

**RECIPROCAL TRANSPLANT AND MACHINE LEARNING STUDY OF OAK MISTLETOE ON
THREE HOST OAK SPECIES IN SANTA MARGARITA, CALIFORNIA**

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by

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mistletoe on three host oak species in Santa Margarita,
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ABSTRACT

RECIPROCAL TRANSPLANT AND MACHINE LEARNING STUDY OF OAK MISTLETOE ON THREE HOST OAK SPECIES IN SANTA MARGARITA, CALIFORNIA

Ella Abelli-Amen

At Santa Margarita Ranch, California, oak mistletoe (*Phoradendron villosum*) parasitizes valley oak and blue oak but cannot be found growing on coast live oak despite its abundance and ability to parasitize coast live oak in other areas. It seems as though this species of mistletoe is specializing on certain host oak trees, but the mechanisms of this specialization are unknown. In order to investigate this pattern, we utilized a type of machine learning in GIS called supervised classification as well as a reciprocal transplant study in the field. The three species of oak trees were classified with 87% accuracy using drone imagery and 95% accuracy using open source NAIP imagery. This classification technique could be applied to the whole state of California as long as ground truth points for each species were collected. This could be extremely useful for large scale forest management projects and ecological questions. Unfortunately, the classifier was unsuccessful at distinguishing mistletoe from host and so the number of mistletoe on each host could not be quantified using this technique. The reciprocal transplant study involved collecting mistletoe fruit from individuals growing on each of the three hosts and experimentally applying them back onto all three hosts. This allowed us to test whether there are host races of mistletoe that specialize at growing on certain hosts. We found that seeds from each host origin germinated equally well regardless of where they were dispersed, and seeds survived best on coast live oak, regardless of where they originated from. Based on these results, there must be some mechanism, other than host races, that explains the lack of mistletoe on coast live oaks at Santa Margarita Ranch. Future projects should investigate whether evidence for host races can be found at a later stage of seedling development and the roll of bird dispersers in creating the pattern.

Keywords: mistletoe, oaks, host specificity, host races, reciprocal transplant, supervised classification

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In loving memory of my mom, Terese.

You always told me I could do anything I set my mind to.

TABLE OF CONTENTS

Page

LIST OF FIGURESviii

CHAPTER I: MECHANISMS OF HOST SPECIFICITY IN *PHORADENDRON VILLOSUM*: A RECIPROCAL TRANSPLANT STUDY

1. INTRODUCTION 1
 1.1 Background 1
 1.2 Purpose of study 3
2. MATERIALS AND METHODS 4
 2.1 Study site 4
 2.2 Study species 5
 2.3 Experimental design: reciprocal transplant study 6
 2.4 Seed fate 7
 2.5 Data analysis 8
 2.6 Climate and timeline 9
3. RESULTS 10
 3.1 Germination 10
 3.2 Survival past one year 11
4. DISCUSSION 12
 4.1 Take-aways 12
 4.2 Germination 12
 4.3 Survival 12
 4.4 Climate 13
 4.5 Future directions 13
 4.6 Conclusion 14

CHAPTER II: SUPERVISED CLASSIFICATION OF THREE OAKS AND OAK MISTLETOE

1. INTRODUCTION 15
 1.1 Background 15
 1.2 Purpose of project 16
2. MATERIALS AND METHODS 16
 2.1 Field work 16
 2.2 Imagery 18
 2.3 Computer analysis 18
3. RESULTS 21
 3.1 Overall results 21
 3.2 Oak Classification Results 22
 3.3 Mistletoe Classification Results 23
4. DISCUSSION/ CONCLUSION 24

CHAPTER III: TUTORIAL

1. USING THE ARROW GOLD GPS WITH ARC GIS ONLINE MAPS TO COLLECT GROUND TRUTH POINTS FOR THE CLASSIFIER.....	27
2. USING THE TRUPULSE LASER AND TRUANGLE WITH THE ARROW GOLD GPS.....	29
3. SUPERVISED CLASSIFICATION OF NAIP IMAGERY	31
4. GETTING APPROVAL FROM CAL POLY TO FLY A DRONE	36
5. FLYING THE DRONE	37
6. PROCESSING DRONE IMAGERY (CREATING AN ORTHOMOSAIC)	40
REFERENCES.....	42
APPENDIX.....	47

LIST OF FIGURES

Figure	Page
1. The range overlap where coast live oak, valley oak, and blue oak co-occur in purple. The Santa Margarita Ranch study site marked by the star.	4
2. <i>Phoradendron villosum</i> herbarium occurrences in California. Green dots are <i>P. villosum</i> growing on <i>Quercus spp.</i> and grey dots are on all other hosts.	6
3. A <i>P. villosum</i> seed germinating on valley oak, a few months after being applied to a branch. The green structure emerging from the seed coat is the haustoria which will, if the seed is to be successful, form a connection with the host tree’s xylem.	8
4. Three examples of <i>P. villosum</i> seeds which survived one year after experimental dispersal: haustoria has become plumper and is clearly still alive (green in color, whereas most other seeds were brown and shriveled up)	8
5. Timeline of actions taken during the experiment overlaid with climate data. The daily range of reported temperatures (gray bars) and 24-hour highs (red ticks) and lows (blue ticks), placed over the daily average high (faint red line) and low (faint blue line) temperature, with 25th to 75th and 10th to 90th percentile bands. Weather data taken from weatherspark.com which sourced the data from the nearby Paso Robles Municipal Airport.....	9
6. Boxplot of observed proportion of seeds germinated by host origin and host destination. If there was evidence for host races at the germination stage, the seeds applied on the same oak (host destination) they were collected from (host origin) would have a higher proportion seed germination, which we did not see (see results section for statistical results).....	10
7. Boxplot of observed proportion of seeds which survived by host origin and host destination. If there was evidence for host races at the survival stage, the seeds applied on the same oak (host destination) they were collected from (host origin) would have a higher proportion of survival, which we did not see (see results section for statistical results). Contrary to our predictions, seeds survived best on coast live oak, despite not occurring on this host naturally at the site.....	11
8. The ground truth points as seen in the collector app for the three oak species. Red are coast live oak, blue are blue oak, and green are valley oak.....	17
9. Canopy Height model made from LiDAR data. Grey pixels are ground level, and everything in color is above the ground (trees).....	19
10. Pixels separated into two bins: taller than 10 ft (white) and shorter than 10 ft (black).....	19
11. The NAIP imagery clipped to the locations of the trees found using the canopy height model from LiDAR data.....	20
12. Classified result with five categories: coast live oak (dark green), valley oak (light green), blue oak (blue green), grass (tan), and bare ground (grey).....	21
13. A comparison of unclassified NAIP imagery to the classified result.....	22

14. Confusion matrix for the drone imagery oak classification. Correct species of oak trees are shown on vertical axis, and predicted oak species are shown on horizontal axis. Correct predictions are found on the diagonal from top left to bottom right. Incorrect predictions are off diagonal.....	22
15. Confusion matrix for the NAIP imagery oak classification. Actual species of oak trees are shown on vertical axis, and predicted oak species are shown on horizontal axis. Correct predictions are found on the diagonal from top left to bottom right. Incorrect predictions are off diagonal.....	23
16. Confusion matrix for the drone imagery mistletoe classification. Whether a tree actually has mistletoe or not is shown on vertical axis, and whether each tree was predicted to have mistletoe or not is shown on horizontal axis. Correct predictions are found on the diagonal from top left to bottom right. Incorrect predictions are off diagonal.....	23
17. Shown is a group of coast live oaks, as classified by the NAIP oak classifier, overlaid with the drone mistletoe classification (light green pixels).....	24
18. Arrow Gold GPS device assembled in the field.....	28
19. A. The fully assembled laser and angle device using all of Dr. Andrew Fricker’s equipment. B. Photo of assembled device from the Laser Offset Mapping with Collector Manual. Some set-up steps are slightly different based on the parts Dr. Fricker has but the overall idea is the same.....	30
20. Canopy Height model made from LiDAR data. Grey pixels are ground level, and everything in color is above the ground (trees).....	32
21. Pixels separated into two bins: taller than 10 ft (white) and shorter than 10 ft (black).....	33
22. The NAIP imagery clipped to the locations of the trees found using the canopy height model from LiDAR data.....	33
23. Classified result with five categories: coast live oak (dark green), valley oak (light green), blue oak (blue green), grass (tan), and bare ground (grey).....	35
24. Confusion matrix for the NAIP imagery oak ID classification. Actual species of oak trees are shown on vertical axis, and predicted oak species are shown on horizontal axis. Correct predictions are found on the diagonal from top left to bottom right. Incorrect predictions are off diagonal.....	36

Chapter 1: MECHANISMS OF HOST SPECIFICITY IN *PHORADENDRON VILLOSUM*: A RECIPROCAL TRANSPLANT STUDY

1. INTRODUCTION

1.1 Background

Mistletoe are a group of independently evolved parasitic plants that includes more than 1,300 species across five families worldwide. Mistletoe grow in the canopy of host trees and shrubs, and, while they make their own sugars via photosynthesis, they steal water and minerals from their host using a specialized root (haustoria) that is able to penetrate the host's vascular system (Nickrent, Daniel, 2000). Although many species of mistletoe are generalist parasites, some exhibit host-specific adaptations which can lead to host specialization and even speciation (Norton and Carpenter, 1998).

Host specificity emerges when a parasite occurs on only a subset of available hosts. This has been documented in a number of mistletoe across two major families, the Loranthaceae and the Viscaceae. For example, a transect study in Kentucky found that *Phoradendron leucarpum* (Viscaceae) parasitizes only four tree species 89% of the time, when eight other potential hosts are also present (Thompson and Poindexters, 2005). A study in Brazil found that 87% of *Phoradendron crassifolium* (Viscaceae) infections were restricted to 27% of the available hosts, all in the Anacardiaceae (Messias et al., 2014). This observational study was followed by a transplant experiment where seeds were applied to both trees commonly parasitized by the mistletoe and trees never before observed with mistletoe infections: the seeds were able to germinate and survive on all hosts. In New Zealand, three of five focal mistletoe in the Loranthaceae were found to primarily parasitize *Nothofagus* (despite many other available hosts), and degree of host specificity was highly correlated with primary host abundance (Norton and Lange, 1999). In California, the oak-mistletoe, *Phoradendron villosum*, is restricted to certain species of *Quercus*, especially in mixed-tree stands (Thomson and Mahall, 1983).

While the patterns of host specificity are well documented around the world, the underlying mechanisms are still being investigated for many mistletoe species. Work on *Plicosepalus acaciae* (Loranthaceae) in Israel has shown that seedlings germinate on their preferred host at higher rates, which they hypothesized is due to chemical cues from the host (Rodl and Ward, 2002). Conversely, a

study in Texas on *Phoradendron tomentosum* (Viscaceae) found that there was no difference in germination when seeds were planted onto three different host species but there was a significant difference in early seedling establishment which explains the pattern of host preference observed in that population (Clay, Dement, and Rejmanek, 1985). Apart from chemical cues from the host, Arruda et al. found that seedling establishment was most successful on twigs with rough bark and less than 1.4 cm in diameter (Arruda et al., 2006).

Even in generalist species, there can be local adaptation to certain host species, i.e., specialist genotypes (also known as host races). For example, a widespread generalist species of mistletoe called *Arceuthobium americanum* was found to be divided into three distinct genotypes based on geography and primary host. Due to the lack of morphological or phenological differentiation, the authors recommend these three genotypes remain a single species (Jerome and Ford, 2002). The California desert mistletoe, *Phoradendron californicum*, has been found to have two genetically and morphologically distinct host races that grow on cat-claw acacia and honey mesquite (Glazner et al., 1988). *Viscum album* in Europe was found to be divided into four genetically distinct lineages, three of which were distinct based on primary host and one which was geographically isolated. The authors recommended the splitting of this species into four new taxa (Zuber and Widmer, 2009).

An alternative mechanism to explain the host specificity could be differential dispersal by birds. With few exceptions, mistletoe fruit are consumed and dispersed to new infection sites by birds: a study done on *Phoradendron californicum* found that birds dispersed mistletoe seeds more frequently to taller, open canopy trees than to short trees with dense and spiny crowns (Caraballo-Ortiz et al., 2017). The pattern of mistletoe infection of trees in Baja California was attributed to bird dispersal and not to mistletoe establishment success: the probability of mistletoe dispersal to a tree increased with tree size (Overton, 1994). A study on an African mistletoe found that birds were more likely to disperse seeds to taller trees *and* they were more likely to survive there (Roxburgh and Nicolson, 2008). There is evidence that the patchy distribution of oak mistletoe (*P. villosum*) is the result of western bluebird (this mistletoe's primary disperser) territoriality around pre-existing mistletoe resources (Wilson et al., 2014). Two mistletoe species and their avian dispersers were studied in Africa: it was found that bird's preference for certain host species shaped the occurrence pattern of

these mistletoe species, not host-mistletoe compatibility (Roxburgh and Nicolson, 2005). Considering all of these examples, birds can have a profound effect on the occurrence patterns of mistletoe and could play a role in creating the patterns of host specificity.

