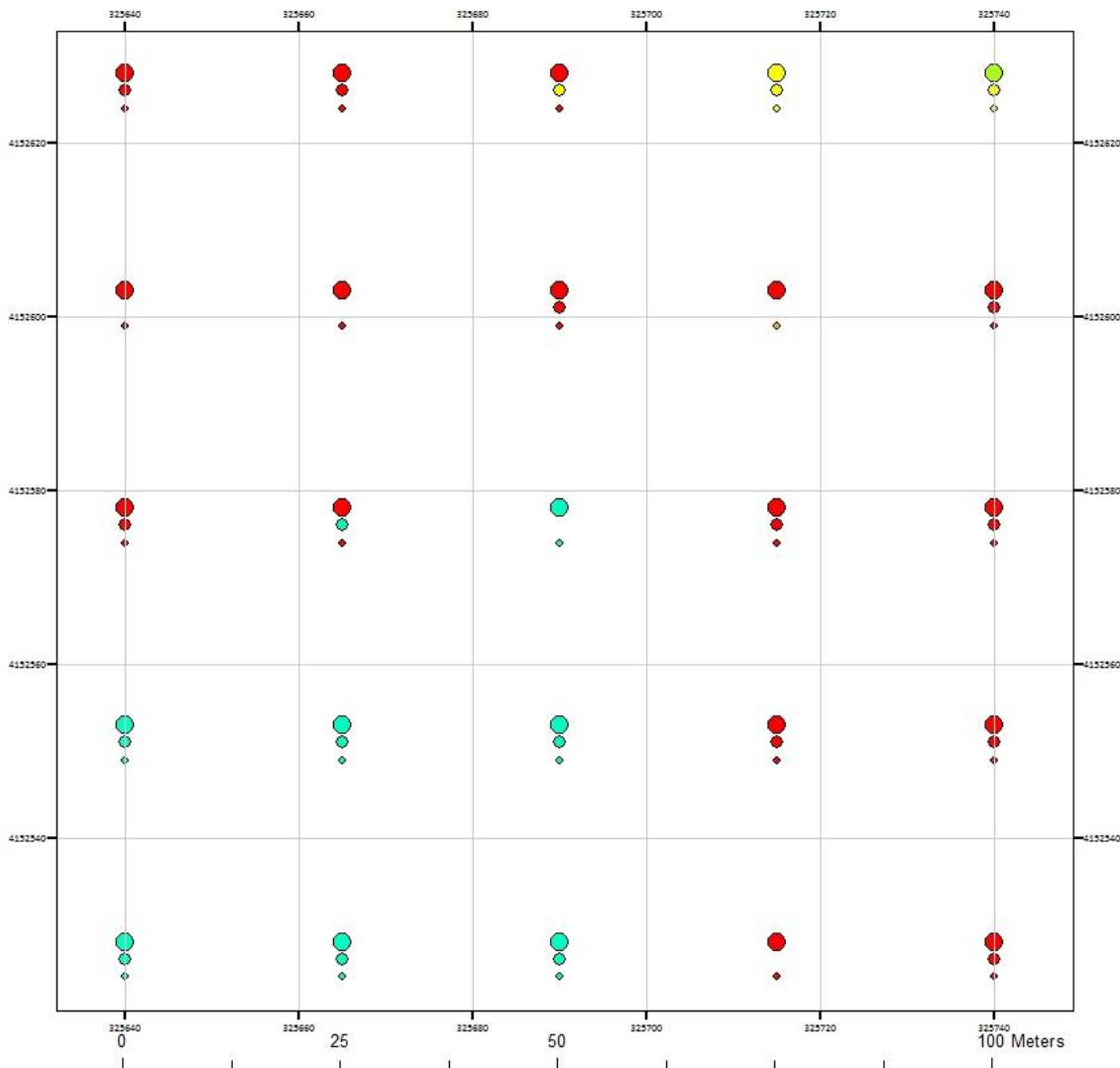
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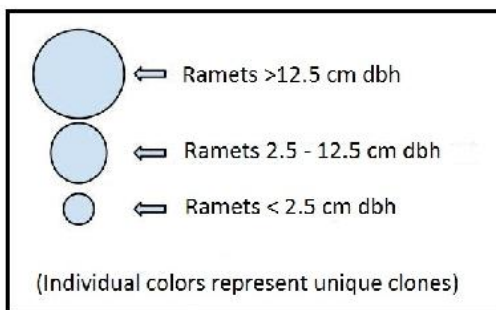
Cedar Mountain Area-  
Red indicates plot location.



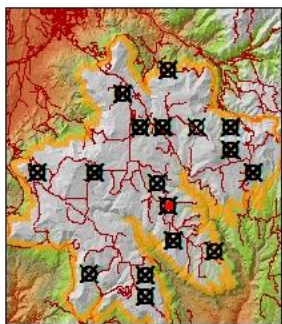
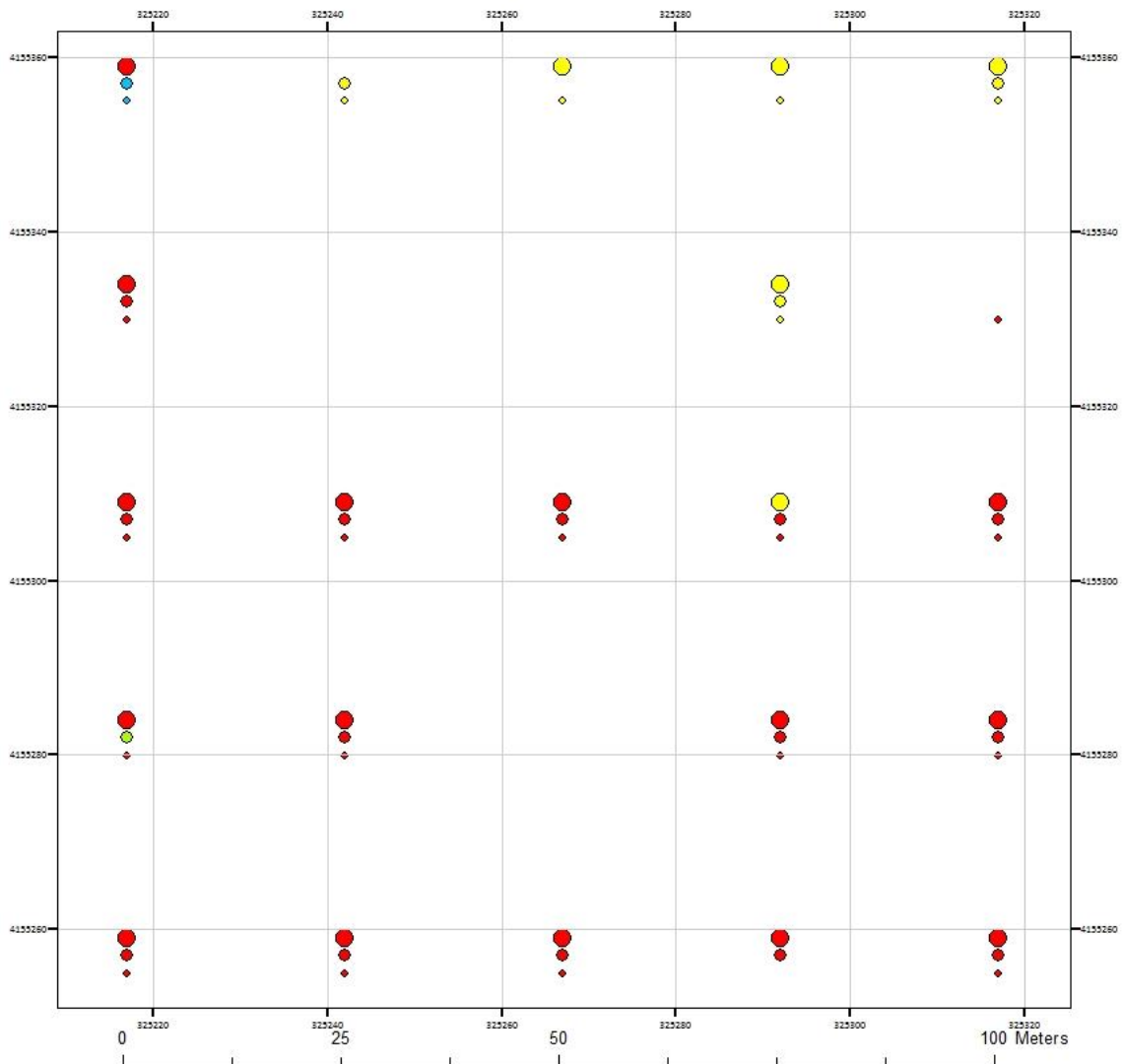
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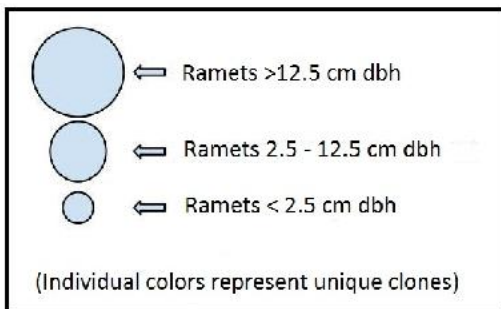
Cedar Mountain Area-  
Red indicates plot location.



