Analysis of Old-Growth Sugar Pine (*Pinus lambertiana*) in the Stanislaus-Tuolumne Experimental Forest, California.

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Introduction

Understanding the mechanics of conifer resource allocation strategies and their relationship to constitutive and induced chemical defenses is an essential component in predicting mortality rates and modeling advanced stand dynamics. Changes in climate, unprecedented accumulations of fuels resulting from fire exclusion, and shifts in the spatial and temporal scales of insects and pathogen activity continue to threaten the longevity of many conifer species. These combined abiotic and biotic factors, which contribute to tree stress, necessitate the ability to quantify factors affecting defense resource allocation strategies (Mattson & Haak 1987; van Mantgem et al. 2004; van Mantgem & Stephenson 2007; Schwandt et al. 2010; Tomback & Achuff 2010, Roy et al. 2014).

Many species of white pine face numerous challenges resulting from complex interactions of climate, fire exclusion, pathogens, and insects (van Mantgem 2004; Schwandt et al. 2010; Tomback & Achuff 2010). Sugar pine (*Pinus lambertiana*) is recognized as a particularly threatened species due to substantial changes in stand structure, species composition, and disturbance regimes within its native ranges (Kitzmiller 2004; van Mantgem 2004). Current high-density forests are more susceptible to insect and pathogen attacks as well as stand-replacing wildfires (Smith et al. 2005; Fettig et al. 2007; Maloney et al. 2008). Increases in average temperatures coupled with recurrent drought years have also increased stress in larger trees (Miller & Urban 1999; van Mantgem 2004; Battles et al. 2007). In addition, changes in climate that have resulted in prolonged summer seasons and decreased winter periods have been correlated with increased bark beetle activity, as many species of bark beetles have been shown to reproduce for longer time periods under these warmer conditions (Williams & Liebhold 2002; Bentz et al. 2010).

Within pine species (*Pinus* spp.) resin duct structures and associative oleoresin flow represent the primary defense mechanism to bark beetles and their fungal associates (Lewinsohn 1991; Klepzig et al. 1995; Paine et al. 1997; Phillips & Croteau 1999; Nagy et al. 2000). Resin duct structures within pines are elaborate and extend in the vertical, radial, and transverse directions throughout the xylem and phloem. These structures form extensive networks capable of quickly transporting large volumes of resin to various areas of the tree in response to physiological demands (Lewisohn et al. 1991; Phillips & Croteau 1999; Perrakis & Agee 2006; Wainhouse et al. 2009). Quantifying resin flow in this regard represents an effective pursuit in the analysis of defensive characteristics as they specifically relate to mortality.

This study looks to specifically investigate potential relationships between thinning treatments, topographic characteristics, and general resin production of old-growth sugar pine in the western Sierra

Nevada, California. Within old-growth (> 200 years) sugar pine we expect to observe an influence from thinning treatments on overall oleoresin production. We believe that trees that have been subjected to thinning treatments produce larger quantities of resin on average than those in the control groups. This increased resin production is expected as thinning should theoretically increase resource availability for the remaining trees, resulting in a greater allocation of these abundant resources to defensive compounds.

Methods

__Site Location__

Data was collected from the Stanislaus-Tuolumne Experimental Forest (38°10.4´ N, 120°0.0´ W), located approximately 50 km east of the town of Sonora, in the western Sierra Nevada, California (Figure 1). In 2009, 25 permanent plots covering approximately 135 acres were established in the forest to track annual change as part of an ongoing study of old-growth pine mortality in relation to silvicultural and prescribed fire treatments (Adams et al. 2004). Twenty of the plots were subjected to thinning treatments in 2011 while the remaining five were left as a control group. Prior to these thinning operations, fire suppression was the only active management across the study site. These plots served as the basis for this study and all measurements were recorded between May and August of 2014.

Topography across the site was moderate with elevations ranging from approximately 1,813 meters to 1,940 meters. Vegetation within the study sites consisted predominantly of sugar pine (*P. lambertiana*), Jeffrey pine (*P. jeffreyi*), ponderosa pine (*P. ponderosa*), incense cedar (*Calocedrus decurrens*), and white fir (*Abies concolor*). Understory vegetation was relatively sparse but contained a wide variety of flora, including deerbrush (*Ceanothus integerrimus*), mountain whitethorn (*Ceanothus cordulatus*), greenleaf manzanita (*Arctostaphylos patula*), and wood rose (*Rosa gymnocarpa*) among other various species of shrubs and grasses.

