Heritable Resistance to Sudden Oak Death in Tanoaks

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ABSTRACT

Sudden Oak Death (causal agent *Phytophthora ramorum*) was first found on the west coast of the United States in 1990. Ever since the oomycete has been spreading up and down the western coastline through a variety of host species. Tanoaks (*Notholithocarpus densiflorus*) are one of the most susceptible hosts to *P.ramorum*. Tanoaks are a keystone species in a large number of forests on the west coast, providing food, shelter, and ecosystem services. Understanding Sudden Oak Death resistance could be critical for the future of tanoaks. This study examines the intra- and inter-familial variation of resistance in tanoaks in hopes of better understanding heritable resistance. This was done through an inoculation study of saplings and detached leaves that were analyzed for variance in resistance. Previous findings have indicated heritability of resistance acting in a polygenetic model, however in this study that is not the case. Based on data from the sapling inoculations there was zero variance within families, indicating no heritability in a polygenetic model. However the variation in responses among the individuals within families to the inoculation indicate resistance through major gene effects. A major gene model might be a more accurate understanding of heritable resistance to Sudden Oak Death in tanoaks.

KEYWORDS

Phytophthora ramorum, *Notholithocarpus densiflorus*, multi-point sapling inoculations, detached leaf inoculations, tanoak clonal propagation.

INTRODUCTION

Invasive diseases have the ability to alter ecosystem stability worldwide (Black 1974, Garnas et al. 2011). Invasive diseases can especially impact trees within forest ecosystems because trees play such a large role in the landscape pattern and function (Condeso and Meentemeyer 2007). An invasive disease thrives on a balanced ecosystem because the ecosystem lacks the developed resistance that is found in the homeland of the disease.

Sudden Oak Death (SOD) is an example of an invasive disease with a large impact on the western forests of the United States. The first finding of the oomycete *Phytophthora ramorum* occurred in Santa Cruz in the 1990s (Ivors et al. 2006, Mascheretti et al. 2008). The impact of SOD was not observed until the year 2000 when large quantities of oaks started to die from an unknown cause (Alexander and Lee 2010). The continued infection and impact of *P.ramorum* on the coastal forest trees lead to the discovery of Sudden Oak Death. The symptoms of SOD include lethal trunk lesions and bleeding cankers (Rizzo and Garbelotto 2003, McPherson et al. 2010), which can eventually lead to tree mortality.

Although SOD has a variety of host species that are not greatly affected by *P.ramorum* infection, tanoaks (*Notholithocarpus densiflorus*) are heavily impacted. Tanoaks are the most susceptible to infection and also have an increased probability of mortality compared to other canker-forming hosts of SOD (Davis et al. 2010, Rizzo and Garbelotto 2003). Mortality rates of tanoaks from SOD in coastal forests have lead to 95% loss of the forest basal area (Mortiz et al 2008). SOD also causes reduced foliar moisture within infected tanoaks (Kuljian and Varner 2010) that can increase likelihood of forest fires. Once the tanoaks are infected they can support sporulation of *P.ramorum* without additional foliar hosts (Rizzo et al. 2005) leading to increased *P.*ramorum spread throughout forests. To help decrease the mortality of tanoaks and the vulnerability of forest ecosystems it is essential to observe the inter- and intra-familial relationships of tanoaks to understand resistance to SOD.

Plant inheritance of resistance is usually understood to follow one of two models, polygenetic model or major gene model. A polygenetic model is characterized by additive effects of heritable resistance, called quantitative traits. This is characterized by continuous variation and differences in degree of response. An example of a polygenetic model of inheritance in humans would be height. This model is expected in resistance because most of the target traits,

like resistance, are quantitative traits (Junyi 2006). The other possible model that could be seen is a major gene model of heritable resistance. This model is characterized by qualitative differences, described through binary yes/no or a categorical response. An example is Mendel's yellow/green peas, or in this study leaf or stem infected/uninfected. Previous work on tanoak resistance has described it in quantitative variation terms (Hayden et al. 2011), however there was a large environmental effect; it was not clear how consistent the response is within a single genotype.

If the environmental effect was decreased and a single genotype was used, would the same results be obtained? In this study, I examine tanoak individuals grown from seed collected from forests along the western coast of the United States. Specifically, I ask: is SOD resistance heritable and if so, which model of heritability does it follow? This research question was approached by examining how individual variation in resistance to *P.ramorum* inoculations in tanoaks compares to variation of resistance among families of tanoaks.

METHODS

This project had three major parts that examined various characteristics of tanoaks, *P.ramorum* and their interactions. First, I investigated a tanoak clonal propagation method. Secondly I selected the isolate of *P.ramorum* for the inoculations from eight candidate isolate strains. Finally, I preformed multiple inoculations of *P.ramorum* on tanoak saplings and detached leaves and recorded their responses to determine the inter- and intra-familial variation components for my study.