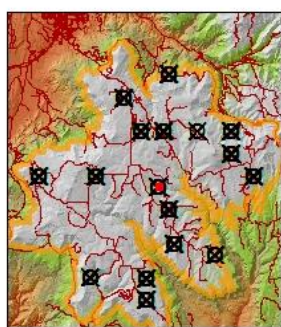
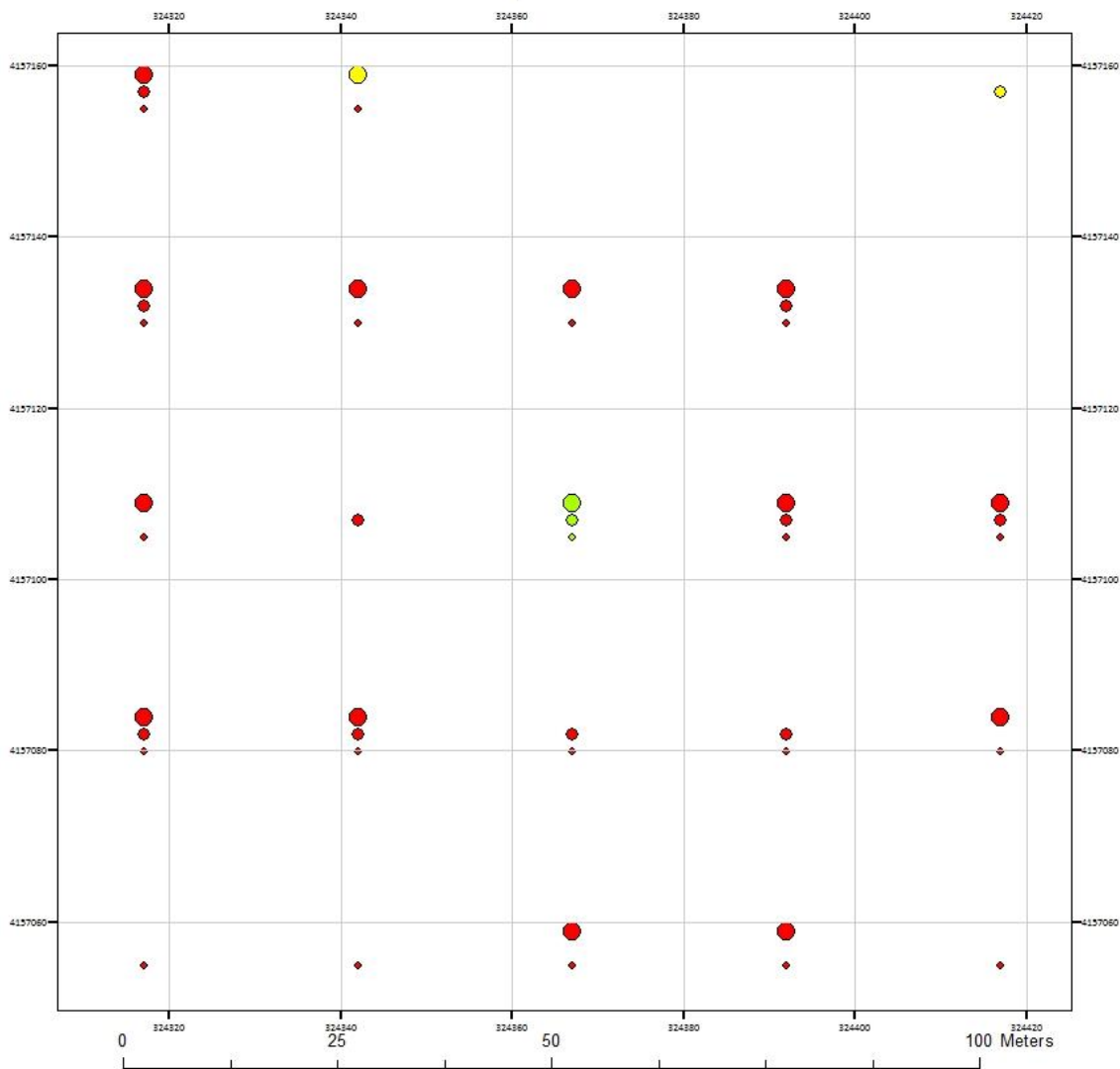
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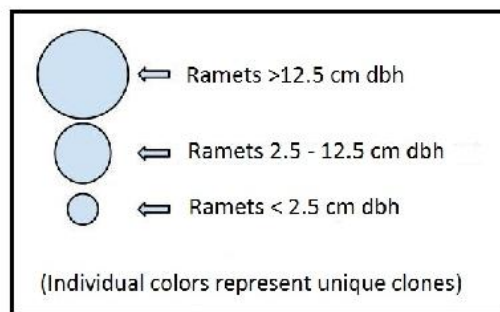
Cedar Mountain Area-  
Red indicates plot location.



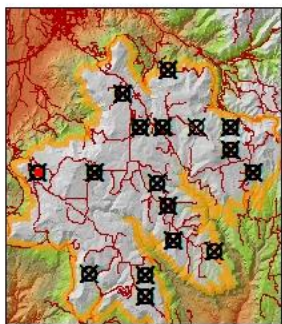
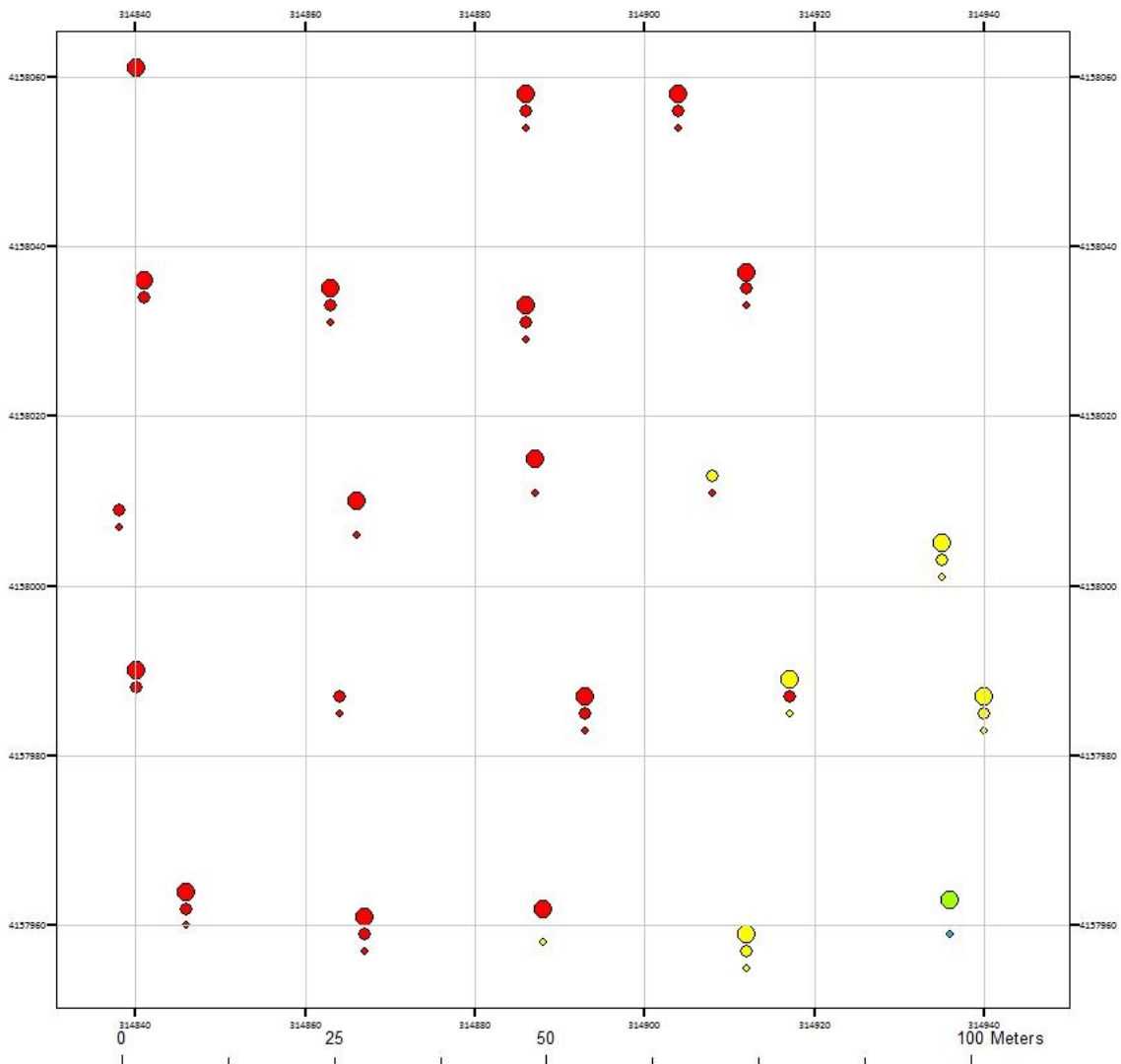
### Clonal Boundary Interactions- Plot 051