Thinned plots were open with predominantly large old-growth trees variably retained based upon their current landscape position. *Pinus* spp. represented the majority of these trees with large white fir and incense cedar scattered throughout the unit. Overall density was significantly greater in the control plot with abundant white fire and incense cedar regeneration.

__Data Collection__

Forty sugar pines were randomly selected across the overall site while ensuring even distribution throughout the study plots to help control for microsite variability. In order to assess the potential influence of silvicultural treatments, 20 trees were selected from control units while an additional 20 were selected from thinned plots. Due to the relatively limited number of control to thin plots (five control vs. 20 thinned), four trees were selected per control plot while only one or two were typically identified within any given thinned plot.

At each tree diameter at breast height (DBH), recorded at approximately 1.37 meters, tree height, canopy base height, canopy width, bark thickness, phloem thickness measurements, and rates of oleoresin flow within a controlled 24-hour period were recorded. Resin was collected by removing a 25 mm section of phloem tissue with the use of a leather punch after which a V-shaped metal funnel constructed from a light tin-alloy was secured to the wounded area via push-pins in the adjacent bark. A

14-mL vial was then placed flush against the bole to collect resin flowing from the site of injury. Resin was collected within a 24 hour period for all trees to help reduce potential influences of climate.

Data Analysis

Data analysis was performed primarily in R and NCSS statistical software package (NCSS Statistics 2009). We looked to initially compare and contrast differences in the properties of resin structures and resin flow between each of the respective treatment types (thinned and control) via simple statistical procedures, including comparative means tests (t-tests) and analysis of variance (ANOVA). For the purpose of this investigation, the total amount of resin flow per tree reflects the dependent variable while phloem area, canopy measures, and treatment type compose the suite of independent variables. We additionally use phloem area as an explanatory variable in our analysis. This potentially represents a novel aspect of our study as this type of approach is not well documented in related literature. We used the equation for area of an annulus to determine the total area of phloem, which can be thought of as an individual ring of tissue located between the inner xylem and the cork cambial layer of the outer bark. While average phloem thickness may decrease with increased radial growth phloem area should theoretically increase (Kolb et al. 1998, 2006; Meinzer et al. 2011) which makes this an ideal metric for correlation to tree diameter. Preliminary tests of assumptions within the resin flow and phloem area data showed they were not normal in distribution. A logarithmic transformation was applied to these variables which resolved this issue and these served as the primary variables for subsequent analysis.

GIS Analysis

ArcMap 10.1 (Esri, Inc.) was used for the geospatial analysis of information, where the synthesis of shapefile and raster data allowed us to quantify the spatial relationships within the study. Each dataset was reviewed for Quality Assurance Quality Control standards and projected, if required, to the North American Dataset 1983, Universal Trans Mercator, Zone 10 North, spatial reference system. Geoprocessing tools provided by Esri, Inc. were used to narrow the scope of the data in our analysis by clipping irrelevant data and emphasizing spatial relationships. Using the “Select by Attributes” tool within ArcMap, samples were organized to describe selected traits and rated based on individual tree measurements to describe diameter at breast height (DBH), tree height, and resin production per tree. The results were then compared to different environmental characteristics such as slope, aspect, and elevation, which were derived from the Digital Elevation Model (DEM) using tools from the Spatial Analyst extension made possible by Humboldt State University. The Spatial Analyst tools were used to delineate landform geomorphology, and by “Reclassifying” the information to describe statistical similarities, we compared the differences in environmental conditions to the selected tree measurements in order to test our hypotheses. Categorical breaks in the ‘Reclass’ tool were calibrated manually to describe different classes of information (ie. 100-ft elevation intervals, Five-foot tree height intervals, DBH, and resin production intervals per milliliter). Additional map traits such as hillshade and contour lines were added to better orient the reader with elevation changes around the study site.

Results

Analysis of Variance (ANOVA) tests between treatment groups did not yield significant differences between treatment type (thinned and control) and overall oleoresin flow ($p < 0.062$; Table 1; Figure 7). However, due to high within- and between-tree variability of resin flow, one could evaluate significance at the 0.1 α level, with the understanding that this will increase the probability of type 1 error compared with a stricter level of α (Zar 1999; Perrakis & Agee 2006). Under such conditions there is a significant difference between treatment groups and oleoresin production with trees in the thinned groups producing more oleoresin on average (0.414 ± 0.097) than trees in the control plots (0.151 ± 0.097).