Tanoak Clones

To create the tanoak clones I used trees that had been grown to saplings in pots from acorns taken in 2006 from five different parent trees (SM 31, 51, 52, 53, and 74) within the San Mateo County, Midpeninsula Regional Open Space District. I took four cuttings to create clones from 10 saplings per acorn parent sapling.

To take the cuttings I first examined the sapling to find the optimal places to take the cutting. The most optimal place to take cuttings is right below a node on a somewhat mature

stem that potentially has woody growth because it gives the clones the best chance of successfully rooting. Once I mapped out the locations for the cuttings I used a sharp pair of clippers to cut right below the node at an angle. I trimmed off any excess leaves; only leaving one healthy leaf for photosynthetic processes. The cut end was then dipped into rooting powder (Hormex rooting powder #1: Indole-3 Butyric Acid 0.1%) to promote root growth. After the rooting powder was applied I placed the cuttings in a clear, 115 mL potting cone (Steuwe & Sons) filled with ProMix potting mix (Sunshine). The ProMix potting mix was drenched with a fungicide a few days prior to the cloning procedure.

To increase the chances of rooting in the clones I placed them in an enclosed misting chamber. Within the misting chamber I placed the cones into shallow trays filled with perlite (a light drainage rock) on top of heating pads and arranged in a randomized order to prevent unequal treatment.

After three months I checked the clones and took note if they were dead of alive. The few cuttings with established roots, that I saw through the clear cones, were moved out of the misting chamber into a greenhouse, where they were watered twice a day and given nutrients once a week. The cuttings in the greenhouse were also set in a randomized pattern to promote even treatment. I continued to note any changes in the clones and any deaths that happened within the misting chamber.

Isolates

To have the most successful inoculations of the treatment individuals, I had to determine the optimal strain of *P.ramorum* for inoculation. I chose the strain based on the detached leaf zoospore drop inoculation of bay laurel (*Umbellularia californica*) and tanoak leaves. I tested 8 different isolates from the Garbelotto lab culture collection. The isolates were labeled PR52, 2089, 2442, PRA32, 1461, MR209A, MR53B, and 127.

To start the inoculation process I transferred a 0.5 cm agar plug from each of the 8 different isolates onto V8 agar (Erwin and Ribeiro 1996) in 3 replicates, using sterile technique in a laminar flow hood (Garbelotto lab, UC Berkeley). These transfers were grown for a week in an 18°C incubator; I then marked them for their growth diameter. From these V8 plates I was able to start the production of zoospores. I used the Hüberli et al. (2003) method for zoospore

production with the exception of using 1% soil extract instead. I used these zoospores to inoculate 4 bay laurel leaves and 4 tanoak leaves for each of the 8 isolates.

To complete the zoospore drop inoculation, I placed the leaves in a plastic box with dampened paper, randomized for which isolate they would be inoculated with. The leaf was lightly scored with tweezers on the midrib and then 30µl of zoospore solution ($5x10^4$ concentration) was placed on the score, and 30µl of 1% soil tea for control. After a week, I scanned the bay laurel leaves (using an Epson Perfection 1650 flatbed scanner) and measured the lesions in imageJ (Abramoff et al. 2004). After 2 weeks I scanned the tanoaks and measured their lesions using imageJ as well. The mean lesion sizes along with the lesion to leaf ratio were used to determine the most optimal isolate to use in the future sapling inoculations. After the scanning of each leaf type, I sampled the inner and outer lesion growths using sterile technique and placed on PARP selective media (Erwin and Ribeiro 1996) to confirm the presence of *P.ramorum*. I did this by taking a 1/16 cm^2 square from the outer region of the lesion, and one from the inner region of the lesion. I kept the plates in a dark drawer at room temperature and checked the plates for growth after three days.

Tanoak Saplings

To examine the inter- and intra-familial variation to resistance I used trees maintained by the Garbelotto research group (University of California, Berkeley) grown from acorns taken from trees located in Point Reyes National Seashore, Marin County, CA. I used twelve different open-pollinated seed families of tanoaks (from wild parents PR-2, PR-4, PR-5, PR-6, PR-8, PR-9, PR-10, PR-11, PR-12, PR-16, PR-18, and PR-19). These families were selected because they each had 8 individuals that looked healthy, appeared to have four possible inoculation points, and were not heavily impacted by sooty mold that was spreading in the greenhouse. From each of the twelve families eight individuals were selected for the inoculation (labeled PR-2-368, etc, Table 1).

I gathered the individuals from the Oxford Track Greenhouse (University of California, Berkeley) on Feb 20th 2012, and moved them into a growth chamber within the Oxford Track Greenhouse (10-hour days, 20°C day, 15°C night, with a misting apparatus that operated for 30

minutes twice a day). I also watered the trees by hand every four days. I randomized the order of the individuals within ten trays in the growth chamber to avoid any unequal treatment.