1.2 Purpose of study

In this study, we test whether host-race specialization explains local patterns of host specificity in *Phoradendron villosum* (J.R. Abbott & R.L. Thomps) at Santa Margarita Ranch, California (Figure 1). This native mistletoe occurs on approximately 45 tree taxa across California (data from California Consortium of Herbaria), and 32 of those taxa are oaks. In the northern 2/3 of its range (from San Luis Obispo to the California/ Oregon boarder), when there is a pure stand of coast live oak (*Quercus agrifolia*, Née) *P. villosum* can be found parasitizing it, but when valley oak (*Q. lobata*, Née) and blue oak (*Q. douglasii*, Hook & Arn) are also present, the mistletoe is restricted to growing on just the latter two deciduous oaks. In order to investigate the mechanism behind this pattern, we tested the hypothesis that there are host races of mistletoe adapted for certain oak species. We answered the study question ‘does *P. villosum* have some barrier to seedling germination and establishment when grown on a host oak species different than the species the seeds were collected from?’

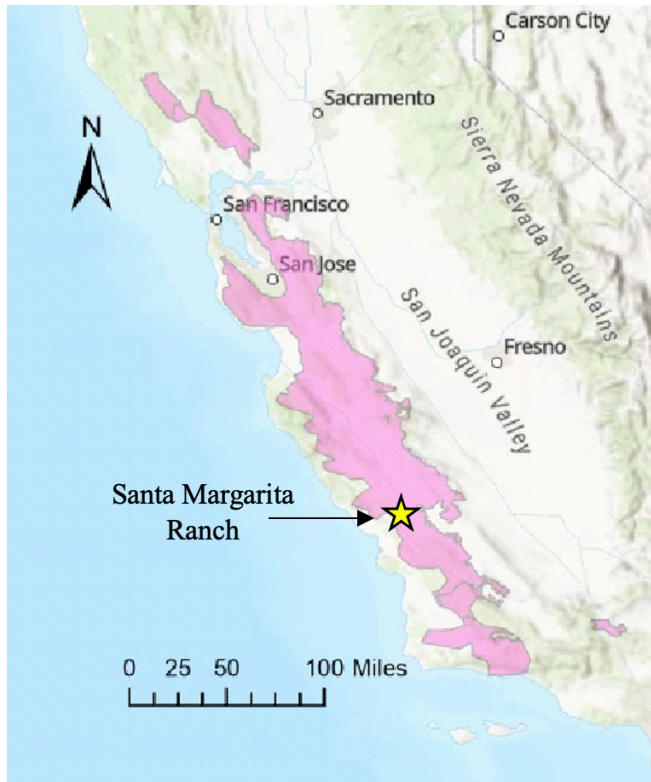


Figure 1. The range overlap where coast live oak, valley oak and blue oak co-occur in purple. The Santa Margarita Ranch study site marked by the star.

2. MATERIALS AND METHODS

2.1 Study site

Field work was carried out in January 2020- April 2021 at the Santa Margarita Ranch located in San Luis Obispo County, California (35°22'53.8"N 120°35'22.7"W, 1,014'). The ranch is characterized by a Mediterranean climate with cool wet winters and hot dry summers. The plant community present at the study site is an old growth oak savanna dominated by coast live oak, valley oak, and blue oak with an understory of non-native grasses and native forbs (Keil, D.J., 2022). This habitat type has been severely reduced across the state to make space for agriculture. At this site, *Phoradendron villosum* parasitizes valley and blue oaks (*Quercus lobata* and *Quercus douglasii*) and exhibits a clumped distribution with many trees unparasitized along with infected trees that often have 10+ mistletoe individuals present. Not a single coast live oak (*Quercus agrifolia*) is infected at this site, despite it being common and interdigitated with the other two oaks.

2.2 Study species

Phoradendron villosum (synonym for *P. leucarpum* sbsp. *tomentosum*) (oak mistletoe) can be found parasitizing trees and shrubs from Baja California and Central Mexico to the Oregon-Washington boarder, but predominately in California where oak trees are dominant (Figure 2). Most often found on *Quercus* sp., but also rarely found other shrubs and trees, *P. villosum* parasitizes at least 45 distinct taxa (data extracted from the California Consortium of Herbaria in Nov 2019, <https://www.cch2.org/portal/collections/list.php>). Oak mistletoe becomes much more conspicuous in the winter months when the deciduous oaks have lost their leaves. Flowers form in late summer and fruits follow in winter and can remain on the plant well into the spring. Fruits are fleshy and one seeded. The seeds pass through the digestive track of birds and are extremely sticky. During the course of this study, bird excrement containing mistletoe seeds was found on branches on several occasions: on *Quercus agrifolia* at Bishop Peak in San Luis Obispo (2020), and on *Quercus douglasii* and a fence post at Taylor Mountain in Santa Rosa (2019). *Phoradendron villosum* germination does not seem to be dependent on passing through the digestive track of a bird (Cannon, 1904). Oak mistletoe is primarily consumed by Western Bluebirds and others occasionally (Coder, 2008). So much is still unknown about this species of mistletoe including sex ratio, why so few individuals are found in fruit, pollinators, and a complete list of avian seed dispersers.

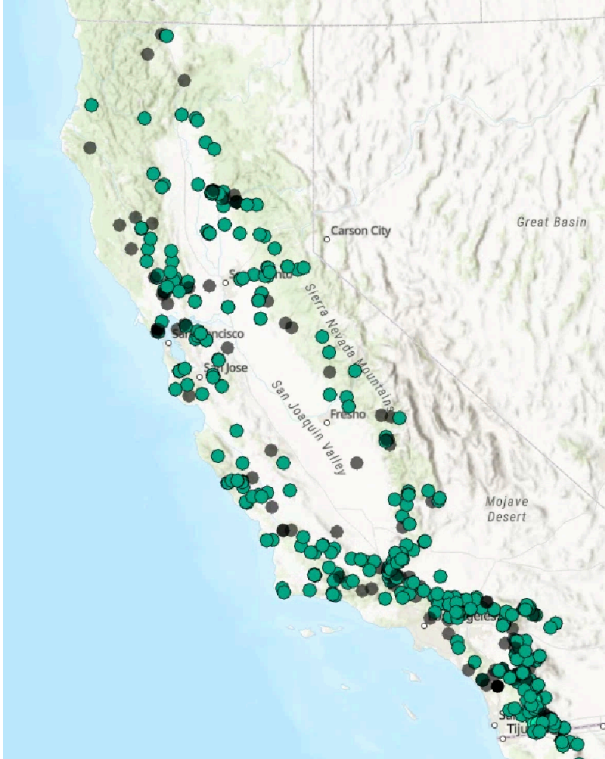


Figure 2. *Phoradendron villosum* herbarium occurrences in California. Green dots are *P. villosum* growing on *Quercus* spp. and grey dots are on all other hosts.

2.3 Experimental design: reciprocal transplant study

Fruits were collected in January 2020 from at least two mistletoe individuals growing on each species of oak using a pole trimmer and added to small petri dishes labeled with their host origin. Mistletoe fruit from two individuals growing on *Q. agrifolia* were collected on Bishop Peak in San Luis Obispo (35.29678, -120.69276); fruit from two mistletoe individuals growing on *Q. douglasii* were collected at Santa Margarita Lake (35.32326, -120.48936); and fruit from three mistletoe on *Q. lobata* were collected at the study site (Santa Margarita Ranch, 35.37815, -120.59199). Collecting fruit from more individuals would have been preferred, but out of hundreds of mistletoe plants observed, only several were found in fruit. These were kept in a refrigerator for a maximum of four weeks while experimental setup was occurring.

Fruit from each host source were transplanted onto branches of all three oak species between February 1, 2020 and February 28, 2020, creating a full 3 by 3 factorial design. Fifteen trees of each oak

species were chosen as experimental hosts at Santa Margarita Ranch. The host trees were selected in a blocked design, so that there was one of each oak species per block (N=15 blocks). Within each block, the trees were chosen opportunistically to minimize microclimate variability and so each was healthy, of similar size, and within ~50 meters of one another. All host trees were marked with a metal tree tag at the start of the study. Whenever an action was performed during this experiment, it was done to an entire block of three experimental host trees on the same day.

On each experimental tree, three branches (with a circumference of 7 cm) were selected for experimental dispersal: these were selected in the North, South, and East cardinal directions whenever possible in case canopy aspect was a confounding factor for germination. In February of 2020, ten seeds (on a few occasions eleven seeds) from each host origin were applied to a branch in three sections that were randomly assigned. For example, on one branch there were 10 seeds from *Q. agrifolia* in a section, followed by 10 seeds from *Q. douglasii*, and then 10 seeds from *Q. lobata*. Seeds within a section were placed approximately one centimeter apart, and the three sections on a branch were separated with a loop of 28-gauge wire wrapped around the branch. For ease of access and because most mature mistletoe at the study site are found in the outer canopy, we selected branches in the mid-lower outer canopy using a 10 ft orchard ladder. Each branch was marked with a metal ID tag and dark green flagging for relocation. Seeds were applied to branches by first removing them from their exocarp and then gently pressing the seed against the branch with a finger. Previous studies have found that naturally sticky mistletoe seeds immediately and effectively adhered to branches (Clay, Dement, and Rejmanek, 1985).

The seeds were experimentally transplanted (also referred to as ‘applied’) during the month of February 2020. The seeds were visited regularly (every two weeks) after that to check if they had germinated, and germination data was recorded for all the seeds in late April 2020. The seeds were observed several times within the next year, but since they did not grow or change much, the final round of survival data was collected in early April 2021.

2.4 Estimating germination and survival after one year

In April 2020, approximately 8 weeks after seeds were applied to branches, seed germination was estimated as the proportion of seeds with visible haustoria out of the total seeds still present on the branch

(mean number of seeds present = 7.15, standard error = 0.14). One year later, in April 2021, survival was estimated as the proportion of seeds with plump and green haustoria out of total the number germinated one year prior.



Figure 3. A *P. villosum* seed germinating on valley oak, 8 weeks after being applied to a branch. The green structure emerging from the seed coat is the haustoria, which will, if the seed is to be successful, form a connection with the host tree's xylem.



Figure 4. Three examples of *P. villosum* seeds which survived one year after experimental dispersal: haustoria has become plumper and is clearly still alive (green in color, whereas most other seeds were brown and shriveled up).

2.5 Data analysis

To test whether germination and survival were predicted by host origin, host destination, or their interaction, we used two separate generalized linear mixed effect models (GLMMs), each with a binomial distribution and the logit link function. Host origin, experimental host, and the interaction of those two were fixed effects, and branch ID was nested within tree ID as a random effect. The response variable was either number of germinates out of number present (model 1) or number of survivals out of germinates (model 2). (R Studio version 1.4.1106, packages ‘lme4’ function ‘glmer’ and ‘car’, function ‘anova’).

2.6 Climate and timeline

The summer of 2020 was unusual in a few ways. First of all, it was the second hottest summer on record in California since 1880 (Climate.gov). The nearest weather station to the field site, the Paso Robles Municipal Airport, recorded three days in a row with temperatures over 110 degrees Fahrenheit (Figure 5). Fires in the area filled the air with smoke, and uncharacteristic heat lightning storms struck. During all this extreme weather, the seeds in this experiment were exposed to the elements.

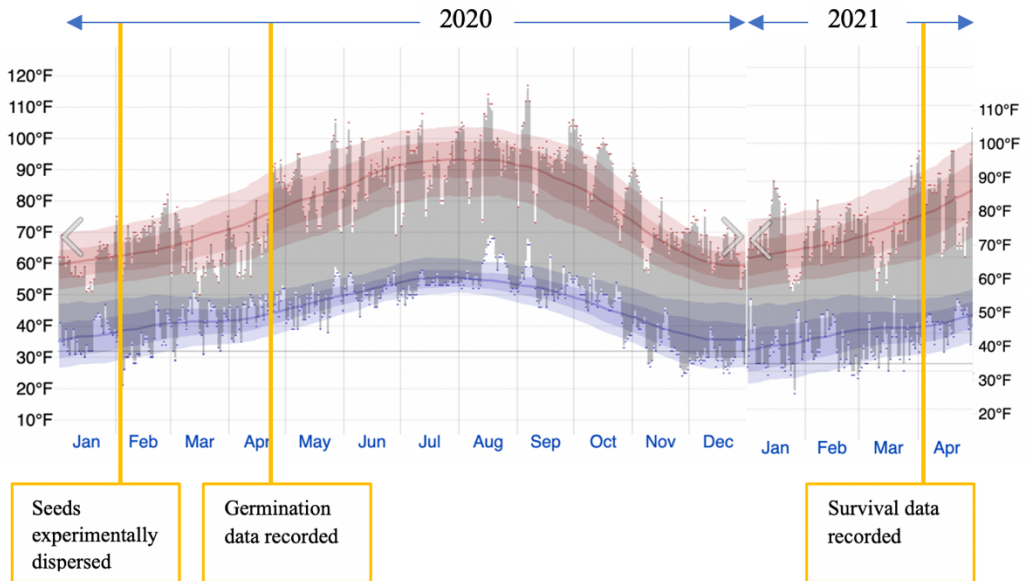


Figure 5. Timeline of actions taken during the experiment overlaid with climate data. The daily range of reported temperatures (gray bars) and 24-hour highs (red ticks) and lows (blue ticks), placed over the daily average high (faint red line) and low (faint blue line) temperature, with 25th to 75th and 10th to 90th percentile bands. Weather data taken from weatherspark.com which sourced the data from the nearby Paso Robles Municipal Airport.