No other factors (DBH, height, canopy base height, bark thickness, or phloem area) were found to be significant between the thinned and control groups (Table 1). This suggests that resin flow and resin production are highly variable and may fluctuate largely independent of commonly associated (and easily measured) tree parameters.

In relation to topographic features, trees with the greatest amount of resin production (> 6.0 mL) were found predominately on north-facing slopes (Figure 2). This same pattern held largely for DBH with trees in the largest DBH classes occurring primarily on north-oriented slopes (Figure 3). Elevation seemed to have minimal influence on overall tree height (Figure 4), although elevation gradients across the study site in general were relatively minimal (127 meters between highest and lowest measured points of elevation). The majority of high resin-producing trees were found within the 1,750 meter elevation gradient and also occurred within thinned units (Figure 5, 6).

Conclusion

In general, results of this particular study did not find statistically significant differences between resin flow in the control and thinned units when evaluating at the traditional 0.5 α level. However, thinning could have a significant influence on resin production if evaluation of these results is conducted at a more permissive α level. Thinning operations reduce competition, theoretically increasing resource availability and resin production for residual trees in thinned units. This study generally supports those assumptions as the majority of high-volume resin producers were found within thinned units and trees within thinned plots had a much higher production rate on average (0.414 ± 0.097) compared to trees within the control plots (0.151 ± 0.097).

However, there are many limitations within this study that warrant consideration. Resin flow samples were collected within a 24 hour period in order to account for environmental variability. However, these rates of resin production could have been measured over an extended temporal scale in order to achieve a more realistic understanding of resin production throughout the year. Resin flow rates are highly correlated with air temperature (Smith 2000; Perrakis & Agee 2006; Baier et al. 2002) so increasing the window of analysis would help to reduce potential variability and provide a more realistic picture of resin production. In addition, topographic gradients across the site were mild and relatively consistent which reduces the effectiveness in extrapolating these results to other regions. Additional research of this nature utilizing a broader list of subjects would help improve on the fundamental design of this study.

Understanding resin production is an important component in tree physiology, forest health, and natural resource management. There is a demonstrated need to continue in our investigation of these unique defensive features and their influence on mortality. This is especially true considering the extensive and highly variable impacts resulting from disturbance interactions which often have complex and unpredictable outcomes. As such, we recommend that additional research on constitutive defense systems is conducted to ensure proper recognition of all potential variables related to the prosperity of old-growth sugar pine. We believe that understanding the implications of silvicultural treatments on resin production is of tremendous importance in the successful management of not only sugar pine, but many other important species throughout the western Sierra Nevada. We hope that our research will serve as an important resource for land managers in the continued stewardship of these unique and valuable ecosystems.

Table 1. Assessment of various physiological tree measures between trees in thinned vs. control plots. Significance is evaluated at the 0.5 α level. Data was collected from old-growth sugar pine (*Pinus lambertiana*) trees in the Stanislaus-Tuolumne Experiment Forest, California.

Factor	F-Ratio	Significance
DBH	0.01	0.944
Height	1.73	0.196
Canopy Base Height	1.56	0.220
Bark Thickness	0.26	0.613
Phloem Area (log)	0.00	0.961
Resin Amount (log)	3.69	0.062

Figure 1. Locator map for study site in the Stanislaus Tuolumne Experimental Forest, Tuolumne County, California.

Source: USGS EarthExplorer / LANDFIRE / Field Research (NAD83, UTM Zone 10 North, Meter)