Table 1: Summary Table of Individuals included in the inoculation study:

Location	Family	Individual	Location	Family	Individual	Location	Family	Individual
PR	2	352	PR	4	318	PR	5	307
		342			353			350
		328			314			345
		320			328			352
		330			306			304
		326			319			348
		315			316			351
		307			332			309
PR	6	374	PR	8	339	PR	9	341
		348			307			356
		332			348			321
		357			359			318
		338			340			334
		362			331			323
		352			373			302
		342			361			303
PR	10	353	PR	11	317	PR	12	327
		313			329			307
		370			316			342
		360			362			373
		310			341			320
		322			352			312
		367			331			304
		371			319			332
PR	16	324	PR	18	333	PR	19	326
		315			311			347
		304			346			318
		320			304			358
		328			307			375
		344			337			304
		325			331			324
		309			320			301

Inoculations

There were two different inoculations that I preformed during this portion of the experiment. First I performed the petiole inoculation on the saplings then the detached leaf inoculation. To prep the individuals for the petiole inoculation, I wrapped para-film (Pechiney

Plastic Packaging Company, Illinois) right below the petiole of the leaf and surrounding the stem to create a cup for the zoospores or control drop. I performed this step on Feb 22-23rd 2012. On each individual I chose three inoculation points and one control. I placed the control at the lowest point on the individual, and then the inoculation numbers increased with height. However if the individual was too small or lacked an adequate location I chose to omit the control. On Feb 24th 2012 I scraped the petiole and the adjacent stem with a scalpel to promote zoospore entry. I pipetted 100µl of $5x10^4$ concentration zoospore solution of isolate 1461, or control (sterile soil extract and deionized water) into the para-film cups. The mister was deactivated on the day prior and day of the inoculation. I watered the individuals generously prior to the inoculation to avoid any water stress. The mister was then reactivated the day following the inoculation.

A detached leaf inoculation was also preformed in conjunction to the sapling inoculation. I took a leaf from each individual prior to the inoculation and labeled it. I also took one control leaf from each of the 10 trays, from a random individual within each tray. I inoculated the leaves using the Hayden et al. (2011) method with mycelia filled V8 plates created on Feb 17th 2012. I placed the inoculated leaves in clamshell cases and then incubated them in a growth chamber with conditions described above, in the Oxford Track Greenhouse, for 2 weeks.

Data Collection

To characterize variation in resistance within and among the saplings, lesion size data were collected from the detached leaves on March 8th 2012, and from the saplings on March 26th through March 28th 2012. To collect the data for the detached leaves I used the same technique, software (imageJ), and pathogen re-isolation methods as in my previous detached leaf zoospore drop inoculation.

From the saplings, the data collected included; a binary evaluation of the infection on the stem and leaves (1 or 0 for displayed infection or no displayed infection respectively), leaf and stem lesion lengths (to the tenths cm), number of neighboring leaves infected, overall status of sapling, leaf coloration, location of any tip dieback, and a sketch of the inoculation numbering for each sapling.

Data Analysis

In JMP10 (Sall 1989) I visualized the familial responses to the inoculations. These included the medians and distributions of the leaf lesion lengths and stem lesion lengths. For the leaf and stem lesion medians, I chose to include the zero values; I thought they were important values in the overall family median because a zero is a valid response of no lesion length. I visualized the overall impact of the inoculation from each family by totaling the individual binary results of the leaf and stem statuses. I used these figures to see if there were more resistant responses displayed in some families than others.

Following the descriptive statistics in JMP I preformed some statistical analysis in R (R Development Core Team 2010). I used the lme4 package (Bates D and Maechler M 2010) to analyze the variance among individuals within their families. The model was: Y ~ InocNum + (1 | Fam/Ind). Where Y is leaf or stem lesion presence or absence, InocNum is the numbered inoculation site, and the Family and Individuals are nested and considered as random effects.

Phytophthora ramorum *is subject to state and federal regulation and quarantine. For that reason, all pathogen propagation and plant inoculations were completed under permit and according to conditions set by the United States Department of Agriculture Animal and Plant Health Inspection Service and the California Department of Food & Agriculture, including sanitation protocols to prevent pathogen escape.*

RESULTS

Tanoak Clones

There was very little difference in clone survivorship among the tanoak families. The families initially started with anywhere from 40 to 45 clones. At the first date all families lost between 15-21 individuals (Fig. 1). Each family had mortality throughout the duration of the sampling period. The total survivorship percentages for the families ranged from 45-59% (Table 2). Family number 52 had the highest survivorship with 59%, and family number 53 had the lowest with 45% survivorship.