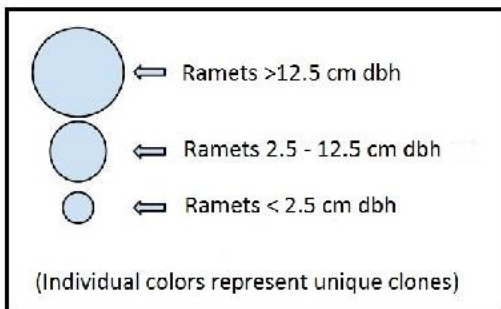
Cedar Mountain Area-  
Red indicates plot location.



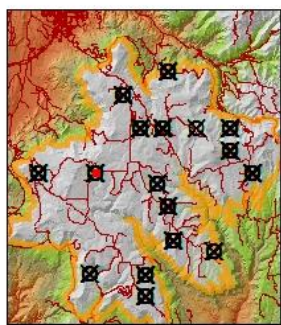
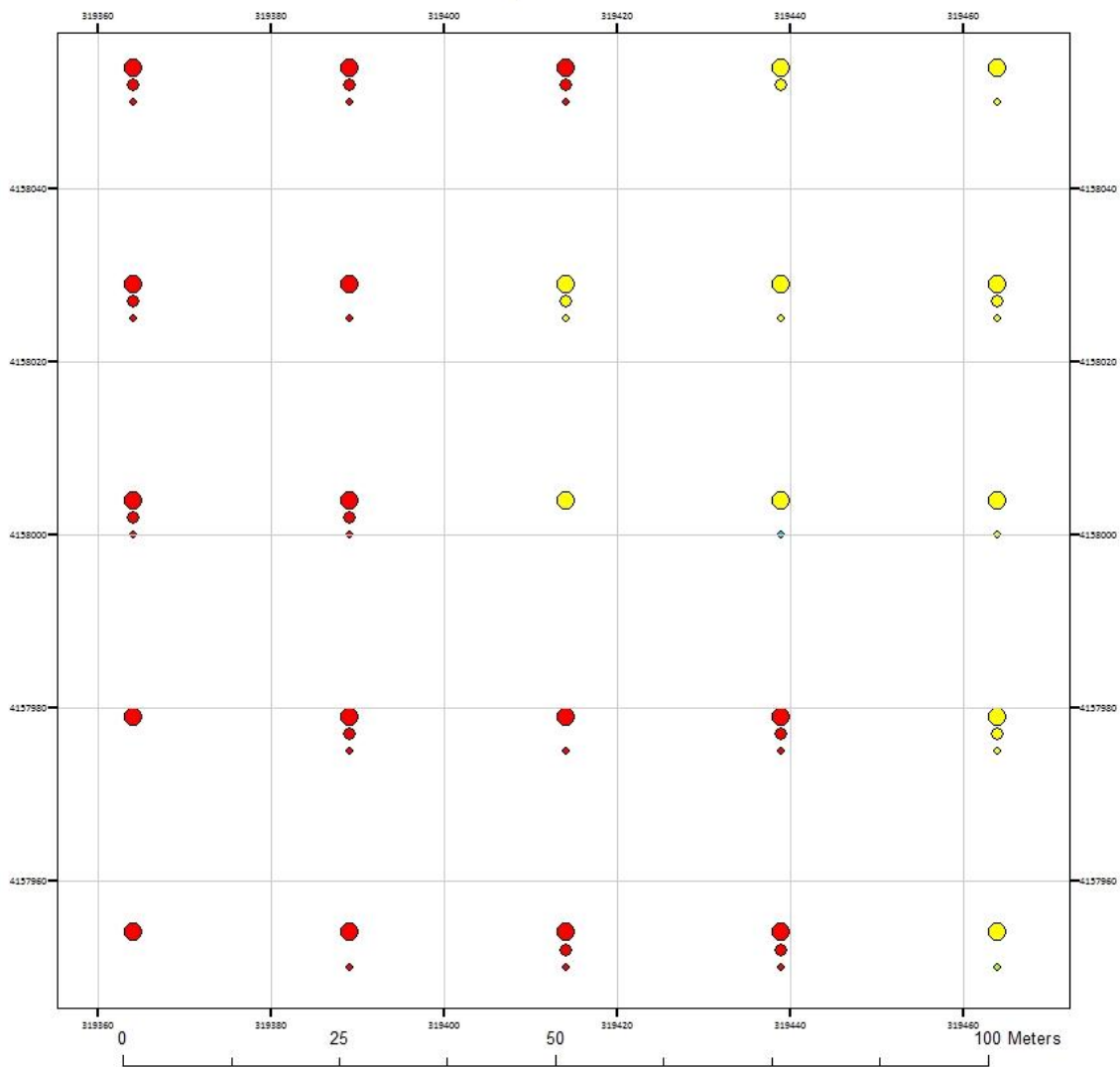
### Clonal Boundary Interactions- Plot 057



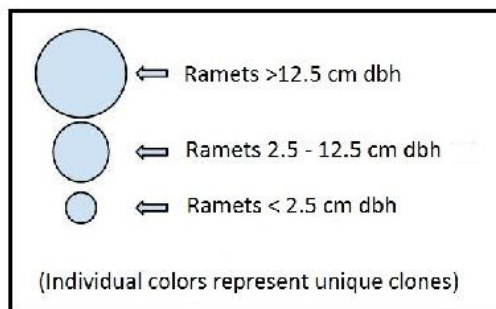
Cedar Mountain Area-  
Red indicates plot location.