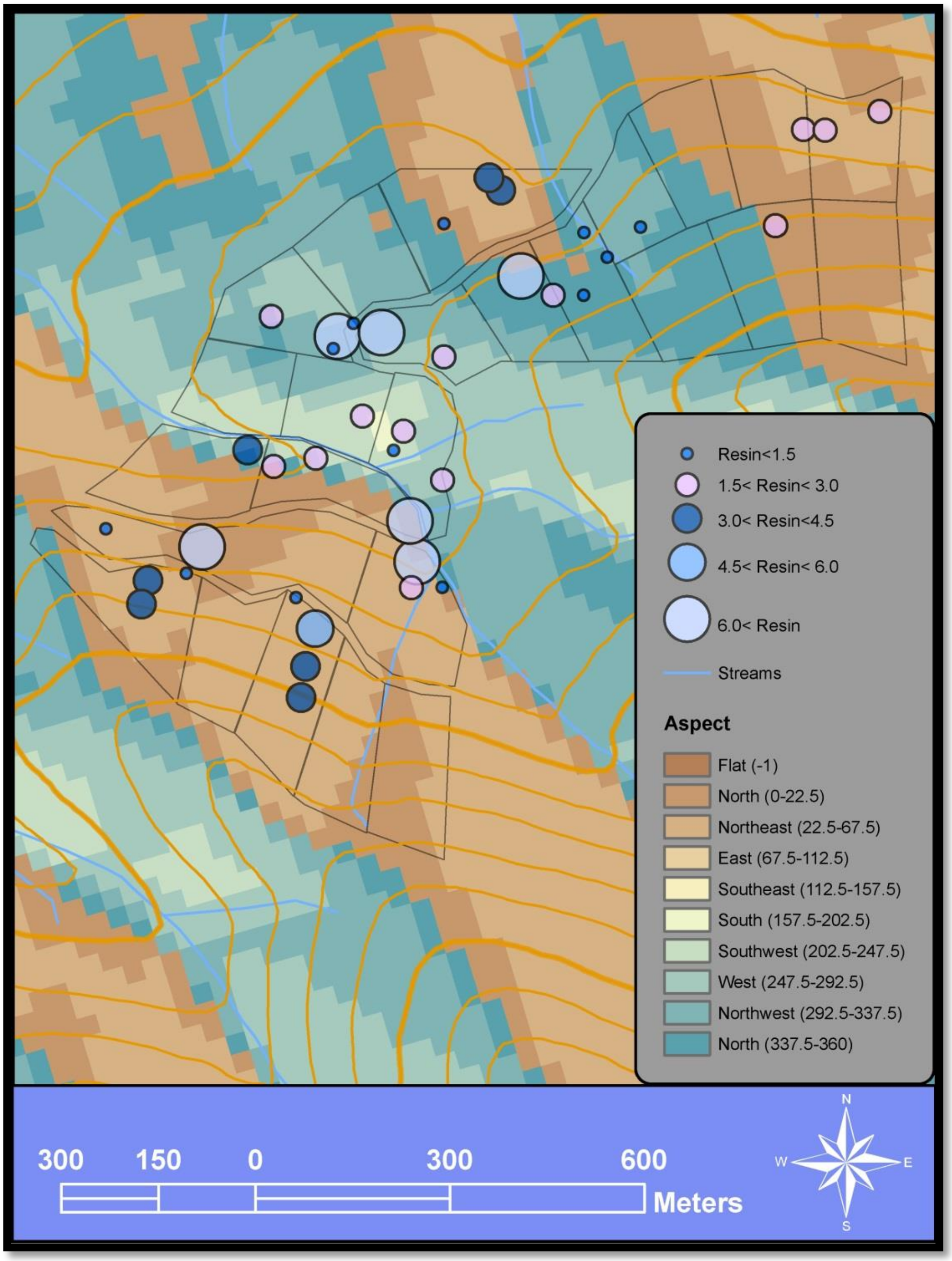

Figure 2. Aspect in relation to overall resin production of sugar pines within study site located in the Stanislaus Tuolumne Experimental Forest, California.

Source: USGS EarthExplorer / LANDFIRE / Field Research (NAD83, UTM Zone 10 North, Meter)