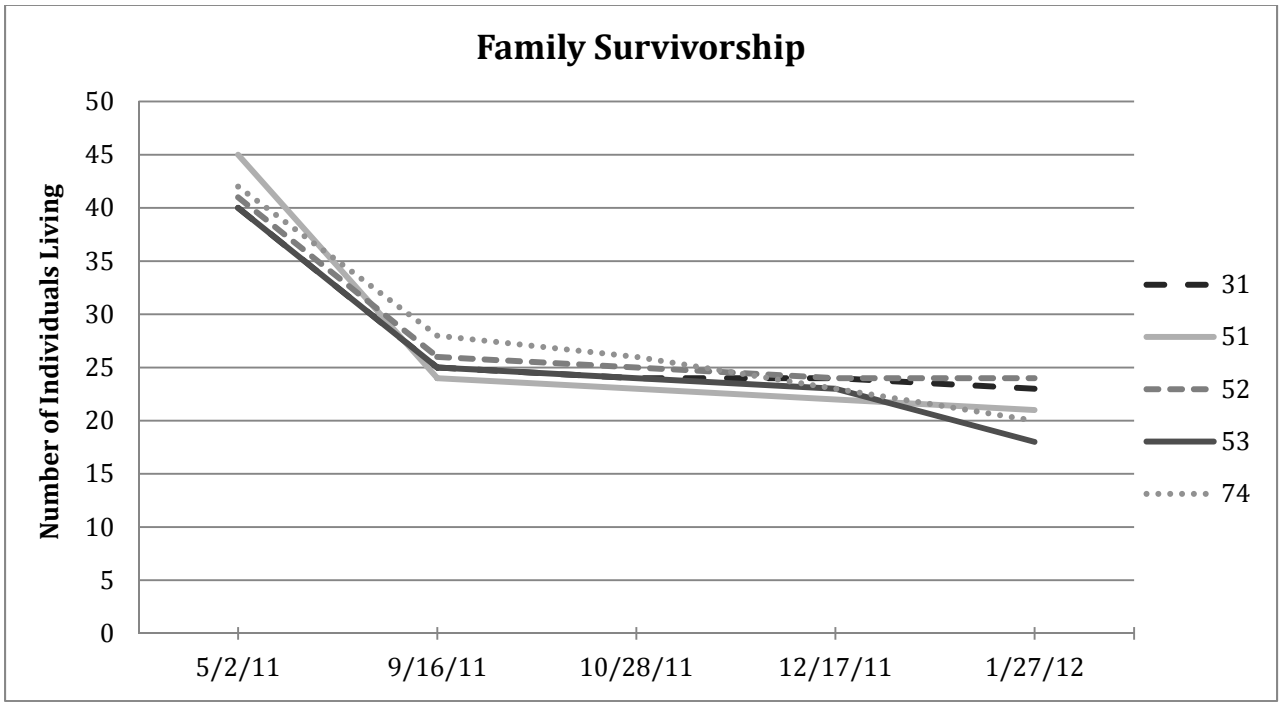

Figure 1: Number of Living Clones in each Family over Time: Initial date of 5/2/11 is when the clones were made.

Table 2: Total Percent of Living Clones: at final assessment on Jan 27th 2012

Family	Total % Survivorship
31	58%
51	47%
52	59%
53	45%
74	48%

Selection of *P.ramorum* Isolate

I determined the isolate for the sapling inoculation using the results from the zoospore drop inoculation of tanoak and bay laurel leaves. Choosing the isolate was based on the medians and distributions of the lesion lengths on the tanoak leaves for each isolate (Fig. 2). The lesion lengths ranged from 1.17- 6.35 centimeters, compared to the control that showed about 0.10-0.33 centimeters (most measurements were from the initial scoring used for the zoospore drop). Compared to the other isolates and the control PR52 had no lesions, making this isolate unusable for the inoculations. The isolates 1461 and 2442 both have the most ideal normal distributions around their median values, which makes them ideal isolates for the inoculation.