3. RESULTS

3.1 Germination

Forty-five percent of seeds that were experimentally dispersed onto the tree oak species successfully germinated. Germination was strongly predicted by host origin, with seeds collected from *Q. douglasii* germinating at lower rates than those collected from *Q. lobata* or *Q. agrifolia* regardless of which host they were planted onto (GLMM, $X^2=738.62$, $df=2$, $P<0.001$). Germination was not predicted by experimental host (GLMM, $X^2=0.5578$, $df=2$, $P<0.7566$). The interaction term (host origin*experimental host) was only marginally significant (GLMM, $X^2=9.46$, $df=4$, $P=0.051$) with seeds originating from *Q. douglasii* germinating at marginally higher rates when experimentally applied to *Q. douglasii* relative to the other two hosts.

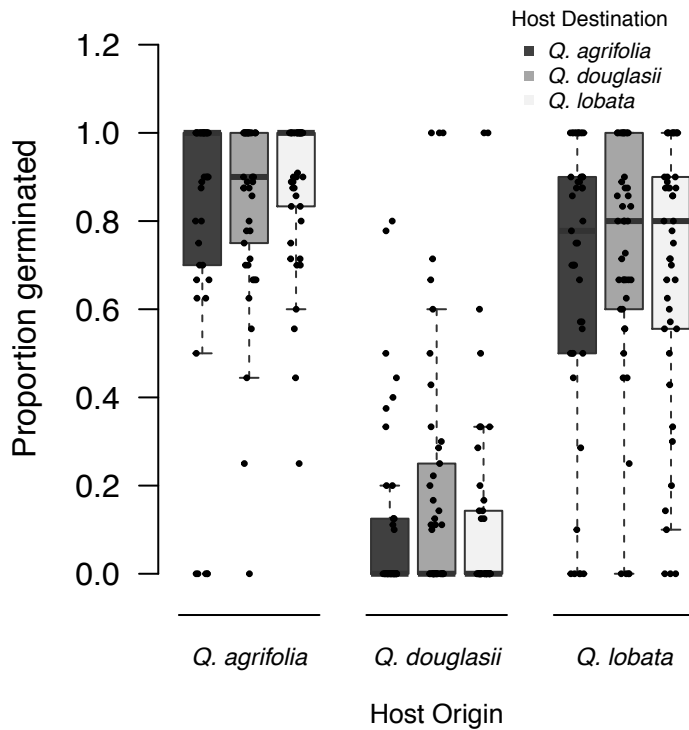


Figure 6. Boxplot of observed proportion of seeds germinated by host origin and host destination. If there was evidence for host races at the germination stage, the seeds applied on the same oak (host destination) they were collected from (host origin) would have a higher proportion seed germination, which we did not see (see results section for statistical results).

3.2 Survival past one year

Only 1.6% of the seeds experimentally dispersed survived one year after application. In contrast to the germination data, survival was not predicted by host origin (GLMM, $X^2= 1.45$, $df= 2$, $P=0.485$) but was predicted by host destination (GLMM, $X^2= 27.39$, $df= 2$, $P<0.001$). Seeds survived at higher rates when applied onto *Q. agrifolia*, the only evergreen host at the site. The interaction term (host origin*experimental host) was not significant (GLMM, $X^2= 0.43$, $df= 4$, $P= 0.980$).

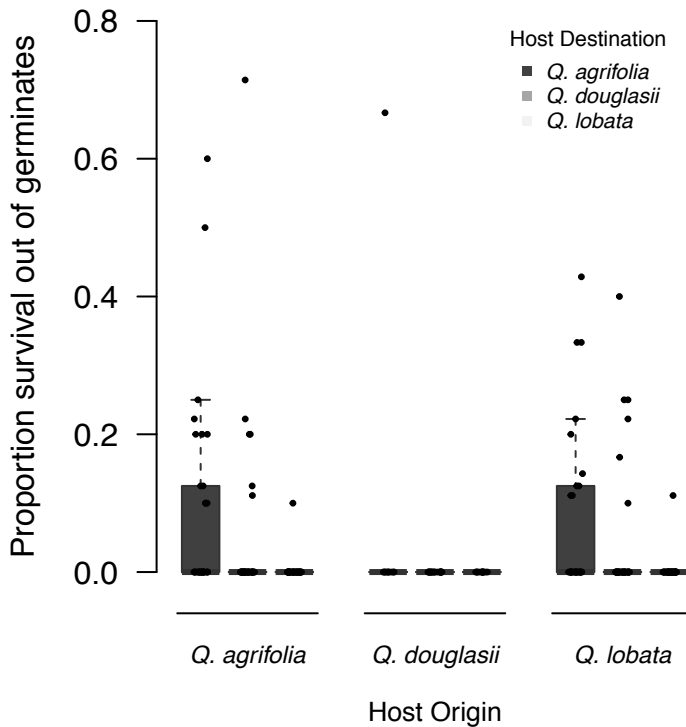


Figure 7. Boxplot of observed proportion of seeds which survived by host origin and host destination. IF there was evidence for host races at the survival stage, the seeds applied on the same oak (host destination) they were collected from (host origin) would have a higher proportion of survival, which we did not see (see results section for statistical results). Contrary to our predictions, seeds survived best on coast live oak, despite not occurring on this host naturally at the site.

4. DISCUSSION

4.1 Take-aways

We find no evidence that *P. villosum* has formed locally adapted host races on the three oak species present at Santa Margarita, California. We did however find that host origin impacted overall germination and host destination impacted overall survival after one year, although in ways that were counterintuitive given the natural occurrence patterns at this site. For example, survival was greatest on the one oak species that *P. villosum* does not locally occur on: *Quercus agrifolia*. Below we discuss the implication of these findings for explaining host specialization in *P. villosum* and place these results in light of the broader literature on host specialization in mistletoes.

4.2 Germination

Germination differed between the three host origins, but regardless of which host they were applied to. Although this experiment did not test for the mechanisms explaining this pattern, we hypothesize the differences in germination could be due to individual mistletoe plant fitness differences: seeds were collected from so few individuals from each host (out of necessity since so few plants were found in fruit). Fruit collected from mistletoe growing on *Q. douglasii* did worst overall in germination. There was no evidence that mistletoe germinated at higher rates when experimentally dispersed onto the same host they were collected from. Future studies of this kind should prioritize collecting fruit from more individuals. These results are similar to work done on a closely related species, *Phoradendron tomentosum*: *Phoradendron spp.* seeds which have been experimentally dispersed seem to germinate readily on all hosts, even if they exhibit some kind of host specificity in their natural populations (Clay, Dement, and Rejmanek, 1985). This is not true across the board for mistletoe: other studies have found that germination differs if the wrong combination of mistletoe genotype and host is experimentally tested (Rodl and Ward, 2002).

4.3 Survival

The survival data also does not support the host races hypothesis: the seeds survived best on *Q. agrifolia* (which had zero natural mistletoe infections at the site) regardless of where they were collected from. This result is very puzzling and could be explained by one or multiple of these hypotheses: 1) the

birds that eat mistletoe fruit do not spend time in *Q. agrifolia* and the seeds are not making it to this potential host, 2) this summer was hotter than average and killed seeds on the two deciduous oaks, but they were able to survive in the dense shade of the evergreen coast live oak, or 3) there really are host races, we just didn't observe the seeds long enough to see the stage where mistletoe on the wrong host are selected against. Other studies have found that seedling establishment is an important phase for mistletoe seedlings to be on their preferred host (Clay et al., 1985). It is possible the seedlings in this experiment were so slow growing that they had not become fully established at the time of final data collection.

4.4 Climate

It is possible that the seedling establishment phase of mistletoe has new selection pressures in the age of climate change (in California specifically, climate warming). Perhaps in the past, mistletoe seeds were able to germinate and survive their first summer on valley and blue oak, but now they need more protection from the heat to survive. Seeds of *Viscum album* were planted and monitored in different climatic conditions and a climate suitability model found that mistletoe fitness and ability to cope with drought stress was lower in the hot and dry areas (Sangüesa-Barreda et al., 2018).

4.5 Future directions

Given our lack of evidence for the host-race hypothesis at Santa Margarita Ranch, we propose that alternative explanations of host specialization should be considered. Differences in seed consumption and dispersal by birds should be investigated for this system. The hypothesis is that mistletoe-consuming birds are not dispersing seeds to coast live oak, which otherwise would be a suitable host. Data should be collected on which birds are consuming mistletoe fruit and what oak species they spend time in. If mistletoe-dispersing birds do not travel to coast live oak, this could explain the lack of mistletoe infections there. Mistletoe in fruit should be observed and after a bird feeds there, data recorded on what tree species the bird travels to within the length of time for fruit to pass through the digestive system.

Future studies should also investigate if there is host specialization in *P. villosum* at a later stage in seedling development. Now that germination and survival rates are more well understood for *P. villosum* a larger-scale, long-term repetition of these methods is recommended. This type of study could shed light on whether a later stage of mistletoe establishment or growth might require host-mistletoe compatibility for

survival. If such a study is attempted, it is highly recommended that fruit be collected from at least 10 different individuals growing on each species and the number of seeds applied in each treatment increased significantly.

4.6 Conclusions

Phoradendron villosum exhibits host specificity at Santa Margarita Ranch and beyond: it parasitizes valley oak and blue oak but leaves coast live oak untouched. This study ruled out the hypothesis that mistletoe has evolved host races that specialize at parasitizing valley and blue oak, at least at the stages of germination and early seedling survival (one year after dispersal). The door is open to future studies to uncover the mystery of oak mistletoe.

Chapter II: SUPERVISED CLASSIFICATION OF THREE OAKS AND OAK MISTLETOE

1. INTRODUCTION

1.1 Background

One step in trying to understand the relationship between oak trees (*Quercus* spp.) and a parasite that grows on them, mistletoe (*Phoradendron villosum*), is to confirm a pattern of mistletoe preference for growing on valley oak and blue oak, and not coast live oak where all three species occur together. There is one paper published about this pattern (Thomson and Mahall, 1983) which investigated mistletoe occurrences using field surveys in just a handful of sites where all three oaks grow together. To determine if those results are reproducible in Santa Margarita, California, we will use supervised classification in GIS. We also hope that these methods could be applied to other ecological questions and forestry management applications.

Supervised classification has been used to differentiate vegetation types, clusters of trees belonging to a single species, and even individual trees across the landscape. Joy et al. (2003) used a supervised classifier to model vegetation types over a larger area to a 10-meter resolution, with 74% accuracy. While the 10-meter resolution data performed well for identifying vegetation type (e.g., forest vs grassland), it is likely not adequate for distinguishing individual trees. Carleer and Wolff (2004) were able to identify clusters of trees in a forest to the species, including oak trees using a classifier on orthorectified data, NDVI transformed data, and principal components imagery. There has also been work done at a global scale to classify vegetation (Friedl, Brodley, and Strahler, 1999). Fricker et al. 2019 and team tagged trees in a mixed conifer forest, used LiDAR to pick out trees from shorter vegetation, and then used NAIP imagery to compare the spectral differences between tree species. They then used a supervised image classifier to apply this training data set to new trees, with an average of 87% accuracy (Fricker et al., 2019). A similar approach was used in Panama to assess the impacts of an imbalanced training datasets when classifying 20 tree species. The species with more training points were over-predicted while the underrepresented species were under-predicted. Their classifier worked with 62% accuracy (Graves et al., 2016).