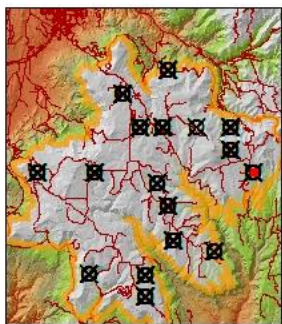
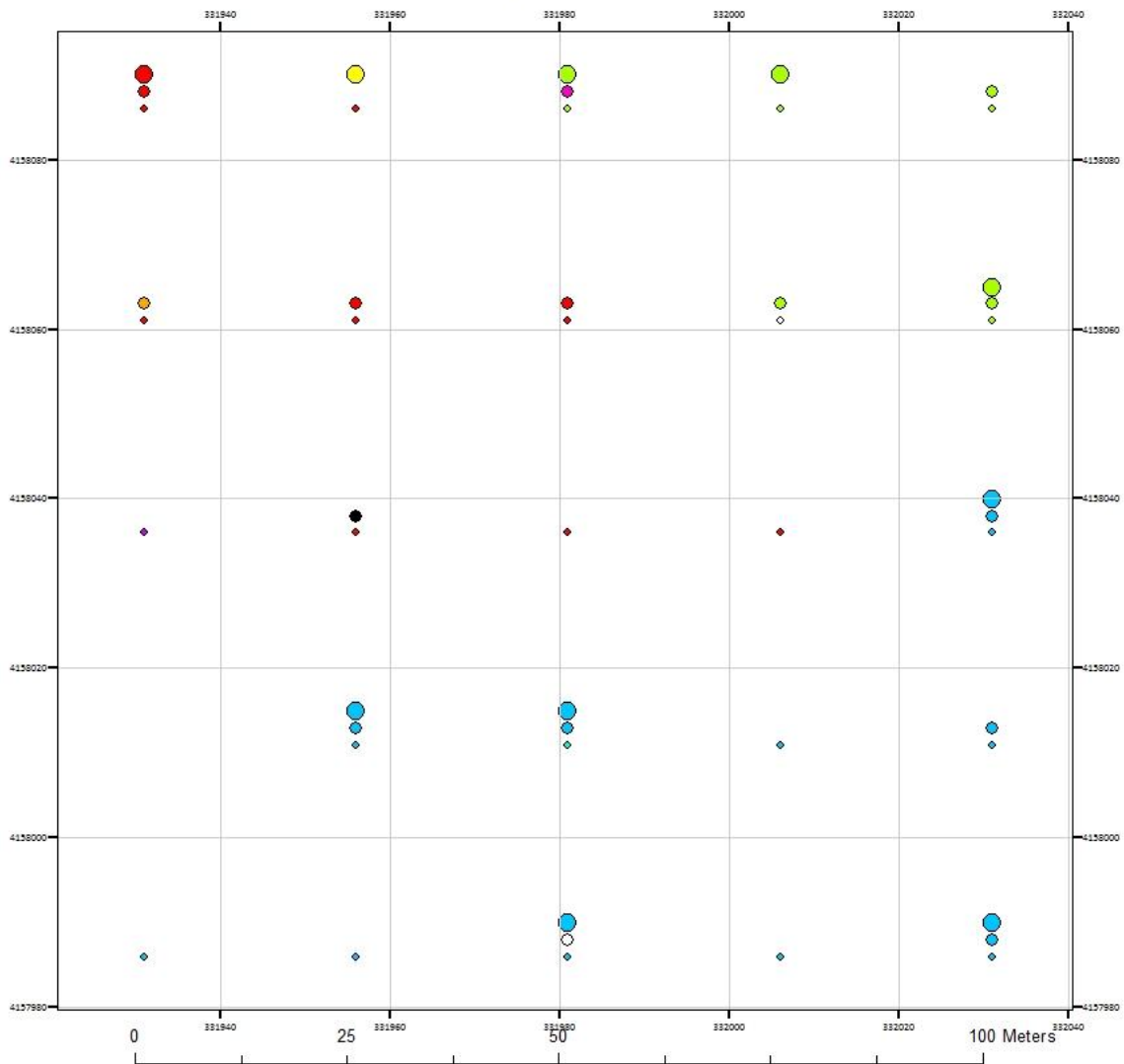
### Clonal Boundary Interactions- Plot 061



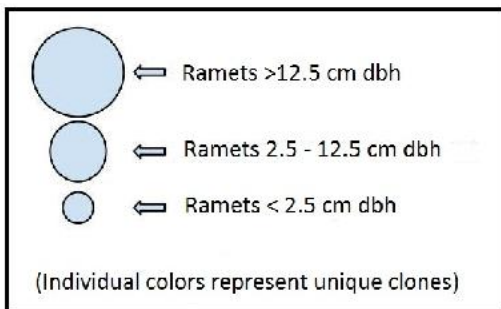
Cedar Mountain Area-  
Red indicates plot location.



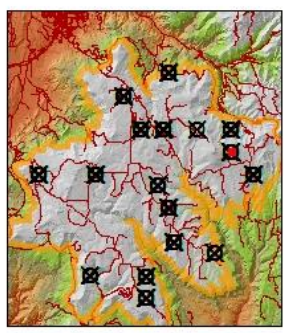
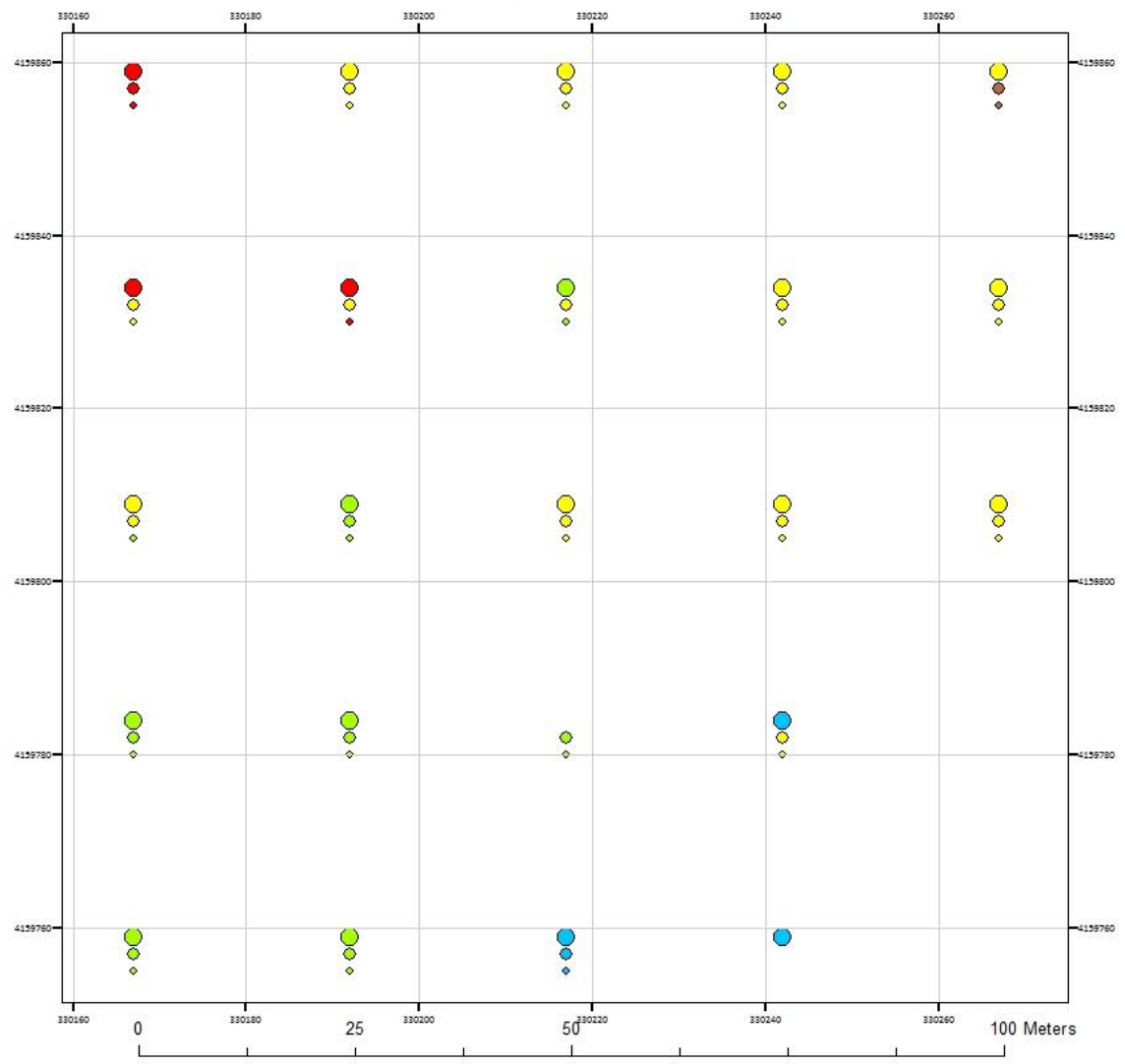
### Clonal Boundary Interactions- Plot 068



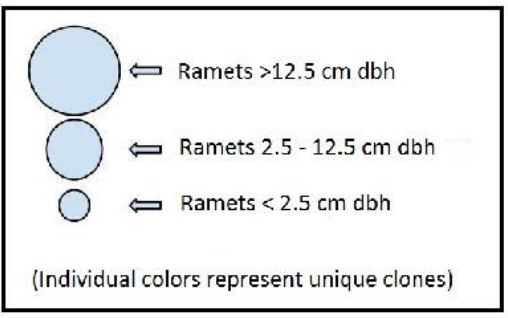
Cedar Mountain Area-  
Red indicates plot location.