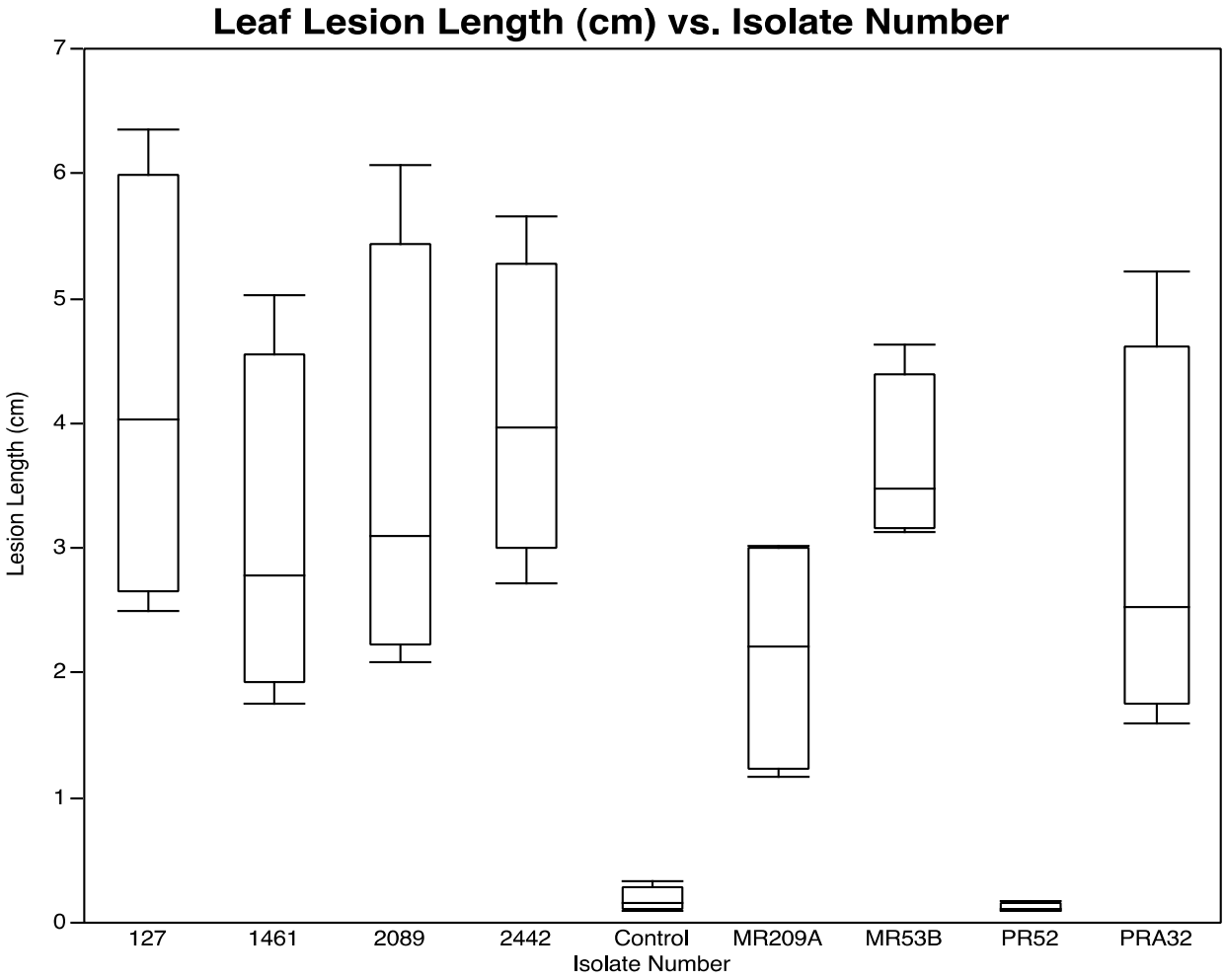

Figure 2: The Median Lesion Lengths for each Isolate: on tanoak leaves measured in centimeters.

Choosing an isolate was also based on the medians and distributions of the lesion area to leaf area ratio. Isolate 1461 and 2442 had quite normal distributions in this assessment as well as the previous (Fig. 3). Isolate 1461 also had a higher median value than 2442 in the lesion to leaf area ratio. Both isolates had growth in all of their pathogen re-isolation inner and outer growth samples (Table 3). After considering all of these factions, I chose to use isolate 1461 for the sapling inoculations, in hopes of yielding the best inoculation results.