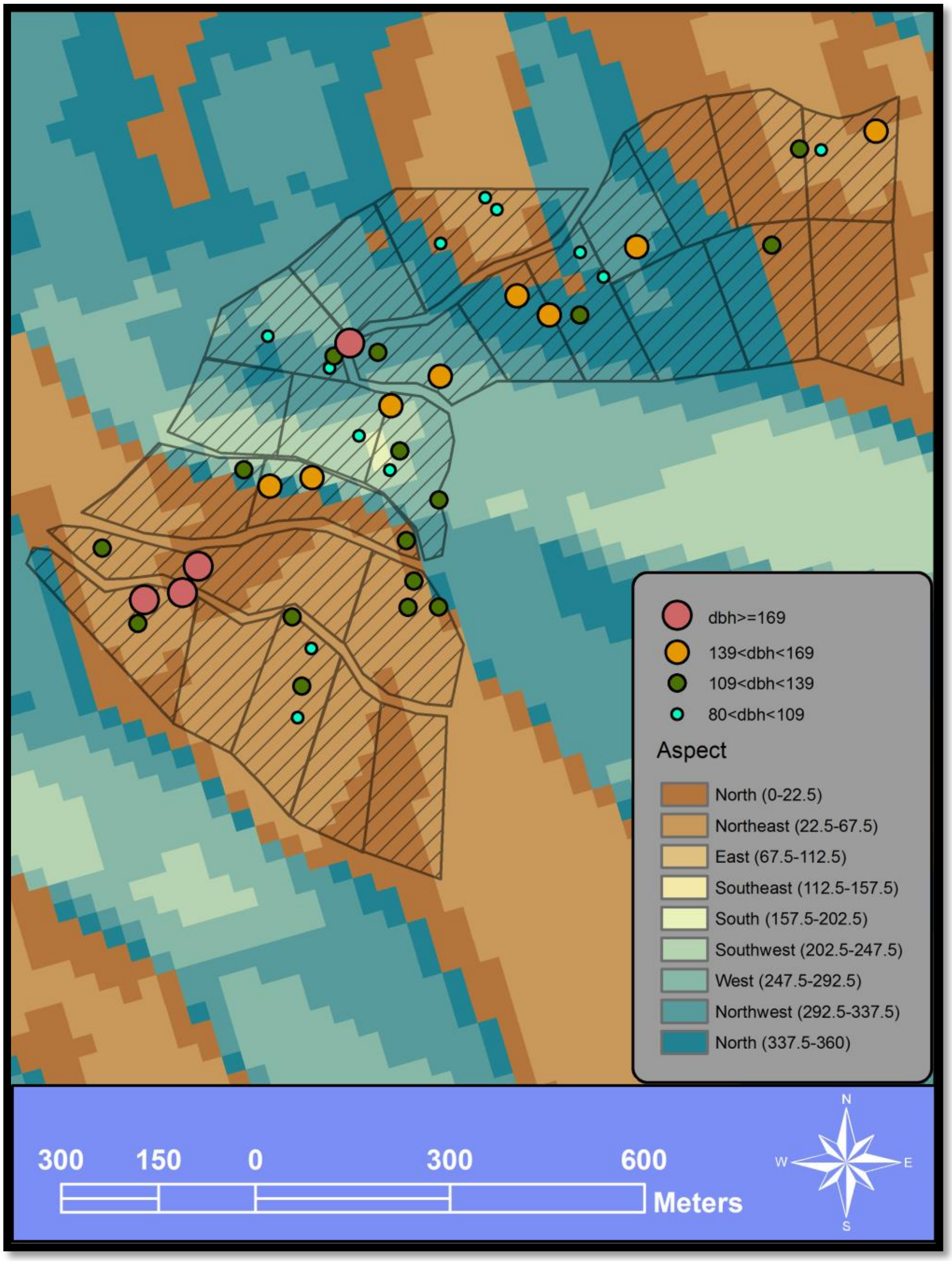

Figure 3. Aspect in relation to DBH of sugar pines within study site located in the Stanislaus Tuolumne Experimental Forest, California.

Source: USGS EarthExplorer / LANDFIRE / Field Research (NAD83, UTM Zone 10 North, Meter)