### Clonal Boundary Interactions- Plot 084

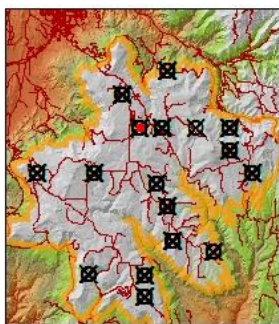
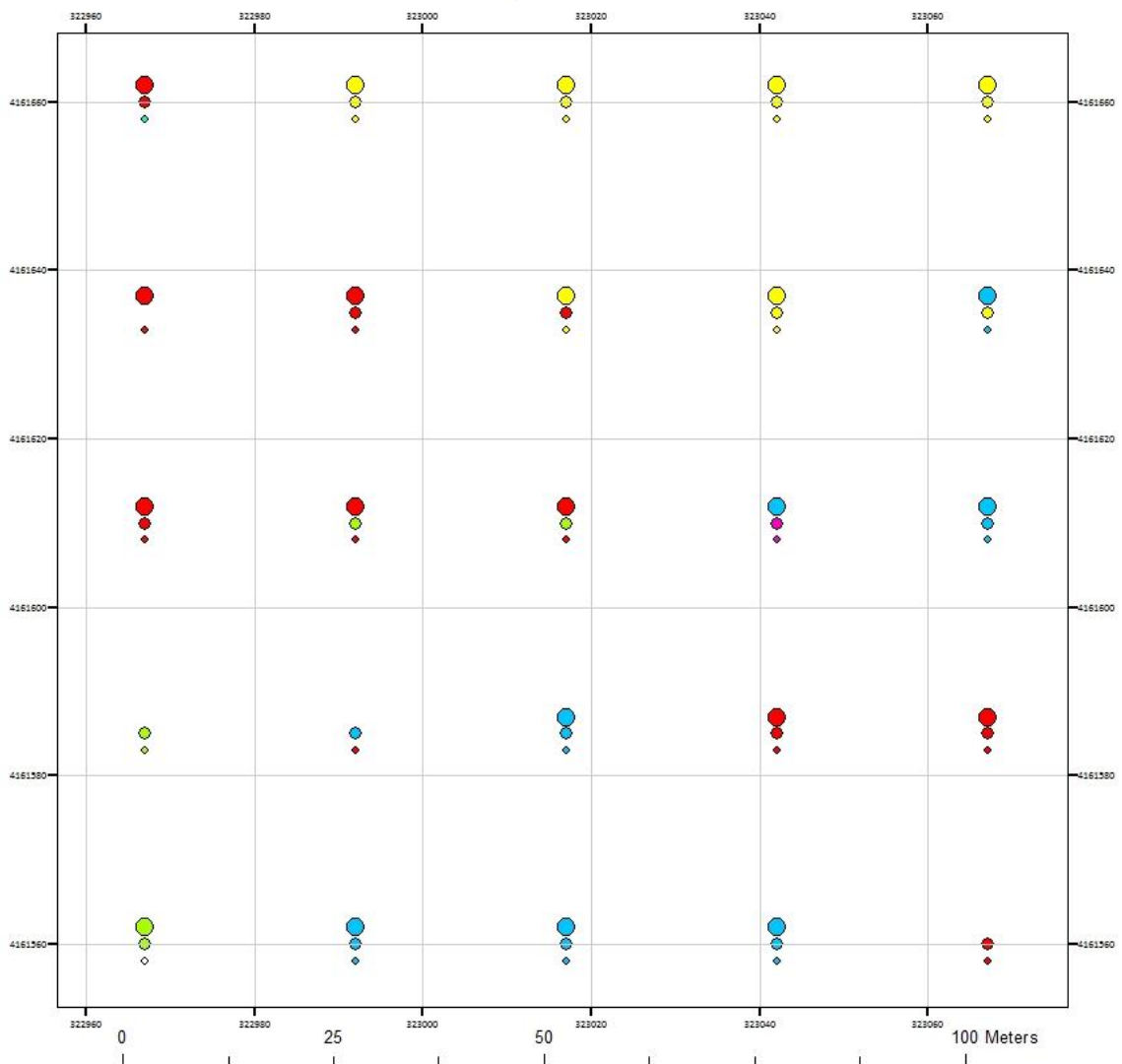


Cedar Mountain Area-  
Red indicates plot location.

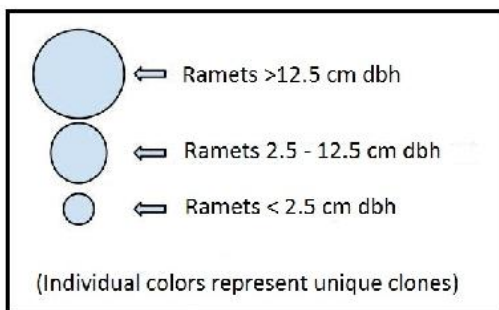




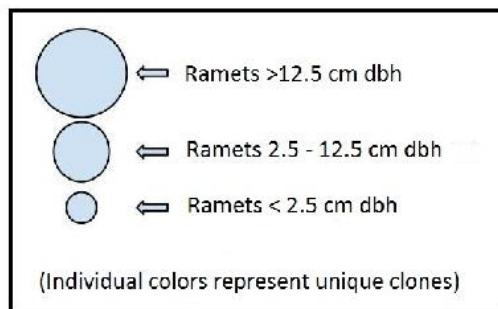
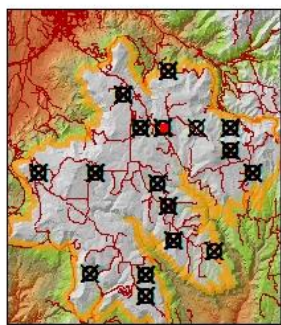
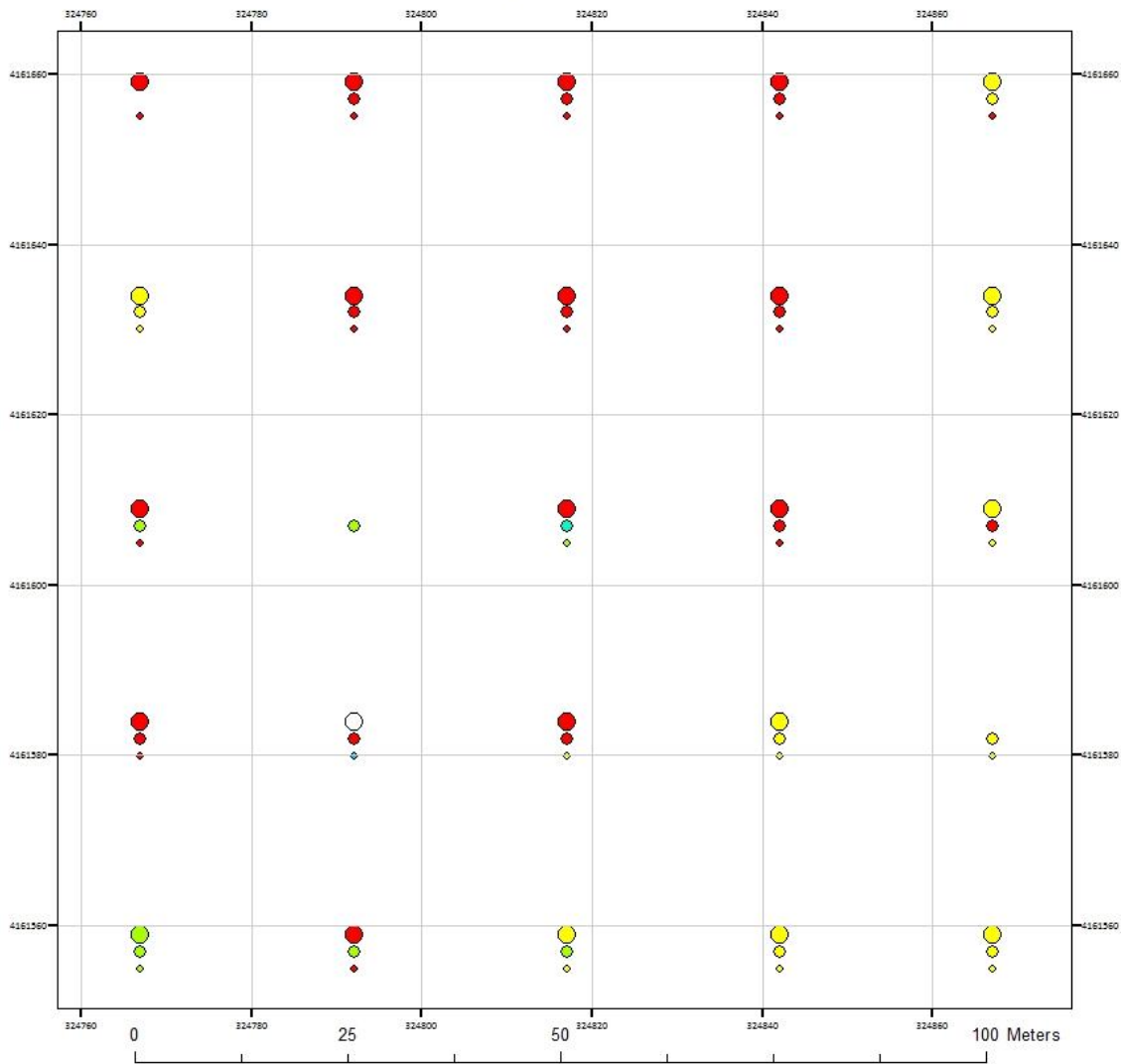
### Clonal Boundary Interactions- Plot 096