1.2 Purpose of project

Oak mistletoe, *Phoradendron villosum*, parasitizes valley oak and blue oak but not coast live oak in mixed species stands north of Arroyo Grande, California. South of Arroyo Grande, oak mistletoe grows on coast live oak frequently. Santa Margarita Ranch is a site north of Arroyo Grande where the mistletoe is found growing on valley oak and blue oak but not coast live oak. As a first step towards potentially using imagery classification to investigate this pattern at a larger scale, we used supervised classification technology at Santa Margarita Ranch. The purpose of this project is to investigate whether we can train the computer to distinguish between coast live oak, valley oak, and blue oak using supervised classification of NAIP imagery, whether mistletoe can be tagged and classified using drone imagery, and if the number of mistletoe growing on each species of oak can be quantified.

2. METHODS

2.1 Field work

The study area of interest is Santa Margarita Ranch near Santa Margarita, California (35.37843, -120.58907). Oak tree ground truth points were collected by walking back and forth over an area of about a square mile and opportunistically recording location (using an Arrow Gold GPS device) and identity of 350 oak trees (Figure 8) belonging to the three focal species: *Q. agrifolia*, *Q. douglasii*, *Q. lobata*. For each tree, location was recorded at the base of their trunk using the ESRI collector map. Coast live oak is easily distinguished from the other two oaks in the field by its dark green, cup-shaped leaves with hairs in the leaf vein axils. These trees are also evergreen while the other two species are deciduous. Valley oaks tend to grow in flat areas and alluvial planes while blue oaks keep to the hillsides and drier areas. The bark of these two trees are also distinct: valley oak is characterized by large, squarish chunks of bark while blue oak has thin strips oriented vertically along the trunk. When they have leaves, the blue oak's are smaller with more shallow lobes and a distinctive blue-green waxy color. The valley oak's leaves are larger, deeply lobed and bright green. There are hybrids between valley and blue oak at the ranch which were avoided for the training sample dataset. We aimed to get an even representation of the three species across the dataset but there were fewer valley oaks at the study site. I collected 60 valley oak, 140 blue oak, and 150 coast live oak ground truth points. Upon completion of field work, I used Arc GIS online map (version 20.2.4) to export the final dataset to ArcGIS Pro (version 2.5.0).

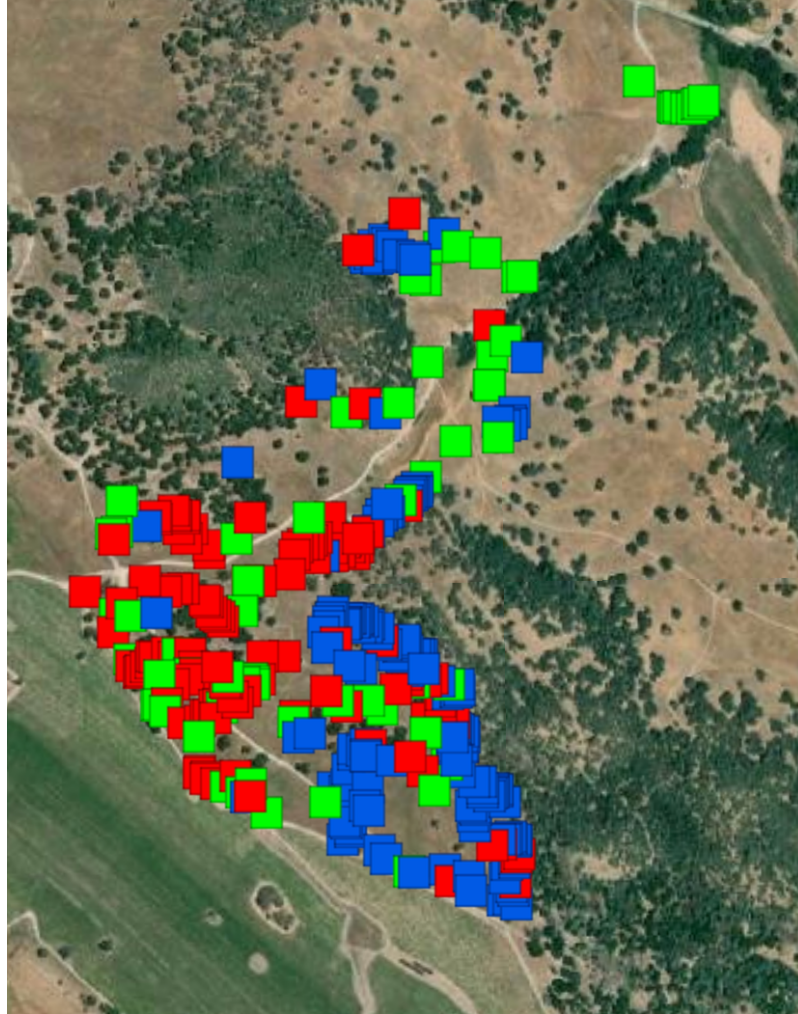


Figure 8. The ground truth points as seen in the collector app for the three oak species. Red are coast live oak, blue are blue oak, and green are valley oak.

Mistletoe ground truth points were collected using the Arrow Gold GPS device in combination with the TruAngle and Trupulse (laser) to create a 3D map (similar to LiDAR) of where the mistletoe plants are located in space (see chapter 3 for more details). In the field, all visible mistletoe within the drone flight area were tagged for this project (~50 individuals). These points were collected in the ESRI collector map and exported into ArcGIS Pro.

Drone imagery was collected at the study site where the three oak species grow together, and some are infested with mistletoe using a p4 Multispectral drone. See chapter 3 for more details about the methods for flying the drone.

2.2 Imagery

The classifier analyzed two forms of imagery: NAIP (National Agricultural Imagery Program) and drone. NAIP imagery from the USDA website was downloaded to use in the classification. NAIP imagery contains the visible spectrum as well as infrared: even if plant material looks identical to us, there may be differences in infrared that could help the computer to distinguish the tree species. Drone imagery has much higher resolution (approximately 3 inches per pixel) and has 5 cameras containing blue, green, red, red edge, and near infrared bands.

Lidar data was used with both the NAIP and the drone imagery to separate the trees from the surrounding grasses, forbs, and shrubs *before* running the classifier. LiDAR data comes in the form of point clouds where millions of lasers bounce back from hitting objects on the earth. Dr. Yun at Cal Poly helped me acquire pre-existing LiDAR data for my study site to make a canopy height model and separate the trees from everything else. This simplified the number of categories the classifier had to choose from but is not required for this process to work.

2.3 Computer analysis

The LiDAR data were used to create a canopy height model which was used to clip both the NAIP imagery and drone imagery to only areas where there are trees, to simplify the classification process. In order to create a canopy height model, the LiDAR data was converted from a *.las* format to a LAS dataset. From the point cloud data, the LAS dataset was converted to a raster. From this raster, the LiDAR can be filtered into points on the ground, and 'first return' points that hit the canopy of a tree. To create the canopy height model, I simply subtracted these two layers from each other. Figure 1 is a map with the ground in grey, and everything above the ground in color.

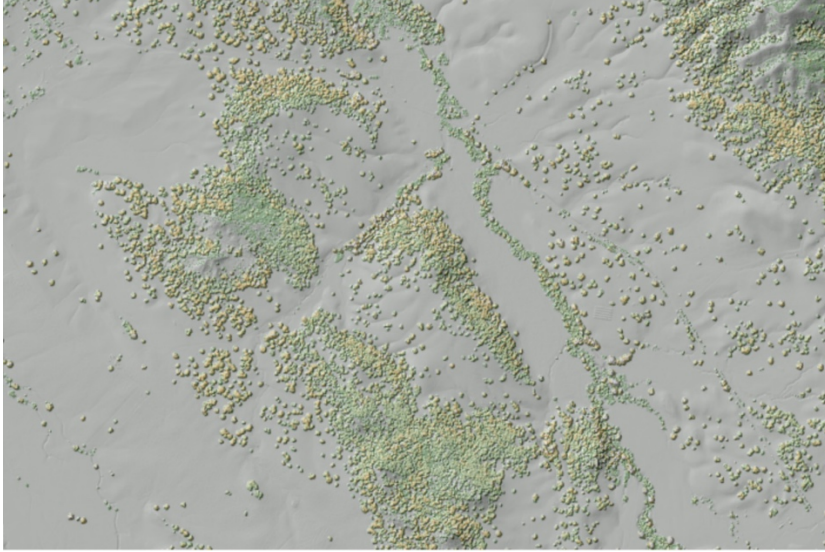


Figure 9. Canopy Height model made from LiDAR data. Grey pixels are ground level, and everything in color is above the ground (trees).

The pixels were separated into two bins: those taller than 10 ft (white) and those shorter than 10 ft (black) (Figure 2) so that the classifier would only be looking at tree pixels.

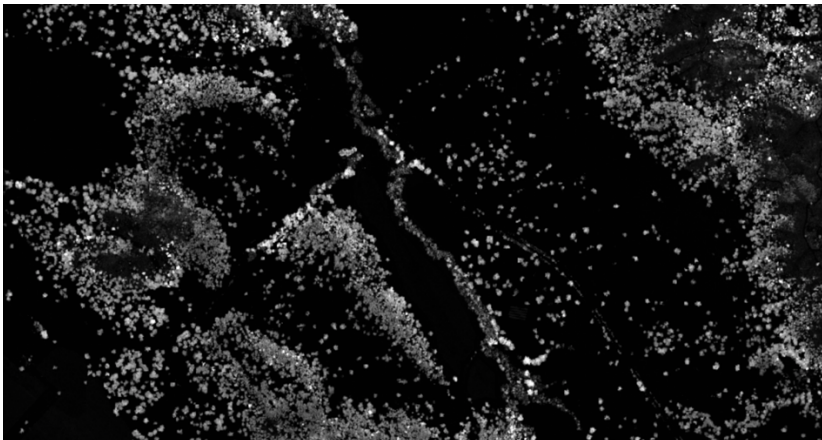


Figure 10. Pixels separated into two bins: taller than 10 ft (white) and shorter than 10 ft (black).

Only the white pixels (trees) were saved for the next step. The NAIP imagery was clipped to the locations of the trees found by the LiDAR canopy height model using the mask by raster tool. The classifier then worked on the patchy imagery layer showed below (only trees).

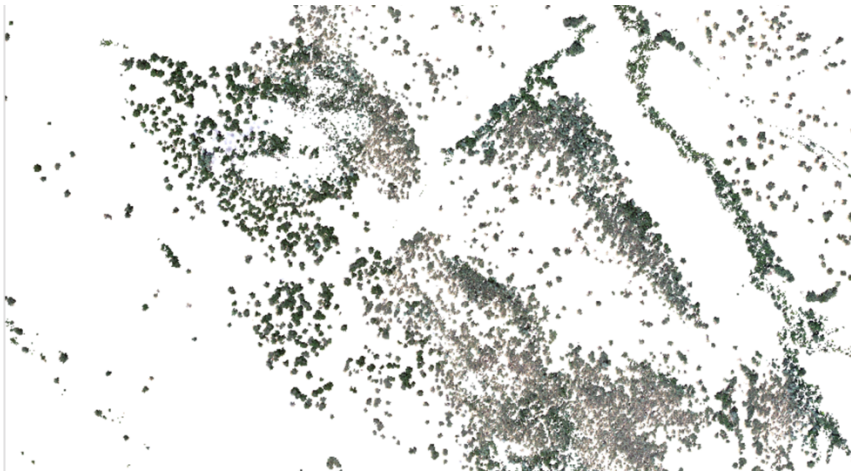


Figure 11. The NAIP imagery clipped to the locations of the trees found using the canopy height model from LiDAR data.

The 350 ground truth points were converted into buffers and fed into a training samples manager to train the classifier. The patchy imagery layer was selected as the imagery layer to classify, and then in the imagery tab there is an option for training samples manager. I added more training polygons under the categories “Grass” and “Bare Ground” to try to improve the classifier because even though I clipped the imagery to the trees, there were still small patches of grass around the edges of the trees, and a few trees had been removed since the LiDAR was collected because there were no trees in the imagery. Using the classification schema and training samples I had created; I ran the support vector machine method of the classifier on the patchy imagery layer. The classifier is a tool in GIS which takes the training pixels and then predicts what new, unknown pixels are and labels them with a hypothesized class.

The drone imagery was processed in Agisoft Metashape Pro to create and an orthomosaic. The photos were stitched together using the flight log data about where the drone was in space and ground control points. The same methods as was used with the NAIP imagery were then used on the drone imagery to create a patchy imagery layer to feed into the classifier.

When adding the mistletoe training polygons, the mistletoe GPS locations were added as a new class in the training samples manager. The GPS points were often slightly off target, so were manually adjusted at this stage for all visible mistletoe. Then the classifier was run again on the patchy drone imagery layer with the added mistletoe training points and a new prediction class called ‘mistletoe’.