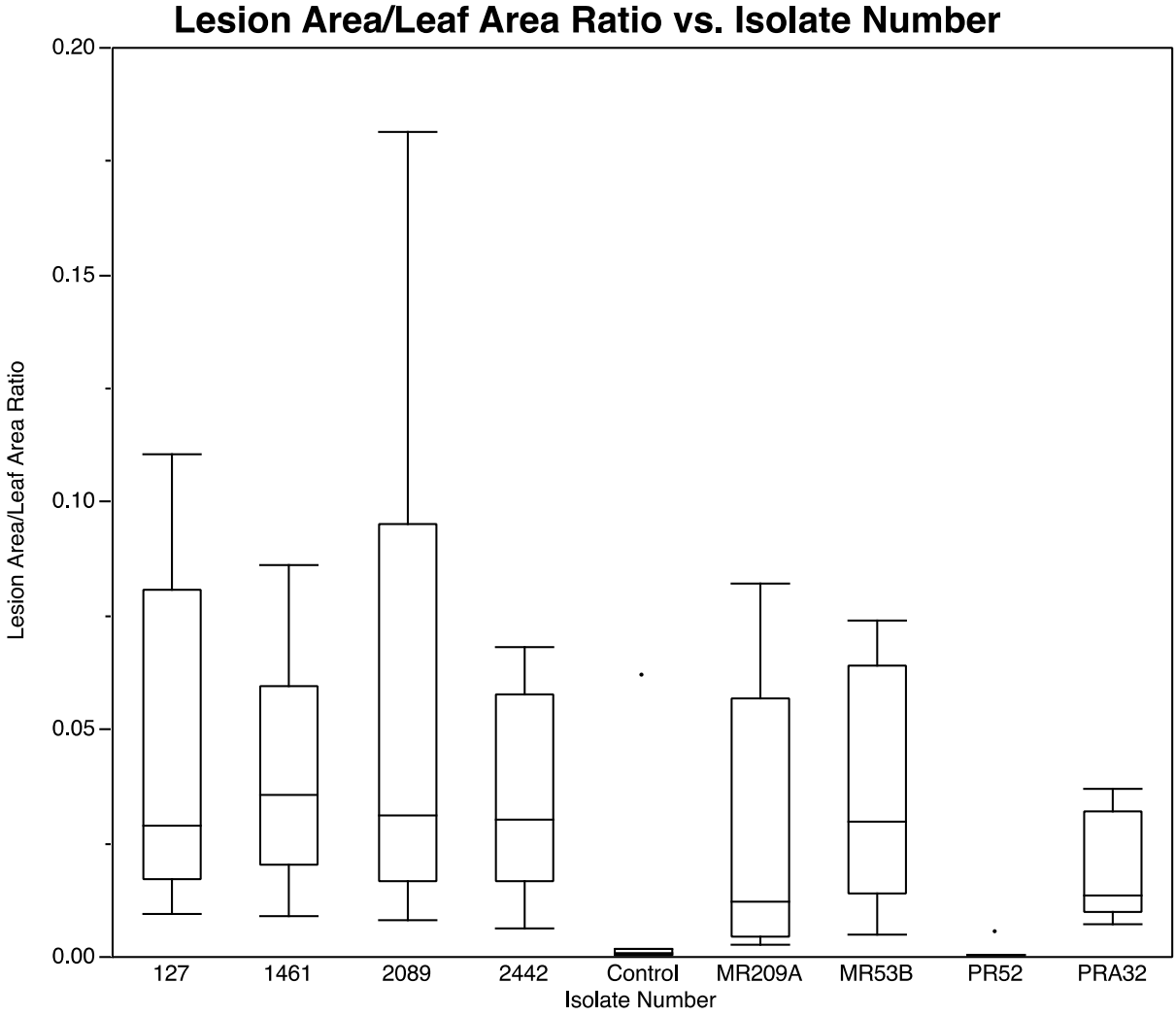

Figure 3: Median values of Lesion Area to Leaf Area Ratio: for Isolates and Control

Table 3: Pathogen Re-isolation Results for each Isolate and Leaf: Inner and Outer Growth presence (YES) or absences (NO)

Pathogen Re-isolation

Bay Leaves				Tanoak Leaves			
Tree #	Isolate #	Inner Growth	Outer Growth	Tree #	Isolate #	Inner Growth	Outer Growth
1	MR53B	YES	YES	5	MR53B	YES	YES
1	1461	YES	YES	5	1461	YES	YES
1	2442	YES	YES	5	2442	YES	YES
1	MR209A	YES	YES	5	MR209A	YES	YES
1	PR52	NO	NO	5	PR52	NO	NO
1	Control	NO	NO	5	Control	NO	NO
1	2089	YES	YES	5	2089	YES	YES
1	PRA32	YES	YES	5	PRA32	YES	NO
1	127	YES	YES	5	127	YES	YES
2	MR53B	YES	YES	6	MR53B	YES	YES
2	1461	YES	YES	6	1461	YES	YES
2	2442	YES	YES	6	2442	YES	YES
2	MR209A	YES	NO	6	MR209A	YES	YES
2	PR52	NO	NO	6	PR52	NO	NO
2	Control	NO	NO	6	Control	NO	NO
2	2089	YES	YES	6	2089	YES	YES
2	PRA32	YES	YES	6	PRA32	YES	NO
2	127	YES	YES	6	127	YES	YES
3	MR53B	YES	YES	7	MR53B	YES	YES
3	1461	YES	YES	7	1461	YES	YES
3	2442	YES	YES	7	2442	YES	YES
3	MR209A	YES	YES	7	MR209A	YES	NO
3	PR52	NO	NO	7	PR52	NO	NO
3	Control	NO	NO	7	Control	NO	NO
3	2089	YES	YES	7	2089	YES	YES
3	PRA32	YES	YES	7	PRA32	YES	YES
3	127	YES	YES	7	127	YES	YES
4	MR53B	YES	YES	8	MR53B	YES	NO
4	1461	YES	YES	8	1461	YES	YES
4	2442	YES	YES	8	2442	YES	YES
4	MR209A	YES	NO	8	MR209A	YES	NO
4	PR52	NO	NO	8	PR52	NO	NO
4	Control	NO	NO	8	Control	NO	NO
4	2089	YES	YES	8	2089	YES	NO
4	PRA32	YES	YES	8	PRA32	YES	YES
4	127	YES	YES	8	127	YES	YES