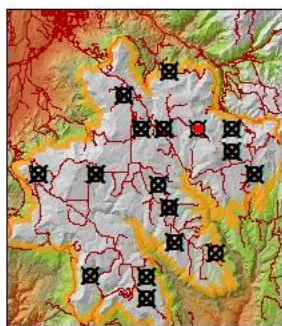
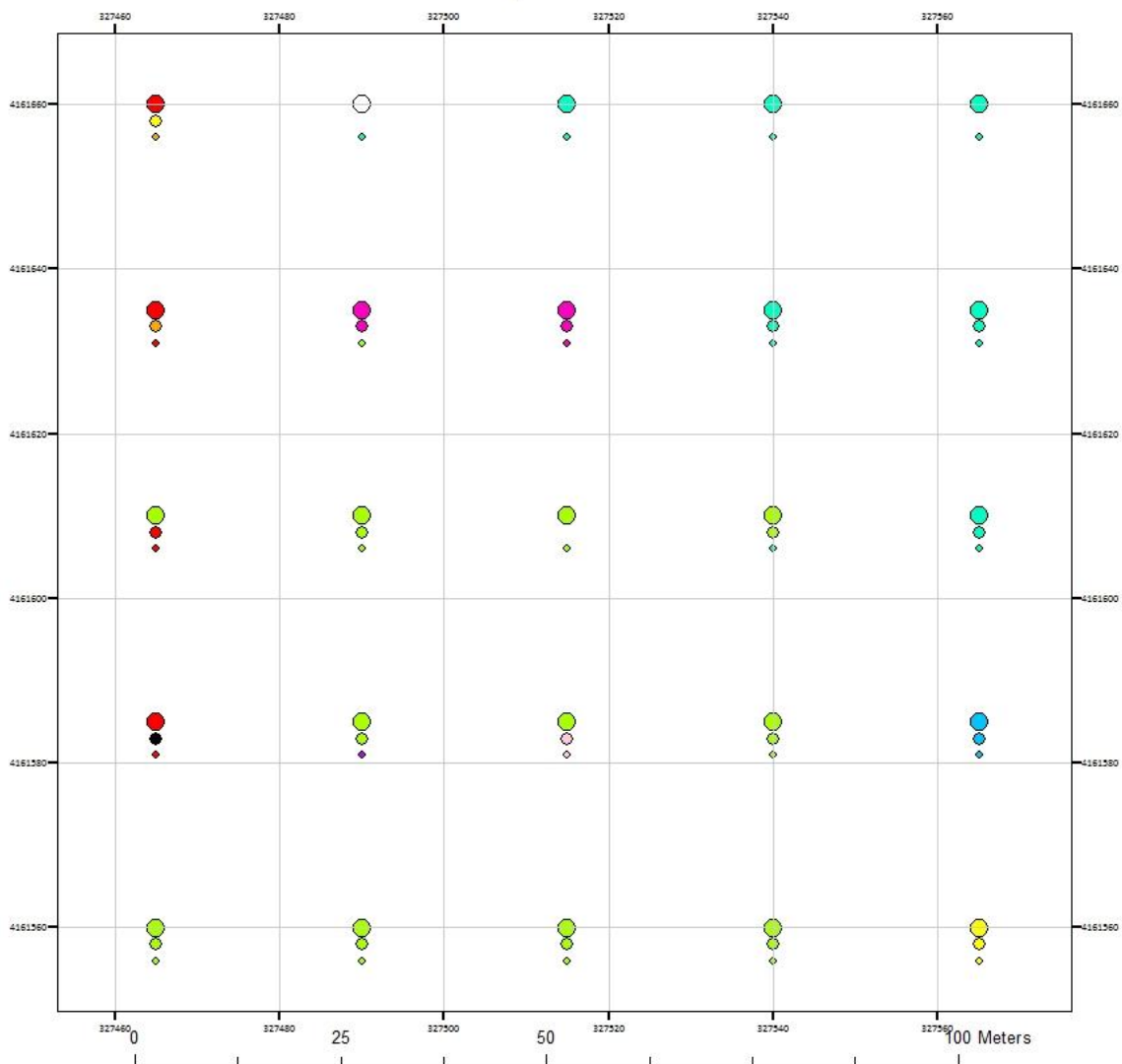
Cedar Mountain Area-  
Red indicates plot location.



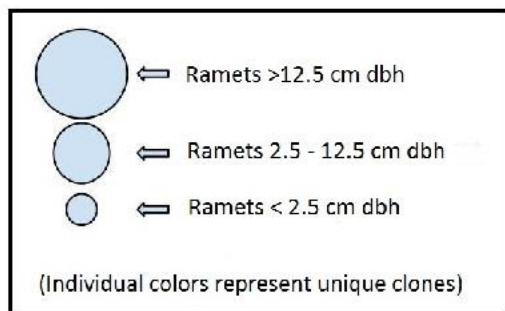
### Clonal Boundary Interactions- Plot 097



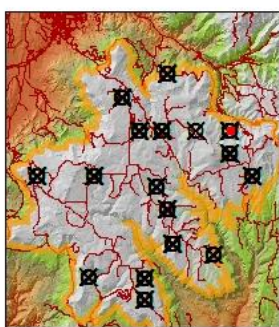
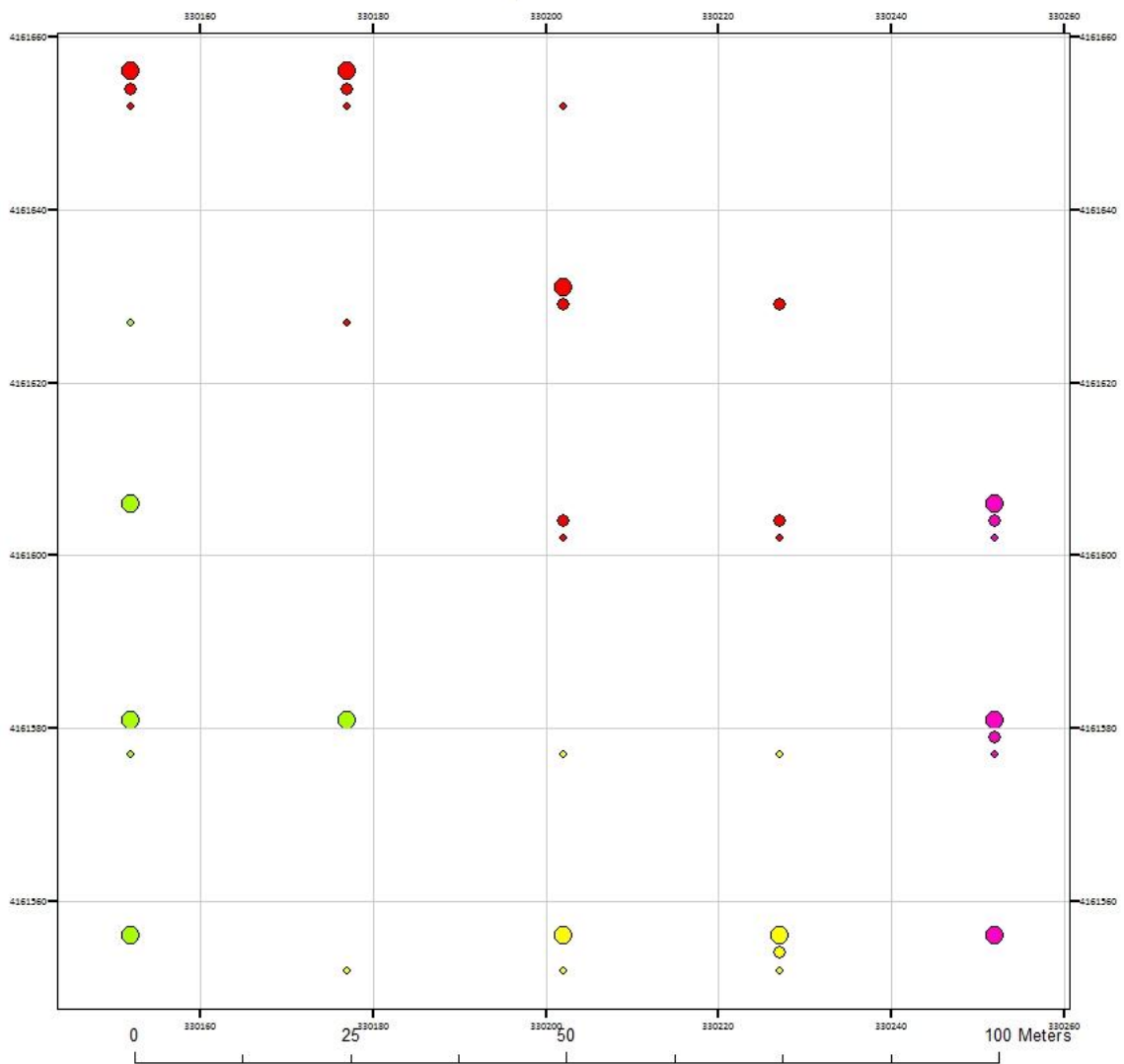
### Clonal Boundary Interactions- Plot 100



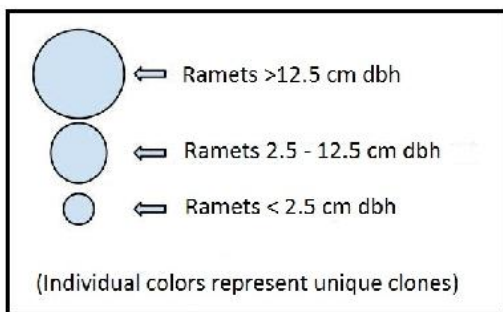
Cedar Mountain Area-  
Red indicates plot location.