3. RESULTS

3.1 Overall results

The classified result was a blotchy raster layer made up of mainly three colors which represented the three oak species. To make sure that each tree was represented by only one color, a few smoothing steps were taken (filter by majority tool). When this layer was overlaid onto imagery, the identified pixels lined up well with the crowns of trees (Figure 12). The smoothed raster result, the base layer imagery, and the ground truth points were then used in the confusion matrices to quantify the results.

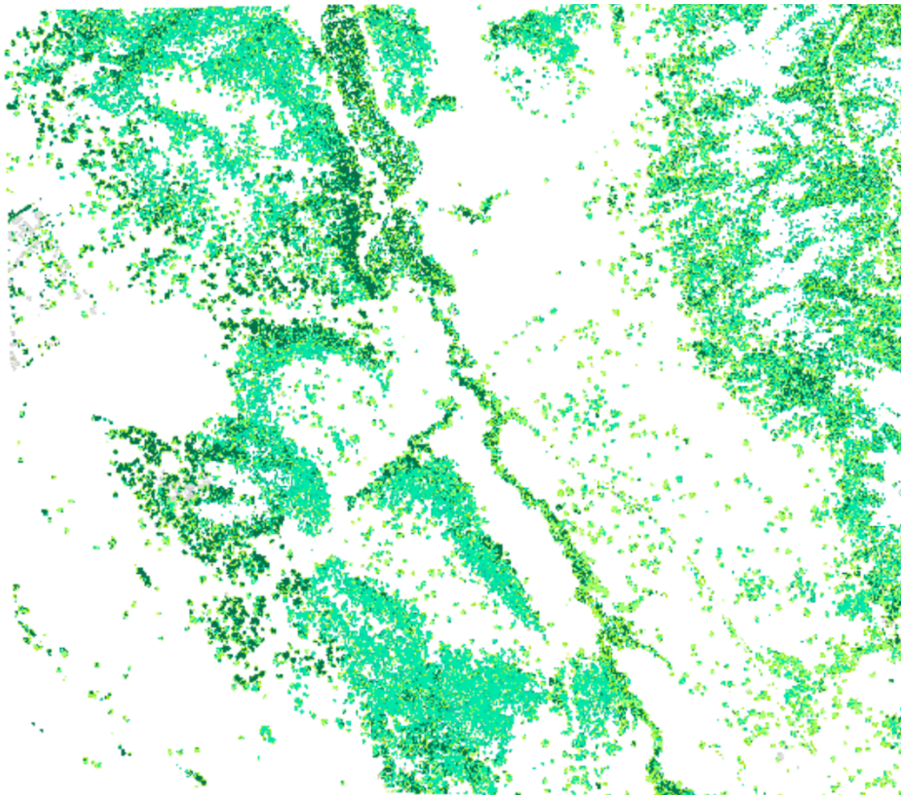


Figure 12. Classified result with five categories: coast live oak (dark green), valley oak (light green), blue oak (blue green), grass (tan), and bare ground (grey).



Figure 13. A comparison of unclassified NAIP imagery to the classified result.

3.2 Oak Classification Results

The oak classification's success was quantified by randomly sampling 65 out of about 175 trees, then checking their true species ID against the classifier's predicted species. I logged the actual and predicted species ID in confusion matrices.

		<u>Predicted</u>		
		<i>Valley</i>	<i>Coast Live</i>	<i>Blue</i>
<u>Actual</u>	<i>Valley</i>	1	0	6
	<i>Coast Live</i>	0	14	0
	<i>Blue</i>	0	2	42

Figure 14. Confusion matrix for the drone imagery oak classification. Actual species of oak trees is shown on vertical axis, and predicted oak species is shown on horizontal axis. Correct predictions are found on the diagonal from top left to bottom right. Incorrect predictions are off diagonal.

		<u>Predicted</u>		
		<i>Valley</i>	<i>Coast Live</i>	<i>Blue</i>
<u>Actual</u>	<i>Valley</i>	5	0	1
	<i>Coast Live</i>	0	16	1
	<i>Blue</i>	1	0	41

Figure 15. Confusion matrix for the NAIP imagery oak classification. Actual species of oak trees is shown on vertical axis, and predicted oak species is shown on horizontal axis. Correct predictions are found on the diagonal from top left to bottom right. Incorrect predictions are off diagonal.

The oak classification on the drone imagery had an overall accuracy of 87.7%. The oak ID classification on the NAIP imagery had an overall accuracy of 95.4%.

3.3 Mistletoe Classification Results

Results of the mistletoe classification could not be assessed by the same methods as the oak ID classification. Trees were randomly selected and assessed for if they actually contained mistletoe, and whether the classifier found mistletoe. The classifier worked with 40.7% accuracy which is largely due to the high number of false positives. The classifier predicted there were mistletoe plants in almost every tree and spread throughout the tree in small clumps of pixels (Figure 17). The mistletoe classification was therefore not successful.

		<u>Predicted</u>	
		<i>No mistletoe</i>	<i>Mistletoe</i>
<u>Actual</u>	<i>No mistletoe</i>	4	15
	<i>Mistletoe</i>	1	7

Figure 16. Confusion matrix for the drone imagery mistletoe classification. Whether a tree actually has mistletoe or not is shown on vertical axis, and whether each tree was predicted to have mistletoe or not is shown on horizontal axis. Correct predictions are found on the diagonal from top left to bottom right. Incorrect predictions are off diagonal.

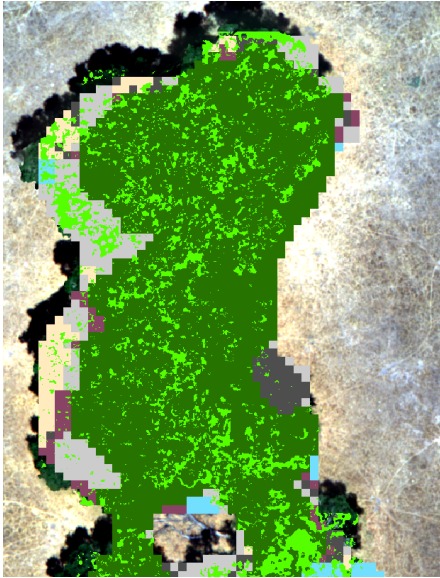


Figure 17. Shown is a group of coast live oaks, as classified by the NAIP oak classifier, overlaid with the drone mistletoe classification (light green pixels).

4. DISCUSSION/ CONCLUSION

The classifier worked beautifully on the oak trees, distinguishing between them with 95% accuracy for the NAIP imagery and 87% accuracy for the drone. Unfortunately, the classifier was unsuccessful at classifying the mistletoe, creating thousands of false positives. We can train the computer to distinguish between coast live oak, valley oak, and blue oak using supervised classification of NAIP imagery and drone imagery, but we are unable to tag and classify mistletoe using drone imagery or quantify the number of mistletoe growing on each species of oak.

To investigate why the classifier was more successful on the lower resolution NAIP imagery, we artificially turned down the resolution of the drone imagery and improved the classifier (results not presented). In some cases, it seems, more information is not necessarily better and taking an average of several pixels might reduce variability. Given this finding, readily available NAIP imagery performs well for classifying individual oak species growing in mixed species stands. Although LiDAR data is not

available everywhere in California, this technique could be applied using only NAIP imagery. If LiDAR is available, this simplifies the classification and makes it so only tree pixels are being sorted into classes. Santa Margarita Ranch is an example of a very simple application of this technique: other areas in California could have more tree or shrub diversity. The classifier works best with many training samples for each class, so uncommon species could pose a problem in some areas. Compared with the Fricker et al. 2019 study which classified a mixed coniferous forest with 87% accuracy, this study had higher accuracy at the level of individual trees which could be due to more training samples per species and fewer species being classified overall (Fricker et al. 2019). The classifier had the hardest time identifying valley oak successfully which confirms the findings of Graves et al. in Panama: fewer training samples leads to under-prediction. This study had the fewest training samples of valley oak, and valley oak was often predicted to be blue oak by the classifier. This points towards the threshold for successful classification being somewhere between 50 (the number of training samples for valley oak) and ~140 (the number of training samples for blue and coast live oak).

The same phenomenon of the classifier working with higher accuracy on the NAIP imagery than the drone imagery could point toward why the classifier did not work on the mistletoe. With so much detail in the drone imagery and with the training polygons being quite small and potentially mixed in with host oak leaves, this led to the classifier seeing “mistletoe” in many places it wasn’t. Another factor that may have contributed to this unsuccessful result is that mistletoe are often times buried in the canopy of the host tree, which can be hard to tell from the ground. Potentially some of our training points for mistletoe contained oak pixels, confusing the classifier. Another factor is that there is serious warping in the drone imagery due to wind during the drone flight. This didn’t seem to impact the oak classification, but a mixing of mistletoe and oak pixels may have been a detriment to the mistletoe classifier. Overall, the number of mistletoe in the drone flight area was probably too low, and future attempts to classify mistletoe should choose an area with high densities of mistletoe in the outer canopy. Due to all these limitations and to the expense and time investment to fly a drone for even a small area, mistletoe classification using drone imagery at this time is not scalable or a viable replacement for field surveys.

This project is informative for future work. For classifying individual trees, NAIP imagery is free and actually works better than drone imagery resulting in highly accurate classifications (>95%). For

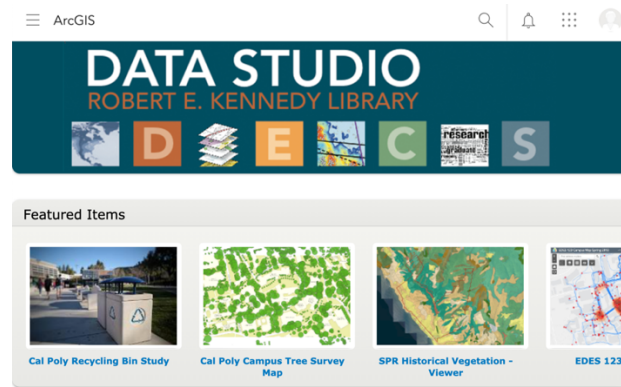
classifying smaller plants (mistletoe ~1-2 feet across), even the finest resolution imagery (drone imagery) was not useful. Thus, machine learning may be useful for identifying patterns of co-occurrence of the oaks and other tree species across California, but patterns of mistletoe infestation will still need to be assessed by field surveys for the time being.

Chapter III: TUTORIAL

1. USING THE ARROW GOLD GPS WITH ARC GIS ONLINE MAPS

These steps and tools are important to collect a set of points that will become the training points for the supervised classifier. For example, since I wanted to train the computer to tell the difference between Coast Live Oak, Valley Oak, and Blue Oak, I collected about 100 ground truth points for each species. I then used these points to create polygons in GIS around each tree, but absolutely crucial to this process is correctly identifying the trees in the field.

1. Make an online map
 - a. Go to ArcGIS online on a laptop or computer, sign in or make an account. You may need someone at Cal Poly to give you authorization to have a free, full-access account through Cal Poly. My account was made for me through a GIS class I took at Cal Poly.
 - b. You should get to a home page that looks like this:



- c. Click the upper left-hand corner to open a drop-down menu and select “Map”
 - d. Follow the instructions on the left-hand side, but essentially zoom to the area of interest and select a base map. Save your map.
 - e. Now download the ArcGIS collector app on your ipad or smartphone. The icon is blue with a little white clipboard with an arrow. Log in to the same ArcGIS online account you just made. It may take ~30 minutes for your new map to appear in the app, be patient. Make sure the map is there before heading out to do your field data collection.

2. Set up the Arrow Gold GPS unit

- a. Make sure it is fully charged. It takes a long time to charge, but a full battery should last a whole day of data collection.



Figure 18. Arrow Gold GPS device assembled in the field.

- b. Screw the saucer shaped piece to the top of the long pole and attach the box to the pole just below it so that you can connect them with the wires. Turn it on and allow it a few minutes to connect to the satellites and improve its location accuracy.
- c. Open the collector app and click on the little person icon in a circle at the top left-hand corner. Look for the header called “Location” and click on “Provider”. Go to the Bluetooth settings in your device to connect to the Arrow Gold for the first time. After the initial connection, it should appear in the app and connect automatically when it is turned on and in range. The Arrow Gold will show up as the “Eos Positioning System”. The other option in the location provider section is “Integrated” which uses the GPS location of your phone (at much lower accuracy).

- d. Go back to the maps menu and click on the map you made on your computer. You can see your location accuracy at the top of the screen, which if the Arrow Gold is connected properly should be less than 10 inches. When you want to collect a data point, make sure the GPS is right on top of it, and press the blue + button. It will ask you for a category: you should put each of your classes or species in a different category. These will all end up being part of the same shapefile later but with different subcategories in ArcGIS Pro.
- e. Once you have collected all your points in the field, the best way to get your points into ArcGIS Pro is to pull up the Arc GIS Pro program and log into your ArcGIS online account. Then click on the Map tab, then “Add Data”. This will bring up a finder menu, where you can look under “Portal” to find “My content” which should include all your ArcGIS online content. Select the layer with your field data to add it to your map.