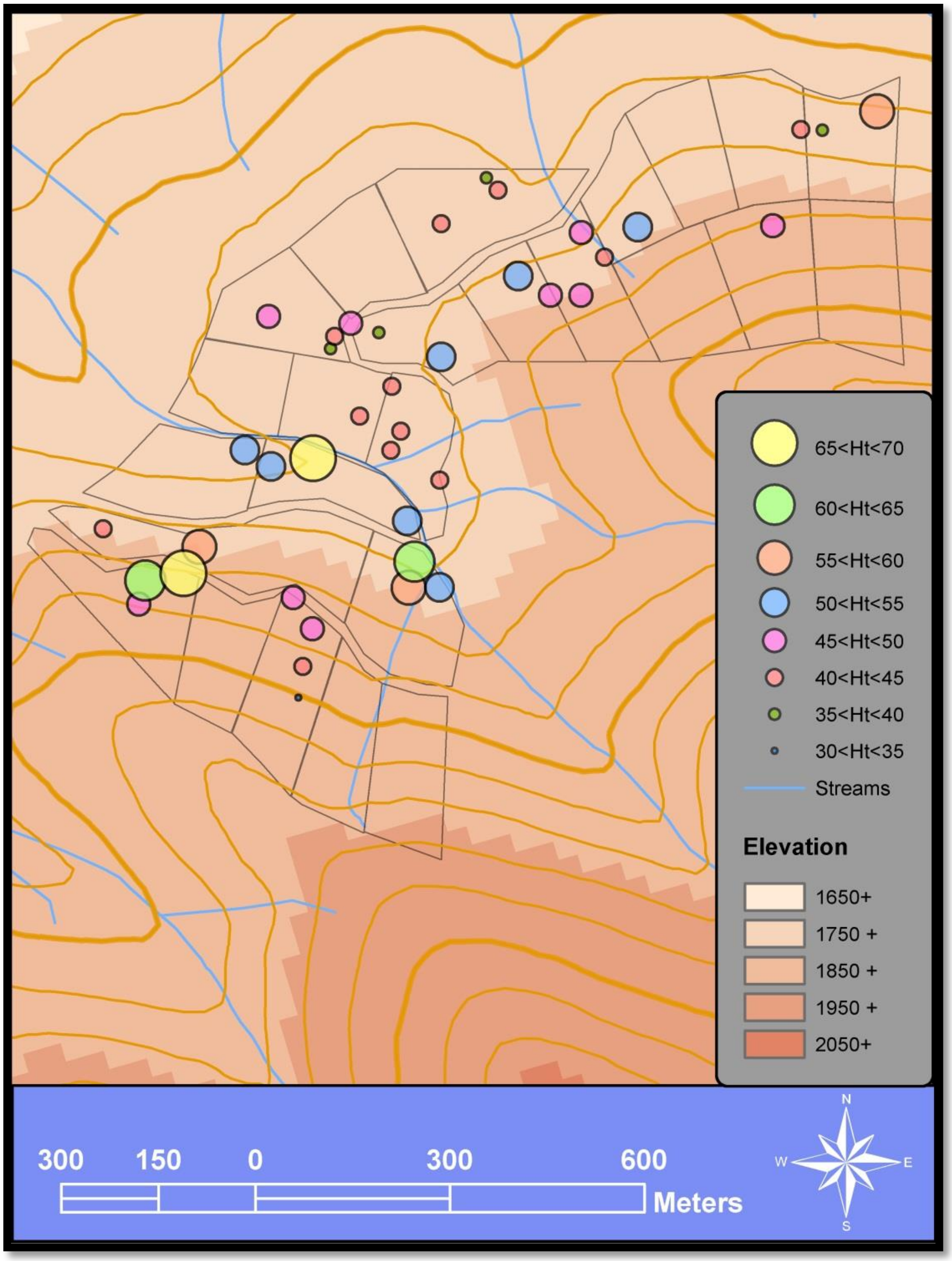

Figure 4. Elevation in relation to overall height of sugar pines within study site located in the Stanislaus Tuolumne Experimental Forest, California.

Source: USGS EarthExplorer / LANDFIRE / Field Research (NAD83, UTM Zone 10 North, Meter)