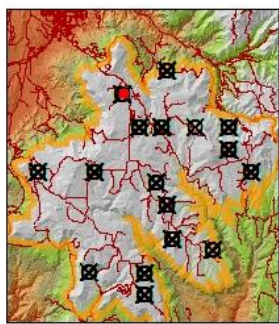
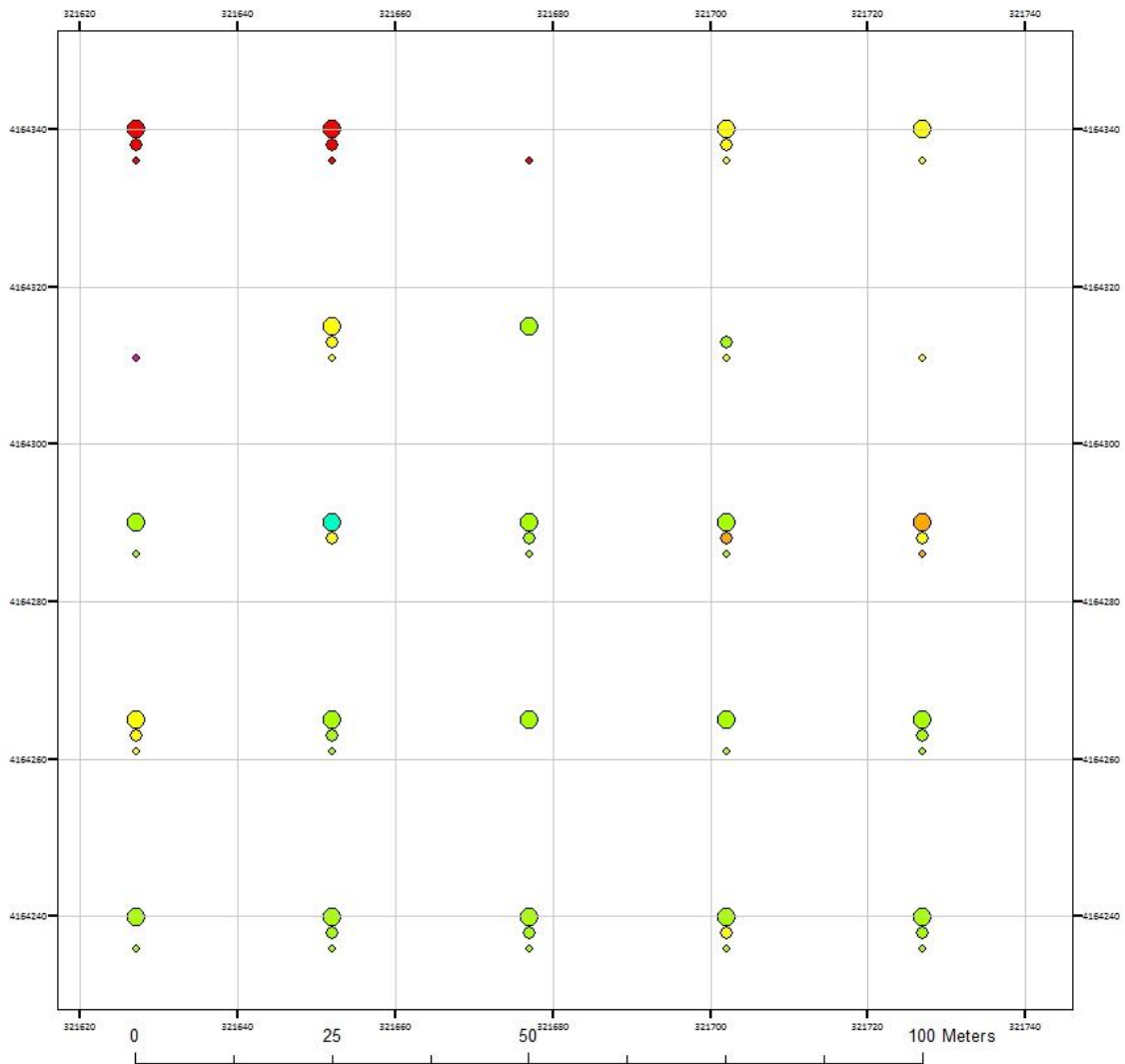
### Clonal Boundary Interactions- Plot 102



Cedar Mountain Area-  
Red indicates plot location.



### Clonal Boundary Interactions- Plot 119



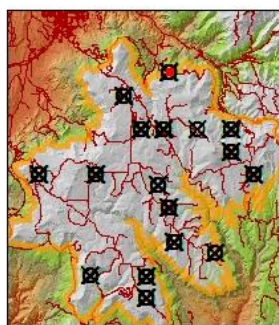
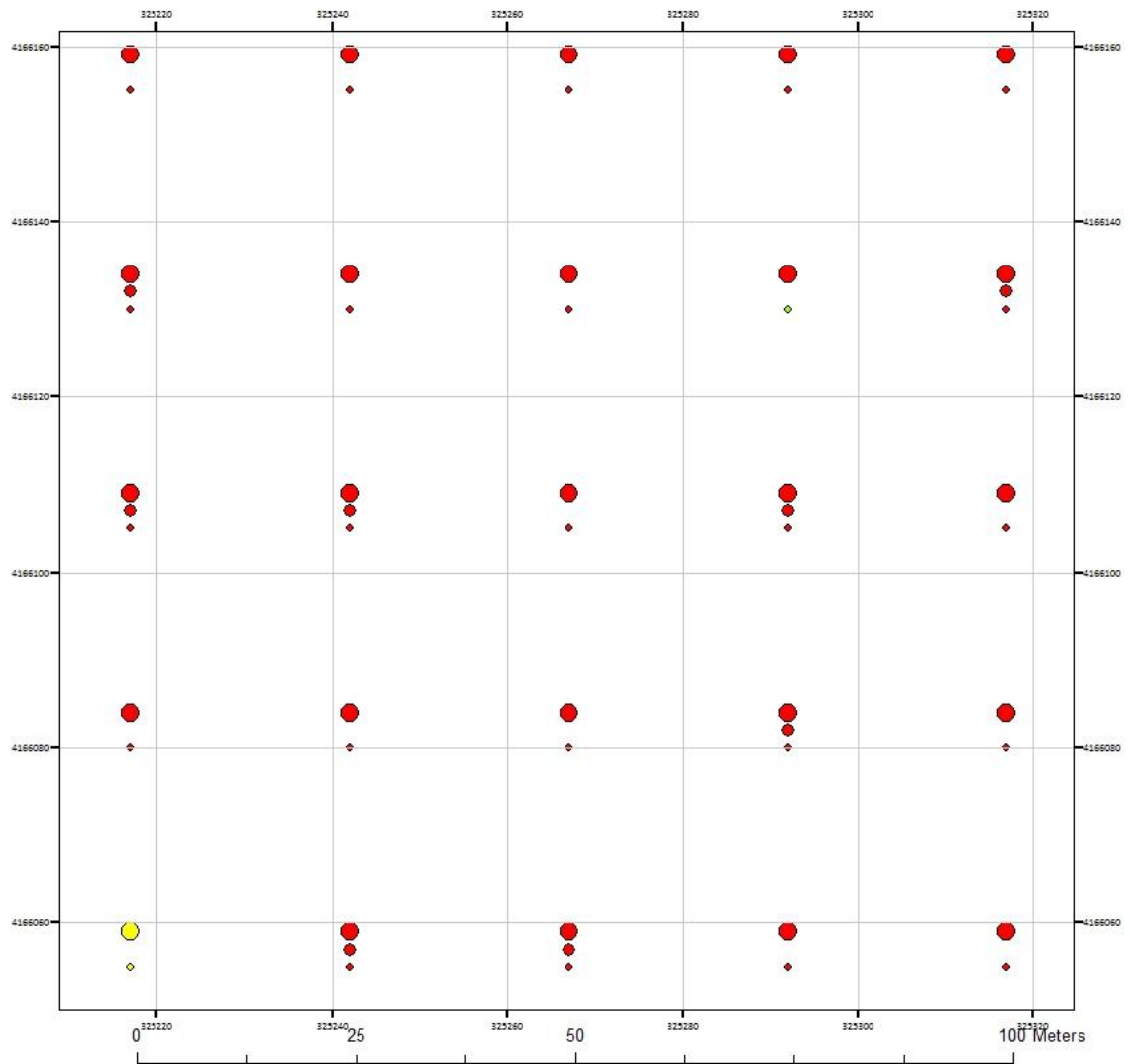
**Cedar Mountain Area-**  
Red indicates plot location.