2. USING THE TRUPULSE LASER AND TRUANGLE WITH THE ARROW GOLD GPS

To add very accurate GPS points for mistletoe plants, we used these laser and angle tools to find points in 3D space (similar to liDAR). Mistletoe grow in the canopy of oak trees, so this was the best option for our project. We spent a day figuring out this system before going out to the field, which I highly recommend.

- a. Create a map in the collector app (see instructions in part I.)
- b. Download the Eos Tools Pro app



Figure 19. A. The fully assembled laser and angle device using all of Dr. Andrew Fricker's equipment. B. Photo of assembled device from the Laser Offset Mapping with Collector Manual. Some set-up steps are slightly different based on the parts Dr. Fricker has but the overall idea is the same.

- c. You will need: A tripod, the pole mount that comes with the Arrow Gold, the Tru Angle, the arm that attaches the True Angle to the laser (the red part in the photo), and the Arrow Gold box and saucer.
 - i. Start by connecting the black pole to the tri-pod. Then connect all the other parts to each other before connecting them to the tripod. It is helpful to have the arrow gold, laser, and triangle laying down on a soft surface when learning how to connect them, and then once they are connected BE VERY CAREFUL because the plastic arm that holds on the laser and arrow gold isn't very strong and will break if you walk with it at an angle and too much torque on that joint. I

recommend carrying it upright or even disconnecting it if you need to walk to your next location for point collection.

- ii. You will need to connect wires between the arrow gold GPS and the triangle and the true angle and the laser.
- iii. The Triangle screws onto the top of the black pole. Make sure the triangle can rotate side to side, and the laser can rotate up and down. If either cannot move, you may need to loosen the screws holding them in place.
- iv. There are three supplemental tutorials for using the laser, the triangle, and for using the whole system together with the collector app which go into more detail than I will here.
- v. Download the Eos Tools Pro app. Use the “Laser offset mapping guide for collector” steps 1-5 for configuring the Eos Tools Pro app and collector with the triangle and laser. Read all the steps carefully! Some things to watch out for:
 1. Make sure you are looking through the correct end of the laser! If you don't see any markings on the screen when you turn it on you may want to try looking in the other end.
 2. There is a Bluetooth password to connect the laser (trupulse). By default it is 1234.
 3. Each time the triangle is powered on you will see “ind” which stands for indexing the encoder. Rotate the triangle 360 degrees in either direction. Then 0.00 will show on the screen and you can start the back sight angle process.

3. SUPERVISED CLASSIFICATION OF NAIP IMAGERY

The first step in the classification was to use the 350 ground truth points collected in the field to train the computer to distinguish between the three oak species using publicly available imagery at a low resolution. This was successful for our project with 95% accuracy.

- a. Get the NAIP imagery for your study area from the USDA website to use in the classification. This may involve getting multiple tiles of imagery and stitching them together in GIS. NAIP imagery contains the visible spectrum as well as infrared: plant material that looks identical to our eyes may be distinguishable in infrared.
- b. Investigate whether your study area has pre-existing lidar data. This will improve the accuracy of the classification. Dr. Yun at Cal Poly helped me acquire LiDAR data for my study site in SLO county. LiDAR data comes in the form of point clouds where millions of lasers bounce back from hitting objects on the earth.
- c. Convert the files from .las format to .lasd or las dataset format using the create las dataset tool.
- d. Then use the LAS dataset to raster tool to create a raster from the point cloud data.
- e. From this raster, filter the LiDAR data into two categories: points on the ground, and 'first return' points that hit the canopy of a tree.
- f. To create the canopy height model, you simply subtract these two layers from each other, resulting in a map where every pixel has data about how high off the ground it is. Since there are no buildings or other objects above the ground besides plants and trees, this created a Canopy Height Model.

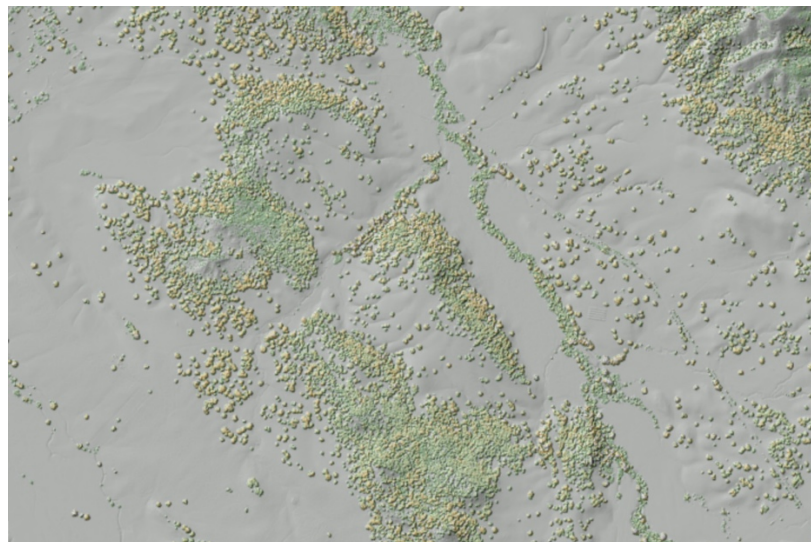


Figure 20. Canopy Height model made from LiDAR data. Grey pixels are ground level, and everything in color is above the ground (trees).

- g. Separate the pixels into two bins: those taller than 10 ft (white) and those shorter than 10 ft (black) (Figure 4).

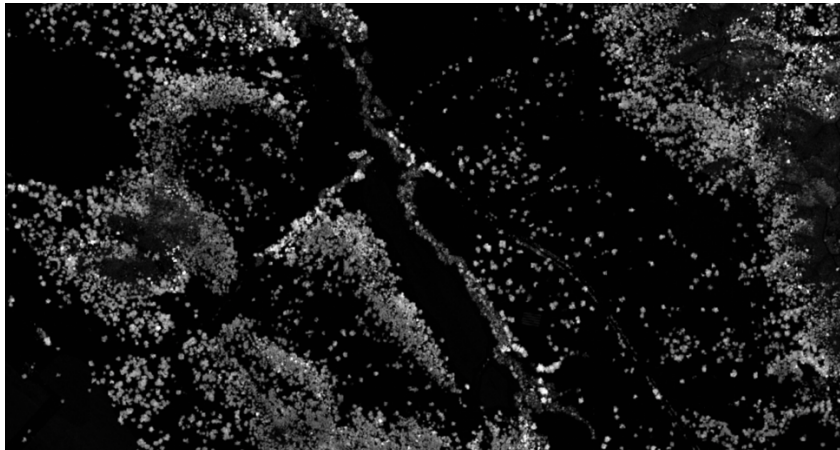


Figure 21. Pixels separated into two bins: taller than 10 ft (white) and shorter than 10 ft (black)

- h. Save only the white pixels as a new layer for the next step. Clip the NAIP imagery to the locations of the trees found by the LiDAR canopy height model using the mask by raster tool. Instead of using all the imagery, use the patchy imagery layer showed below (only trees) in the classifier.



Figure 22. The NAIP imagery clipped to the locations of the trees found using the canopy height model from LiDAR data.

- i. Make your ground truth points into buffers and feed those into a **training samples manager** to train the classifier.
 - i. Select the imagery layer you want to classify, and then in the imagery tab there is an option for training samples manager. I imported the 350 buffers as the training dataset, and as the training schema so that the two categories would match perfectly. This can cause errors when you run the classifier if the training dataset and training schema don't match.
 - ii. If there are more categories besides just your ground truth buffer polygons, you can add more training polygons under new categories manually. For example, I added "Grass" and "Bare Ground" to try to improve the classifier because even though I clipped the imagery to the trees, there were still small patches of grass around the edges of the trees, and a few trees had been removed since the LiDAR was collected because there were no trees in the imagery. I zoomed way in and manually created 10 or more new polygons for each new category. More so than number of polygons, the number of example pixels needs to be high enough to train the classifier.
 - iii. Check all the buffer polygons to make sure they lined up with the trees in the imagery and fix them manually if they don't (this improves the accuracy of the classifier).
 - iv. Save everything.
- ii. Open the classify tool under the imagery toolbar.
 - a) Use the classification schema and training samples you created.
 - b) Run support vector machine method of the classifier on the patchy imagery layer.
- j. Use the tool called filter by majority (or Majority Filter) (which can be repeated multiple times) to clean up the pixilation of the classifier raster, which will help with each tree being covered by one class and not multiple.

- i. Each time you run the tool a new layer will be produced. Use the most recently created layer each time you run the tool again.
- k. Then use a tool called 'Boundary Clean' once.

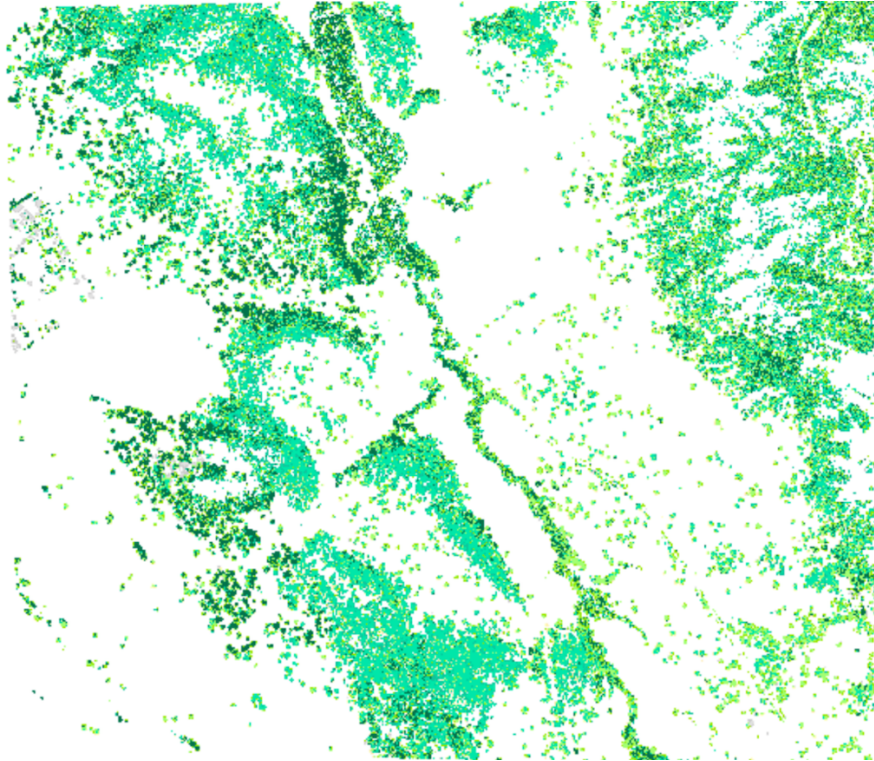


Figure 23. Classified result with five categories: coast live oak (dark green), valley oak (light green), blue oak (blue green), grass (tan), and bare ground (grey).

- l. To figure out how well the classifier worked, randomly sample 125 of your original ground truth points to compare what you identified the tree as to what the computer classifier identified each tree as. There are multiple ways to do this but I did it by visually assessing each point and seeing what the dominant pixel color was. After using the filter by majority tool, most trees were clearly classified as one species. Either...
 - i. Make a new column in the attributes table in ArcPro to add what the computer labeled each tree as and then export this to Excel when you've finished or
 - ii. Export the attribute table first and fill in a new column in excel with two tabs open.

- m. Once you have your data from the attribute table containing your sampled points from Arc GIS in an excel file, and then use the COUNTIFS function to create a confusion matrix. I found that my classifier worked at 95.4 % accuracy! The confusion matrix allowed me to go further than just stating a percent accuracy though: it allows me to see which species were more likely to be confused with each other (Figure 5). This is valuable information moving forward, because I can find the weaknesses of the classifier and improve them. For example, the classifier was more likely to confuse valley oak and blue oak, so in the future I can collect more training samples for those species.

		<u>Predicted</u>		
		<i>Valley</i>	<i>Coast Live</i>	<i>Blue</i>
<u>Actual</u>	<i>Valley</i>	5	0	1
	<i>Coast Live</i>	0	16	1
	<i>Blue</i>	1	0	41

Figure 24. Confusion matrix for the NAIP imagery oak ID classification. Actual species of oak trees are shown on vertical axis, and predicted oak species are shown on horizontal axis. Correct predictions are found on the diagonal from top left to bottom right. Incorrect predictions are off diagonal.

4. GETTING APPROVAL FROM CAL POLY

In order to fly a drone as a Cal Poly student for a project associated with Cal Poly you must go through this process!