Inoculations

From the sapling inoculation we were able to see that some families had smaller median leaf lesion lengths (Fig. 4). The medians of the leaf lesion lengths ranged from 0 to 2.15cm, with family number 16 holding the highest median. Family 12 had a very low leaf lesion median suggesting that some resistance might be occurring within that family. This finding is also reflected in the median stem lesion lengths for each family (Fig. 5). Family 12 also had one of the smallest medians of stem lesion length. Median stem lesion lengths ranged from 0 to 1.65cm.

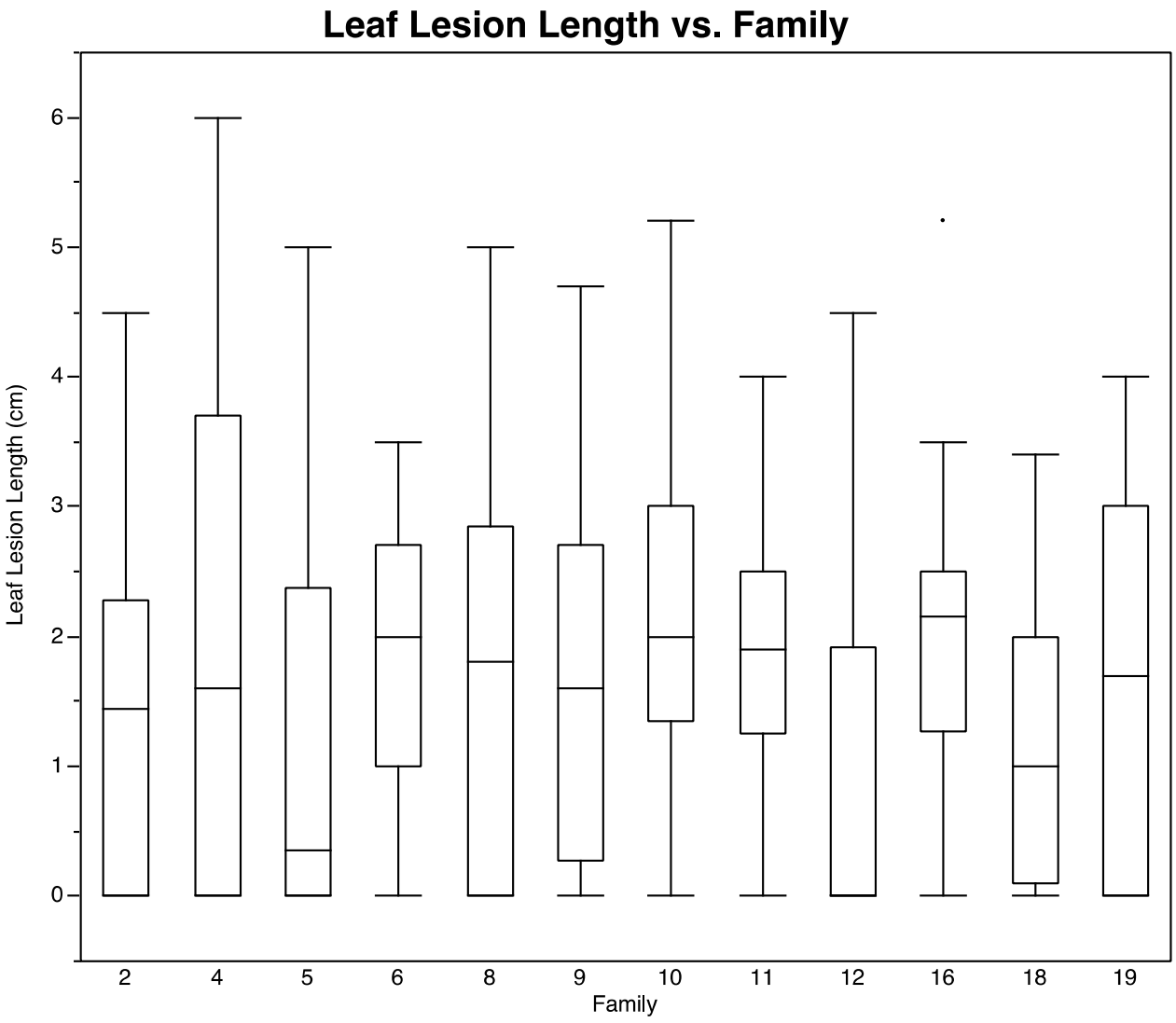

Figure 4: Median Leaf Lesion Length (cm) for each Family: zeros have been included in the mean values for lesion lengths.