	← Ramets >12.5 cm dbh
	← Ramets 2.5 - 12.5 cm dbh
	← Ramets < 2.5 cm dbh

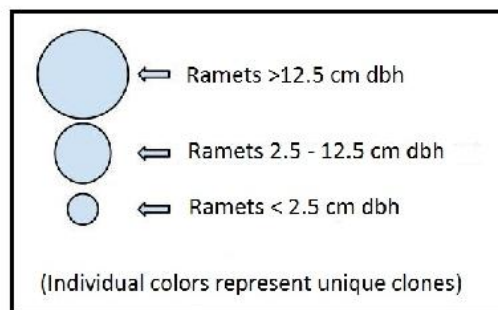
(Individual colors represent unique clones)



### Clonal Boundary Interactions- Plot 131



Cedar Mountain Area-  
Red indicates plot location.



**Appendix B** Table summarizing clones which are encroaching neighboring clones. The colors are consistent with those on the individual plot maps in Appendix 2.a. Any row containing “NA” represents a clone that did not meet the summary requirements defined on pages 12-13.

<b>Plot</b>	<b>Color</b>	<b>Genet</b>	<b># of Occurrences</b>	<b>Potential Sites to Encroach</b>	<b>Sites Encroached</b>	<b>Encroacher</b>
007	Yellow	4	13	16	4	Yes
007	Red	12	1	NA	NA	NA
007	Orange	13	5	20	1	No
007	Green	54	10	15	0	No
011	Red	14	23	2	NA	NA
011	Green	95	2	NA	NA	NA
011	Blue	98	1	NA	NA	NA
015	Green	36	16	8	2	Yes
015	Red	51	8	14	0	No
022	Red	3	7	15	1	No
022	Yellow	7	1	NA	NA	NA
022	Blue	74	13	8	0	No
022	Green	97	1	NA	NA	NA
023	Yellow	55	3	NA	NA	NA
023	Turquoise	56	8	18	1	No
023	Green	65	1	NA	NA	NA
023	Red	85	16	9	0	No
039	Green	47	1	NA	NA	NA
039	Blue	71	1	NA	NA	NA
039	Red	77	17	6	1	No
039	Yellow	86	6	18	0	No
051	Yellow	21	2	NA	NA	NA
051	Green	37	1	NA	NA	NA
051	Red	90	20	3	1	No
057	Red	15	18	6	2	Yes
057	Blue	46	1	NA	NA	NA

<b>057</b>	Yellow	68	6	16	1	No
<b>057</b>	Green	88	1	NA	NA	NA
<b>061</b>	Blue	1	1	NA	NA	NA
<b>061</b>	Yellow	5	10	15	0	No
<b>061</b>	Red	67	15	10	0	No
<b>068</b>	Purple	27	1	NA	NA	NA
<b>068</b>	Turquoise	28	1	NA	NA	NA
<b>068</b>	White	30	2	NA	NA	NA
<b>068</b>	Yellow	31	1	NA	NA	NA
<b>068</b>	Blue	35	10	11	0	No
<b>068</b>	Orange	72	1	NA	NA	NA
<b>068</b>	Black	73	1	NA	NA	NA
<b>068</b>	Red	79	8	14	3	Yes
<b>068</b>	Fuschia	82	1	NA	NA	NA
<b>068</b>	Green	87	5	12	0	No
<b>084</b>	Brown	29	1	NA	NA	NA
<b>084</b>	Yellow	38	14	13	4	Yes
<b>084</b>	Green	43	7	16	1	No
<b>084</b>	Red	91	3	NA	NA	NA
<b>096</b>	Yellow	8	7	19	1	No
<b>096</b>	Green	32	4	23	2	Yes
<b>096</b>	Fuschia	33	1	NA	NA	NA
<b>096</b>	Turquoise	42	1	NA	NA	NA
<b>096</b>	Red	53	10	16	1	No
<b>096</b>	Blue	61	8	17	0	No
<b>096</b>	White	89	1	NA	NA	NA
<b>097</b>	Red	18	15	12	3	Yes
<b>097</b>	Green	19	6	23	4	Yes
<b>097</b>	White	40	1	NA	NA	NA
<b>097</b>	Blue	44	1	NA	NA	NA
<b>097</b>	Yellow	49	10	16	1	No



<b>097</b>	Turquoise	79	1	NA	NA	NA
<b>100</b>	Yellow	34	2	NA	NA	NA
<b>100</b>	Red	45	4	23	1	No
<b>100</b>	Green	48	11	14	0	No
<b>100</b>	Pink	50	1	25	1	No
<b>100</b>	Turquoise	57	8	19	2	Yes
<b>100</b>	Black	58	1	25	1	No
<b>100</b>	Fuschia	63	2	23	0	No
<b>100</b>	White	78	1	24	NA	NA
<b>100</b>	Purple	92	1	25	1	No
<b>100</b>	Orange	93	2	NA	NA	NA
<b>102</b>	Red	9	8	8	0	No
<b>102</b>	Yellow	17	5	12	0	No
<b>102</b>	Fuschia	59	3	12	0	No
<b>102</b>	Green	66	5	11	0	No
<b>119</b>	Red	20	4	21	0	No
<b>119</b>	Orange	70	2	NA	NA	NA
<b>119</b>	Green	75	14	11	0	No
<b>119</b>	Turquoise	76	1	NA	NA	NA
<b>119</b>	Yellow	80	9	22	4	Yes
<b>131</b>	Green	2	1	NA	NA	NA
<b>131</b>	Yellow	69	1	NA	NA	NA
<b>131</b>	Red	84	24	1	NA	NA

**Appendix C** Letters from coauthors on Chapter 2, granting me the permission to publish their work in this thesis.

April 22, 2013

Richard Scott Gardner  
 Department of Wildland Resources  
 5230 Old Main Hill  
 Utah State University  
 Logan, UT 84322-5230  
 615-351-5410/[r.gar@aggiemail.usu.edu](mailto:r.gar@aggiemail.usu.edu)

Richard L. Lindroth  
 University of Wisconsin-Madison  
 Department of Entomology  
 1630 Linden Drive  
 Madison, WI 53706 U.S.A.

Dear Dr. Lindroth:

I am in the process of preparing my thesis in the Department of Wildland Resources at Utah State University. I hope to complete in the Spring of 2013.

I am requesting your permission to include your contributions as a co-author in my thesis to be published by Utah State University.

Please indicate your approval of this request by signing in the space provided below. If you have any questions, please call me at the number above.

Thank you for your cooperation,

Richard Scott Gardner

---

I have contributed as a co-author on Chapter Three of Richard Scott Gardner's thesis. I grant him permission to publish my work in his thesis and to include me as a co-author.

*Richard L. Lindroth*

4/22/2013

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April 22, 2013

Richard Scott Gardner  
Department of Wildland Resources  
5230 Old Main Hill  
Utah State University  
Logan, UT 84322-5230  
615-351-5410 / r.gar@aggiemail.usu.edu

Robert Justin DeRose  
Rocky Mountain Research Station, Forest Inventory and Analysis  
507 25th Street  
Ogden, UT, 84401

Dear Dr. DeRose:

I am in the process of preparing my thesis in the Department of Wildland Resources at Utah State University. I hope to complete in the Spring of 2013.

I am requesting your permission to include your contributions as a co-author in my thesis to be published by Utah State University.

Please indicate your approval of this request by signing in the space provided below. If you have any questions, please call me at the number above.

Thank you for your cooperation,

Richard Scott Gardner

---

I have contributed as a co-author on Chapter Three of Richard Scott Gardner's thesis. I grant him permission to publish my work in his thesis and to include me as a co-author.

  
Signature

  
Date