- a. You will be emailing primarily with Cal Poly’s Director of Risk Management who is currently: Melissa Bullaro (mbullaro@calpoly.edu). Send her an email with a brief description of your flight plan and when you hope to fly. **This process can take a long time (months) so start this as soon as possible!!** Technically you cannot fly a drone with Cal Poly without going through this process.
- b. She will then send you a pdf application to fly a UAS. This is only about a page long, but unfortunately could take some time to get approved.

- c. Once you get the initial approval to start the review process, you will be asked to fill out a template PowerPoint that is the Flight Readiness Review (FRR). The review team is friendly and open to questions if you have them, but my experience was that you don't need to fill out every little detail they suggest you include, and some estimations are fine. Feel free to use my approved FRR PowerPoint as a starting point (especially if you are flying Dr. Fricker's p4 drone. (See Appendix)
 - i. You will be more likely to be approved if you not flying over any people or buildings.
 - ii. Make sure you have adequately addressed the risk of fire.
- d. Once you finish the powerpoint and email back and forth with Melissa about any changes she wants you to make, she will schedule a meeting with you and the review board. This team includes the Director of Risk Management, Cal Poly Chief of Police, and a professor from the Aero Space Department. Be prepared to discuss all aspects of your slides, but it is more of a conversation than a presentation. They will ask you questions about all the safety precautions you are taking and make suggestions for ways you could improve. Make notes or changes to your slides as you talk, because you will need to submit the final slides after the presentation and those become a commitment of what you plan to do. They will send approval shortly after.

5. FLYING THE DRONE

The following steps are specific to the p4 multispectral drone and RTK set up that Dr. Andrew Fricker has. We used an ipad connected to the drone controller with the DJI GS Pro app.

- a. Before you leave:
 - i. Turn on everything at home and make sure all components can connect and are updated.
 - ii. Check airspace: to see if there are restrictions and get flight authorization if necessary: <https://www.dji.com/flysafe/self-unlock>
 - iii. See maximum flight altitude <https://www.airmap.com/>
 - iv. Charge all components

- v. Format SD card through the drone in the settings of the app: click the slider bars, setting wheel, then click format SD card
- vi. Plan flight:
 1. Go onto the ipad and find the DJI GIS Pro app.
 2. Click 'My Missions' at the bottom then click the blue plus sign
 3. Zoom to area, click to create a box, and move one apex at a time to cover your area
 4. Change camera model to Phantom 4 multispec
 5. Set to capture at equal distance
 6. Side overlap ratio set to 60 or 70%
 7. Front overlap may be important for canopy height models (can build a CHM from drone photos)
 8. Play with height
 9. Optimal flight conditions: Mid-day, sunny with no clouds
- vii. Make sure you bring any mitigation measures discussed in the Flight Readiness Review meeting such as shovels and fire extinguishers.
- viii. Bring the Arrow Gold GPS device and find or make some ground control points to place on the ground in your flight area. We used red square pieces of wood ~1 foot x 1 foot with black duct tape in an "X" but we had some trouble locating them in the drone imagery so larger GCPs would be better (3'x3' or larger).
- ix. Practice flying drone at home so you know how to manually operate it in case of an emergency.
- b. Out in the field:
 - i. Prepare safety measures (clear grass around landing pad etc.)
 - ii. Place ground control points (we used about 8) evenly spread throughout flight area and make GPS points on a new ArcGIS Online map with collector. You may want to do some research as to the best method for placing these points in your flight area, as we did it randomly (which seemed to work fine).

- iii. Turn on drone. Short click then long click
- iv. Open the DJI GIS Pro app
- v. Set up RTK and figure out where you will leave it for the whole flight duration before you turn it on (a spot clear from trees overhead, a good path for the drone to take off right nearby, and ideally on one of the highest points of your flight area). Short click then long click
- vi. Connect to RTK by clicking the top area on the app which says 'GPS' and has other icons, then select D-RTK-2 and hit connect.
- vii. Go to missions
- viii. P S or A mode on controller- use P mode for planned flight. P=Program, S=Sport. Use sport to take back control in case of emergency.
- ix. Click on My missions, select the pre-planned mission you want to fly.
- x. Click edit, check settings. Click the blue plane button to fly. Check the warnings in the prepare for flight popup: There is a max of 99 waypoints, so if you have more you will have to land drone and restart to finish the flight pattern. In the waypoint section, it will tell you a range to set the course angle to, hit cancel and go change the course angle in the flight plan. CAUTION: only change the course angle at the beginning, once you start to fly, maintain course angle and hit "still fly" to override the warning.
- xi. Click the camera preview window to see a larger image of what the camera is seeing. Leave the camera on manual mode.
- xii. Do not fly higher than 400 ft above the ground
- xiii. If the RTK connection is weak, restart drone or restart the RTK (although this takes longer to turn back on).
- xiv. Take off from a high point in the flight area so that the drone doesn't run into a hill because it maintains the same height throughout. If you are in a very hilly area you may want to find a program that can account for hills in the flight plan. It doesn't matter if you are at the start point of the flight plan.

- xv. To start the flight plan, click Fly and be ready for the drone to shoot up into the sky like a rocket. While the drone is in the air, one person should always have eyes on the drone in the sky and another person should watch the iPad screen to see what the drone sees. It does have collision sensors but part of the FRR through Cal Poly will be mitigating for potential airplane collisions. Even though the drone flies itself through the flight plan, you must be ready to take over at any time to land the drone in the case of an emergency.
- xvi. Return to home: click the return to home button on the app and leave plenty of battery for the return. If you don't do this, it will stop what it's doing and come back with just enough battery to spare.
- xvii. Keep tablet in shade-overheats easily and lose progress: tablet is the brain.
- xviii. When the battery dies, it will come back to you. Switch battery and hit resume flight plan. DO NOT EXIT OUT OF THE FLIGHT PLAN OR CHANGE ANYTHING ON THE TABLET DURING BATTERY SWITCHES! You could potentially lose where you are in the flight plan and it is very hard to resume in the exact location the previous flight ended.
- xix. After the drone flight is over, collect ground control points.

6. PROCESSING DRONE IMAGERY (CREATING AN ORTHOMOSAIC)

After flying the drone, the individual photographs on the SD card will need to be moved to a computer with Agisoft Metashape Pro and converted to one large, fused photo called an orthomosaic. Use the Tutorial “(Beginner Level): Orthomosaic and DEM Generation with Agisoft Photoscan Pro 1.3 (with Ground Control Points)” alongside this tutorial for the most complete description of the steps.

1. Add Photos
 - a. When prompted, choose multi-camera setup to correctly integrate all five bands (red, green, blue, red edge, and near infrared) into the same orthomosaic.
2. Load Camera Positions

- a. Loading camera positions requires a reference file containing the GPS data for each photo taken by the drone. To create a reference file for camera positions, take the original flight log from the drone and view it online with the *PhantomHelp* DJI Flight Log Viewer.
 - b. From here, export the flight log to a CSV file.
 - c. Open that CSV in Microsoft Excel and edit the contents to only include the time, date, latitude, longitude, altitude, pitch, roll, and yaw of the drone for every datapoint in the flight log.
 - d. Change the names of these data classes according to *Geotagging with ExifTool* Harvey (2020).
 - e. With this edited flight log, follow the commands described in Harvey (2020) to use *EXIFTool* from the command line to geotag all of the drone images based on the time they were taken and the position of the drone at that time.
 - f. Again use the *EXIFTool* to write the GPS data from the newly geotagged photos back into a CSV file. While this may seem like a long and convoluted process, it was the quickest way for me to both geotag all the drone images and generate a partial flight log with position data for only the moments that the drone was taking photos. This partial flight log was imported into the Metashape project as a reference file, allowing for easy georeferencing of the drone photos.
3. Check Camera Calibration
 - a. Enable 'Rolling Shutter Compensation,' as is suggested for DJI drones.
 - b. Set NIR band camera to the master layer because the offset of all five bands is calculated relative to the NIR band in the photo metadata.
 - c. I made sure each camera had a unique layer index.
 - d. In each slave layer, I checked 'Adjust location' under the 'Slave offset' tab. This allows Metashape to automatically calculate and correct the offset between bands of the same image.
 4. Align Photos

5. Optimize Alignment
6. Build Dense Cloud
 - a. I set the quality to High. Even though this setting increases the processing time greatly, I wanted the quality to be as high as possible to increase the number of pixels of mistletoe in the final product.
 - b. I set 'Depth Filtering' to Mild to preserve details on the tops of oak canopies.
7. Build DEM
 - a. I set 'Quality' to High to keep as many mistletoe pixels in the final product as possible.
 - b. I set 'Projection' to WGS 84. This coordinate system is the most compatible with the coordinates from the drone photos.
 - c. I set 'Depth Filtering' to Mild to preserve details on the tops of oak canopies.
8. Build Orthomosaic
 - a. I set 'Surface' to DEM. This builds the highest quality orthomosaic with elevation data from the Digital Elevation Model built in the previous step.
 - b. I set 'Projection' to WGS 84. This coordinate system is the most compatible with the coordinates from the drone photos.
9. Export Orthomosaic
 - a. I set 'Projection' to 'NAD 83/California Zone 5 (ft US).' This differs from the previous projections because this is a projection of the whole orthomosaic onto a new platform, rather than a projection of photos into the orthomosaic. The NAD 83 coordinate system was chosen because it is the same as the coordinate system for the ArcGIS Pro map for this study.
 - b. I set the 'Write BIGTIFF file' option to Yes because I was using a relatively large number (~1100) of photos due to the multiple bands and relatively large area being processed. This might not be necessary for projects with a smaller area and/or single band images.

NOTE: I used batch processing when I built this orthomosaic to save time. This was an important choice for this project, as I was processing a large number of photos, but could be avoided on smaller projects to be able to tweak settings in between steps.

NOTE: For more information see “Tutorial (Beginner Level): Orthomosaic and DEM Generation with Agisoft Photoscan Pro 1.3 (without Ground Control Points).” There are some deviations from this tutorial in this how-to guide to best serve the specific needs of this project.

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APPENDIX:

PowerPoint slides submitted to the Cal Poly Flight Readiness Review Board:

Flight Readiness Review (FRR) for: Classification of Mistletoe on Oak Trees

California Polytechnic State University

9/11/2020

Ella Abelli-Amen and Jack Alexander

CAL POLY
SAN LUIS OBISPO

FRR Scope

- **Aircraft**
 - P4 Multispectral DJI drone, registration number: 118969
 - Location
 - Santa Margarita Ranch, Santa Margarita, California
- **Timeframe**
 - September 2020- March 20, 2020
- **Objectives**
 - Fly the drone over a small section of the ranch in order to run a supervised classifier on the imagery and quantify the number of mistletoe on each species of oak.
- **Other organizations involved (non-Cal Poly)**
 - NA

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Aircraft

Click to add text

**Diagonal Distance
(Propellers Excluded)**
350 mm



CAL POLY
SAN LUIS OBISPO

Cal Poly Flight Readiness Review

Performance specifications

- **Max Service Ceiling Above Sea Level**
 - 19685 ft (6000 m)
- **Max Ascent Speed**
 - 6 m/s (automatic flight); 5 m/s (manual control)
- **Max Descent Speed**
 - 3 m/s
- **Max Speed**
 - 31 mph (50 kph) (P-mode); 36 mph (58 kph) (A-mode)
- **Max Flight Time**
 - Approx. 27 minutes
- **Operating Temperature**
 - 0° to 40° C (32° to 104° F)
- **Operating Frequency**
 - 2.4000 GHz to 2.4835 GHz (Europe, Japan, Korea)
 - 5.725 GHz to 5.850 GHz (Other countries/regions)^[1]
- **Transmission Power (EIRP)**
 - 2.4 GHz: < 20 dBm (CE / MIC / KCC)
 - 5.8 GHz: < 26 dBm (FCC / SRRC / NCC)

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Cal Poly Flight Readiness Review

Vehicle Safety History & Reliability

- **Fleet**
 - In production since: 2019
 - No information available about how many have been built but they are commercially available, relatively affordable, and reliable.
 - Fleet operational flight hours: 0
 - Number of accidents: 0
 - Number of injuries/fatalities: 0
- **P4 Multispectral:**
 - Manufactured in 2020
 - Owned by Cal Poly (Professor Andrew Fricker)
 - Flight hours: 0 (This will be this drone's first official flight)
 - Number of accidents: 0
 - Number of injuries/fatalities: 0