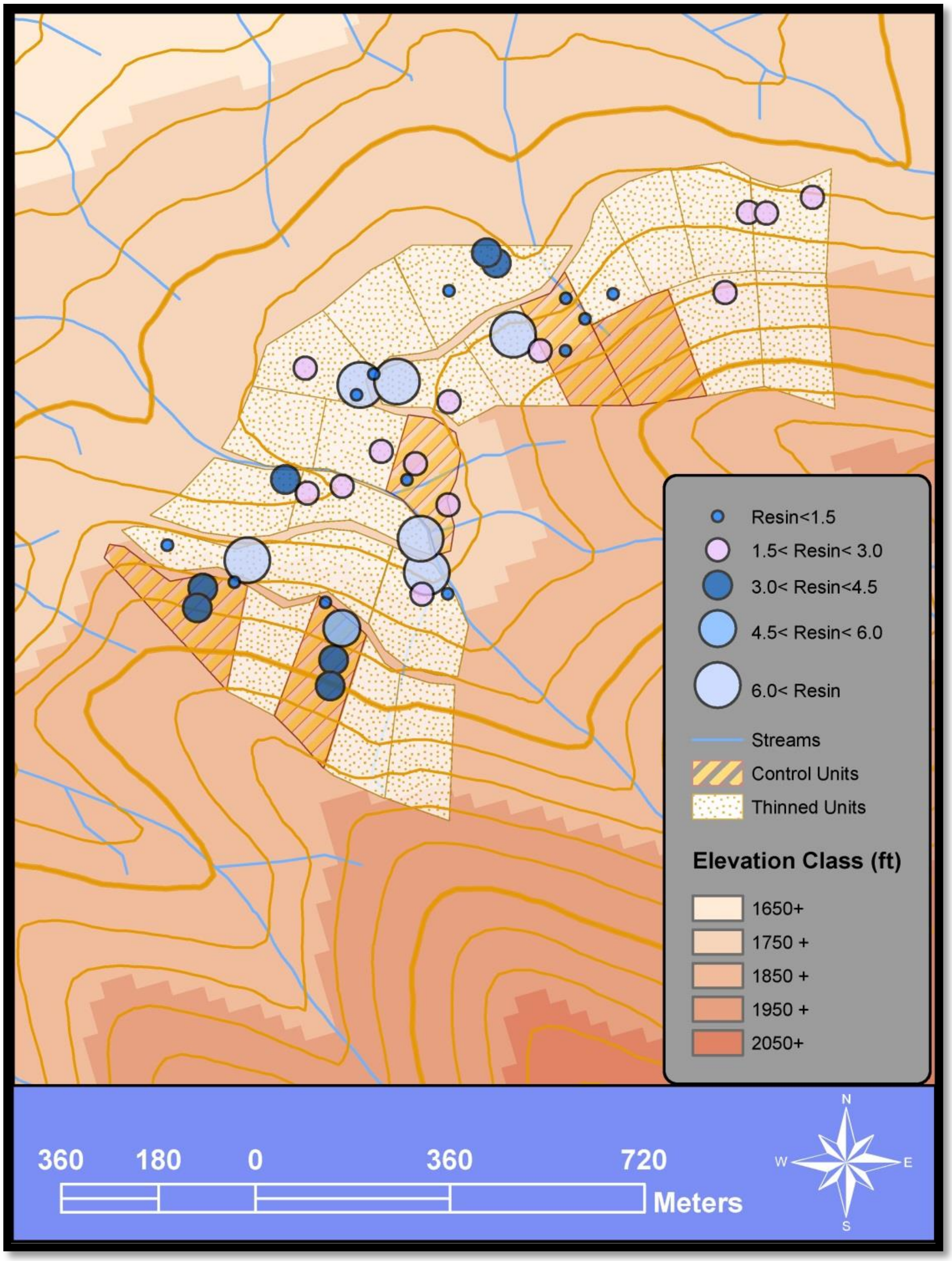

Figure 5. Elevation in relation to resin production and treatment type (thinned vs. control) in study site in the Stanislaus Tuolumne Experimental Forest, California.

Source: USGS EarthExplorer / LANDFIRE / Field Research (NAD83, UTM Zone 10 North, Meter)