Cal Poly Flight Readiness Review

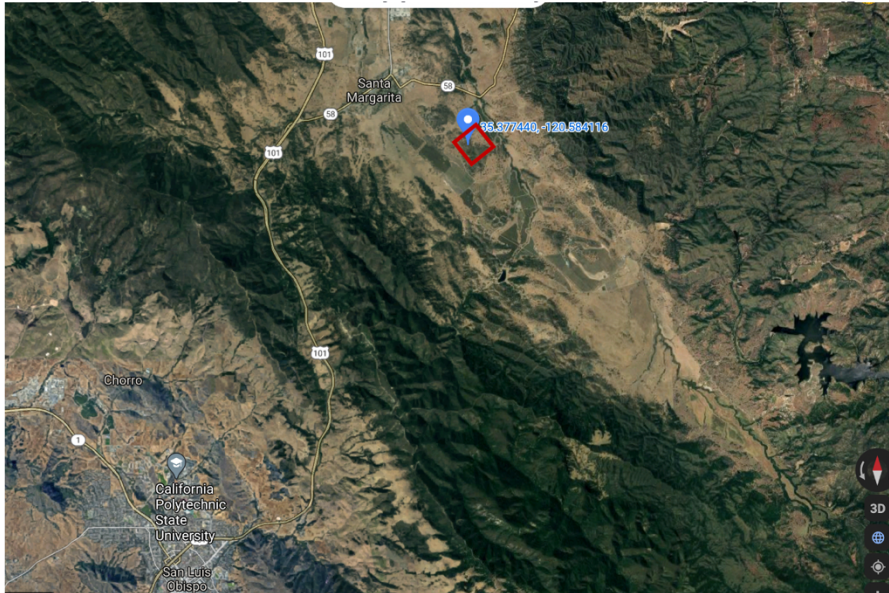
Concept of Operations

- **Purpose:**
 - Fly a small area of the Santa Margarita Ranch on a pre-programmed flight path with photos being taken at regular intervals to later create an orthomosaic. We will then run a supervised classifier on the drone imagery to quantify the number of mistletoe on each species of oak tree.
- **Basic Restrictions**
 - Altitudes limit: 400 ft
 - Distance limits: within sight of drone operators (within half a mile) Operational Profile: Pre-program flight path into DJI Drone Pilot App. Practice flying the drone manually and lowering drone quickly prior to day of flight. Clear grass away from landing zone and backup landing zone. Set up ground RTK unit on tripod, turn on. Set up landing pad. Turn on drone and connect all components. Check flight updates on app. Turn on walkie talkies and test, have ground observation crew move into position. Place drone in the middle of the landing pad, then take off on flight plan.
- Must be a clear, blue sky day for imagery quality reasons
- Must be mid-day to reduce shadows



Cal Poly Flight Readiness Review

Operational Area



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Cal Poly Flight Readiness Review

Detailed Operational Area

- [detailed map showing characteristics of operational area]



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Cal Poly Flight Readiness Review

Flight Crew

- Flight Crew will consist of:
 - Two Air Vehicle Operators (AVO)
 - Before flight: Planning flight, charging batteries, equipment check, set up of equipment.
 - During flight: One AVO will always monitor the tablet to see what the drone sees, while the other will always keep their eyes on it in the sky. Both will help with battery replacement and re-launching.

Two Ground Observers

- Watch for ranch personnel entering flight area. Communicate with AVO's via walkie talkies. Respond to Risks, should they occur. Will be prepared with fire extinguishers and shovels. Keep eyes on drone and watch for incoming aircrafts.

AVO Qualifications and Currency

- Qualifications
 - Flight operators have been trained by an experienced, licensed drone pilot in the environmental consultant industry. Drone flies itself the entire time, so training and skill are not necessary.
- Currency
 - No experience necessary, due to simplicity of flight procedure and approval from drone owner, Dr. Andrew Fricker.

Expected Project Personnel

Personnel	Role	Status
Ella Abelli-Amen	Lead AVO	Cal Poly Masters student in Biology
		(No drone license required due to student status)
Jack Alexander	AVO	Cal Poly Undergraduate in Biology
		(No drone license required due to student status)
Andrew Fricker	Ground Observation Crew	Cal Poly Geography Professor
Sequoia Wade-Dunleavy	Ground Observation Crew	Cal Poly Undergraduate



Minimum Equipment List

- 2 Fire Extinguishers, ABC dry chemical
- First aid kit
- Drone
- ipad and controller
- RTK and tripod
- Landing pad
- Ground truth points (3-gallon bucket lids)
- Walkie talkies for all crew members
- 3 shovels
- Anemometer
- Cell phones and location description for emergency responders



Weather Limits

- Ceiling
 - 1000 ft
- Visibility
 - 3 miles
- Winds
 - > 10 mph (surface winds)
 - > 15 mph gust component
 - DJI P4 can handle 30 mph winds, to be cautious we will only fly in winds half as strong as that.

Required ATC Coordination

- No Coordination is necessary due to the location of the ranch. It is not within the radius that requires communication or approval from any airports or other sensitive areas.

Risk Management: Probability

- Qualitative or quantitative assessment, as feasible
- Time basis is full aircraft design lifetime
 - ~ 200+ hour design lifetime
 - Assess probability as if planned project activities continued for the entire lifetime of the system

Probability	Definition
A. Frequent	If this test went on for the entire life of the system, this hazard would occur on a regular basis.
B. Occasional	If this test went on for the entire life of the system, this hazard would occur multiple times, though only on a minority of individual operations.
C. Likely	If this test went on for the entire life of the system, this hazard would occur at least once.
D. Unlikely	If this test went on for the entire life of the system, this hazard would be expected to not occur at all, though its occurrence is possible.
E. Remote	Physically-possible, but sufficiently unlikely that to expect its occurrence could be considered irrational.



Risk Management: Severity

Severity	Definition			
	Crew/ Test Team	Uninvolved Persons	Aircraft/System	Other Property
I. Catastrophic	Loss of life	Loss of life; or Severe/permanent injury	Loss of manned aircraft; or loss of UAV with value > \$1M	Loss of facility or expensive/unique system
II. Critical	Severe/permanent injury	Minor injury (medical treatment requiring no lost <u>work days</u> , and no permanent injury)	Severe manned aircraft damage; or loss of UAV with value > \$250k	Severe equipment or property damage
III. Minor	Minor injury (medical treatment requiring no lost <u>work days</u> , and no permanent injury)	No injury potential	Minor manned aircraft damage; or Severe damage to UAV with value > \$250k; or loss of UAV with value up to \$250k	Minor equipment or property damage
IV. Negligible	Superficial injury (first aid may be required)	No injury potential	Minor damage insufficient to compromise project objectives	Incidental damage comparable to normal wear-and-tear



Risk 1: Passenger plane or helicopter enters area

- **Description:** The ranch is not in any known flight paths or near airports but there is a small chance a larger, manned aircraft could enter the flight area.
- **Effect(s):** If this occurred, our flight plan would be terminated immediately, lowering the drone as fast as possible.
- **Emergency Procedures:** Switch to sport mode and send drone straight down.
- **Mitigations:** Eyes and ears will always be directed toward the drone in order to detect the approach of an airplane long before it becomes a serious risk.
- **Severity:** Negligible
 - Our aircraft's small size and top speed of 30 mph make it of no serious risk to an airplane, so the worst damage would be the drone itself which has a value of less than \$10,000.
- **Probability:** Unlikely
 - Due to the infrequency of airplane traffic over the ranch and the rarity of this occurring in other circumstances, this risk is unlikely.

Risk 2: Bird Interferes with Aircraft

- **Description:** There are large birds of prey at the ranch that could try to attack the drone.
- **Effect(s):** If this occurred, our flight plan would be terminated immediately, lowering the drone as fast as possible.
- **Emergency Procedures:** Switch to sport mode and send drone straight down.
- **Mitigations:** Eyes and ears will always be directed toward the drone in order to detect the approach of bird long before it becomes a serious risk.
- **Severity:** Negligible
 - The worst-case scenario for this risk is that the bird dies and the aircraft crashes. The damage to the drone would be less than \$10,000, and there is no risk to human life.
- **Probability:** Unlikely
 - There have been no known instances of collisions, only of birds attacking the drone and even this is extremely rare.

Risk 3: Loss of GPS data link

- **Description:** The drone's pre-programmed flight is controlled by the GPS data link and so is the automatic return to home function.
- **Effect(s):** If this occurred, the drone would need to be manually landed.
- **Emergency Procedures:** The pilot could fly back to the home point manually by sight, or if that is not possible, land where the drone lost GPS connection.
- **Mitigations:** Know where the drone is at all times and keep eyes on it. This way if the GPS link is lost, it can be returned manually or retrieved.
- **Severity:** Negligible
 - There is no risk to property or operators.
- **Probability:** Likely
 - This could happen at some point during the life of the drone, but even if it does, the drone would still be able to land safely.

Risk 4: Loss of signal to UAS

- **Description:** During the programmed flight, the UAV suddenly loses signal and cannot be controlled by the ground person.
- **Effect(s):** Drone will be out of our control temporarily.
- **Emergency Procedures:** Drone will be programmed to hover in place until the connection is regained or until it reaches the critical battery threshold, at which point it will return to home automatically.
- **Mitigations:** Pre-programming a safe response plan.
- **Severity:** Negligible
 - The worst-case scenario for this risk is that a bird or plane approaches the drone while we have lost connection to it and cannot perform our preventative measures for those risks.
- **Probability:** Unlikely
 - Just this risk alone is likely to occur, but the the probability that this risk occurs in combination with risk 1 and risk 2 is unlikely.

Risk 6: Battery exhaustion in flight

- **Description:** Battery runs out during flight and drone lands automatically.
- **Effect(s):** Planned flight is paused, drone automatically returns to home point.
- **Emergency Procedures:** Drone starts to return home automatically with plenty of time when battery is low.
- **Mitigations:** Do not push drone to continue flying mission longer than is recommended.
- **Severity:** Negligible
 - The worst-case scenario for this risk is that the drone does not have enough battery to make it back to the home point and it lands automatically somewhere else.
- **Probability:** Unlikely
 - The battery does become exhausted frequently, but as long as the drone is allowed to return to the home point when the battery warning starts to flash, the worst-case scenario is unlikely to occur.



Cal Poly Flight Readiness Review

Risk 7: Battery fire

- **Description:** Battery and drone catch fire.
- **Effect(s):** Would create a wildfire risk.
- **Emergency Procedures:** Try to land drone as quickly as possible and get to it to put out the fire with fire extinguisher. Call for help right away. Description of where we are written down.
- **Mitigations:** Battery management: never overcharge batteries, store at room temperature, and keep them clean and dry. Always have a fire extinguisher handy when flying. Inspect for damage. Mile markers.
- **Severity:** Critical
 - The worst-case scenario for this risk is that the battery fire catches some dry grass on fire and creates a wildfire at the ranch. There are no buildings nearby, but there could be damage to property.
- **Probability:** Unlikely
 - If drone batteries are well taken care of and there are no collisions, this risk is very unlikely.



Cal Poly Flight Readiness Review

Risk 8: Personnel interaction with drone

- **Description:** Ranchers or hunters might see drone and shoot it down or come into flight area.
- **Effect(s):** If this occurred, drone would be damaged and battery fire could possibly begin.
- **Emergency Procedures:** Quickly locate drone with fire extinguisher to assess damage and check for fire.
- **Mitigations:** All personnel at the ranch will be notified of the flight and approval from managers has already been attained.
- **Severity:** Critical
 - The worst-case scenario for this risk is similar to Risk 7: shot causes fire.
- **Probability:** Remote
 - Due to communication with ranch personnel and awareness of fire risk, this risk will not occur

Risk 9: Obstructed landing zone

- **Description:** A tree falls into the landing zone.
- **Effect(s):** If this occurred, we would be able to land the drone somewhere safe nearby in a pre-determined location that has been cleared of grass and debris.
- **Emergency Procedures:** Switch to sport mode and manually land drone in a safe and flat area.
- **Mitigations:** We will make the home point in an area away from trees and traffic so that this is very unlikely to occur and scope out a back up landing location prior to take off.
- **Severity:** Negligible
 - The worst-case scenario for this risk very minor, with no danger to personnel or property.
 - **Probability:** Unlikely
 - Due to mitigation measures this is very unlikely to occur.

Risk Assessment Matrix

		Mishap Severity Category			
		I. Catastrophic	II. Critical	III. Minor	IV. Negligible
Mishap Probability Category	A. Frequent				
	B. Occasional				
	C. Likely	[put risk numbers in boxes]			Risk 3
	D. Unlikely		Risk 7		Risk 1 and Risk 2, Risk 4, Risk 5, Risk 6, Risk 9
	E. Remote		Risk 8		

Flight Termination Methodology

- The flight ends when the aircraft runs out of battery and automatically returns to the home point, without any intervention from the AVOs.

Program Status & Schedule

- This flight plan will only take about 5 hours in total, with about 1 hour of actual flight time, over the course of one day. The day that it occurs is flexible, but weather conditions must be met, and ideally the flight can happen as soon as possible before the deciduous oak trees drop their leaves. We plan to have a practice flight day to get familiar with drone controls and the flight area.