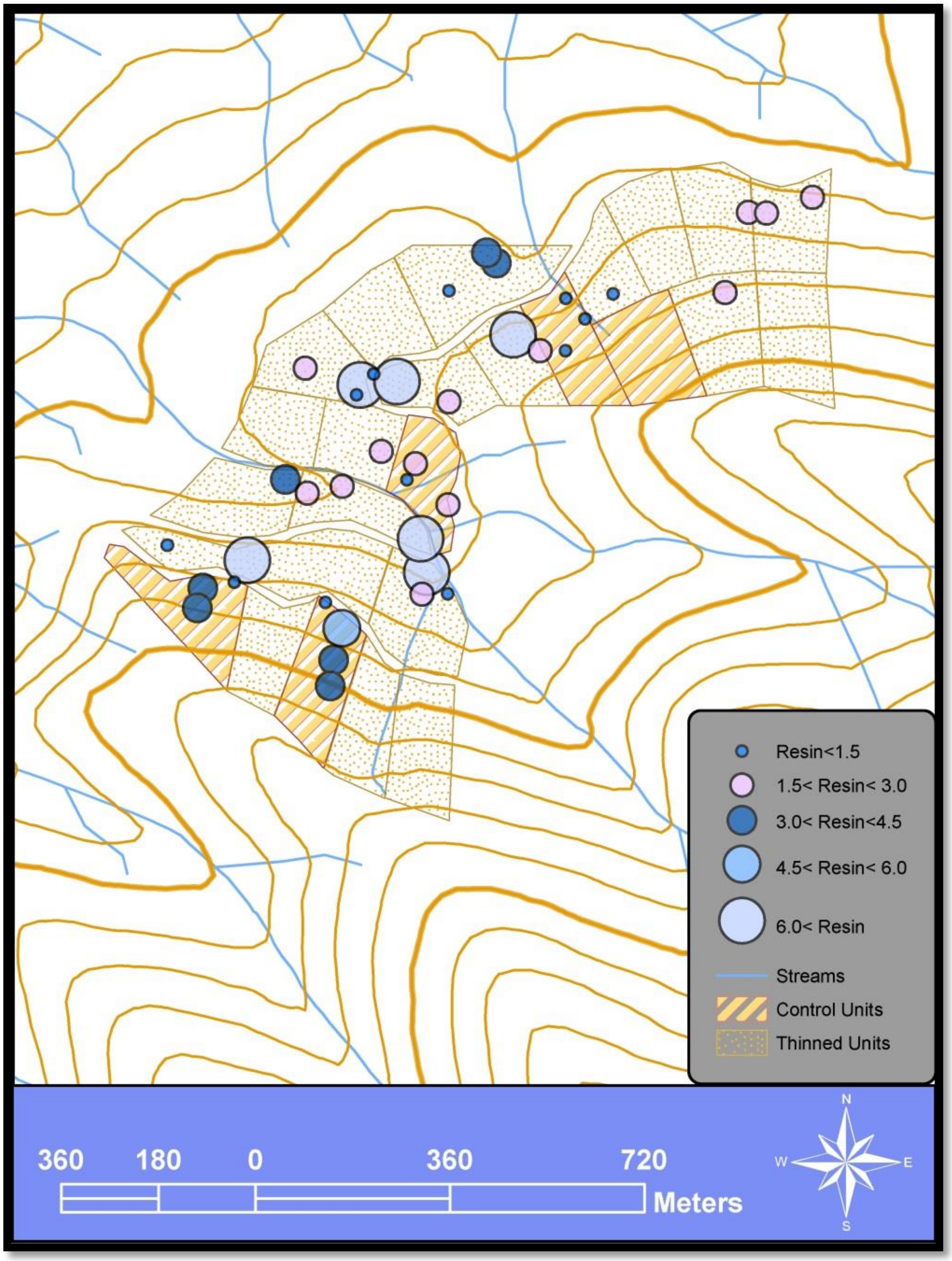

Figure 6. Resin production in relation to treatment type (thinned vs. control) in study site in the Stanislaus Tuolumne Experimental Forest, California.

Source: USGS EarthExplorer / LANDFIRE / Field Research (NAD83, UTM Zone 10 North, Meter)

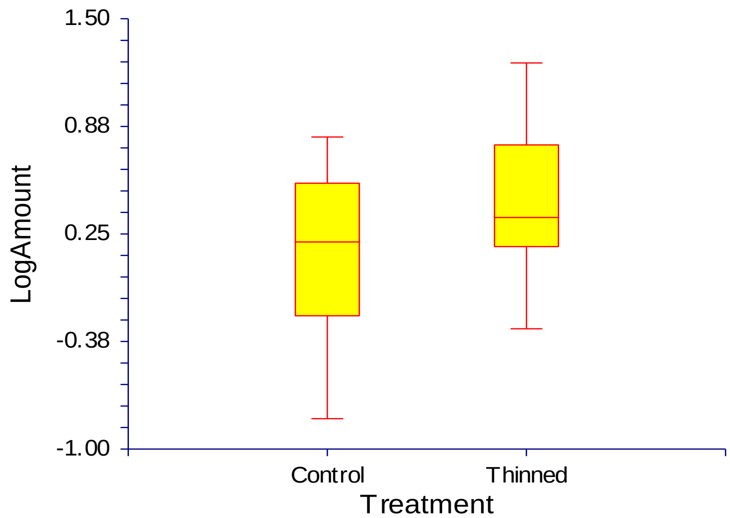

Figure 7. Standard box plot depicting difference between treatment (control and thinned) and resin flow amount (subjected to logarithmic transformation to accommodate for irregular distribution in empirical data). $P < 0.062$.

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