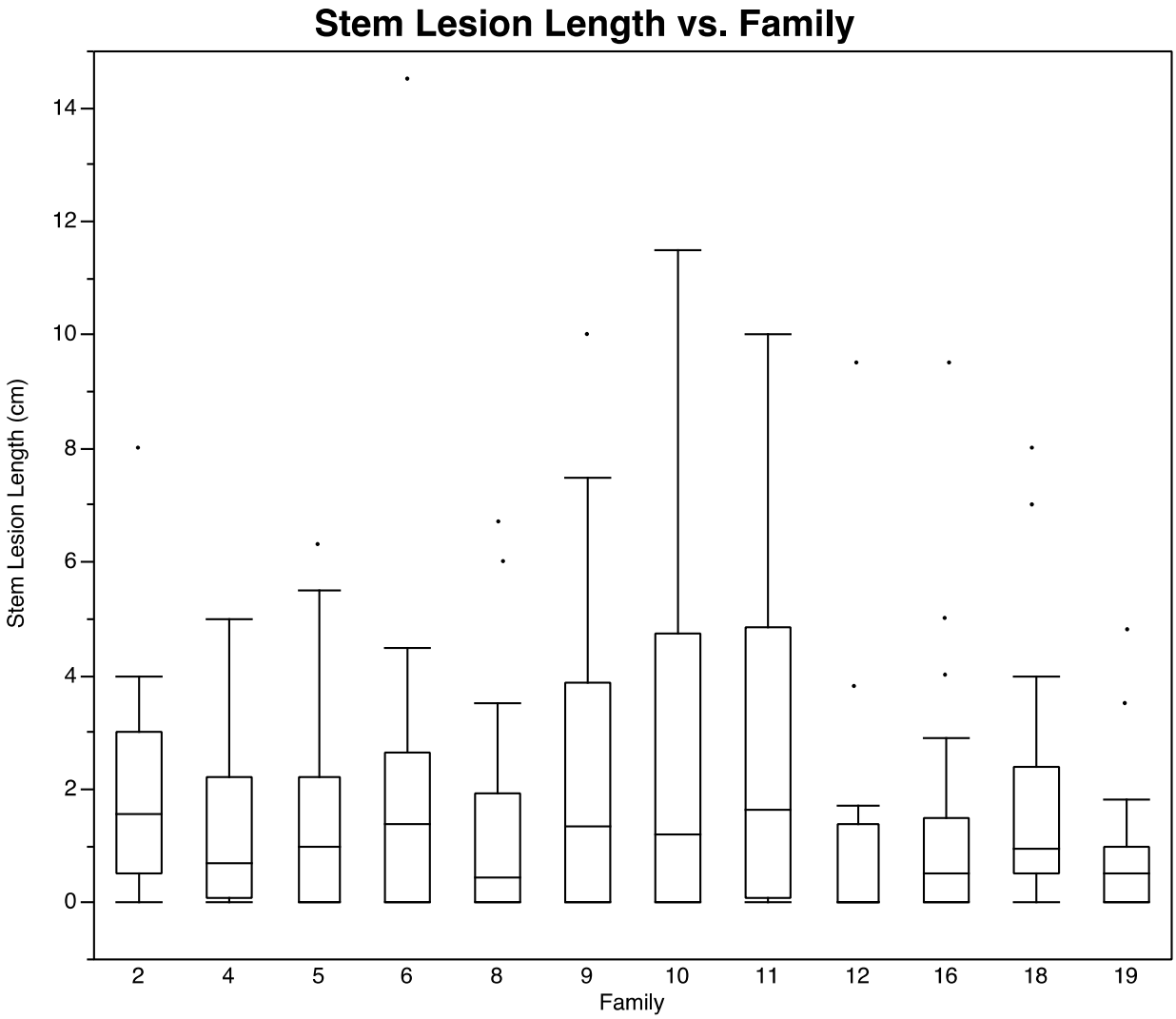

Figure 5: Median Stem Lesion Length (cm) for each Family: zeros have been included in the mean values for lesion lengths

After finding the lowest median values for both stem and leaf lesion length in family 12 I expected to see some variance among families further indicating heritable resistance. However, a generalized linear mixed model indicated little variation among families, but quite a bit of variation among individuals within their family (Table 4). The model also showed that there was a statistically significant difference between Inoculation Point #2 and Inoculation Point #3 in the leaf infection, but this was not the case in stem infection.

Table 4: Generalized Linear Mixed Model of Leaf and Stem Infection Presence

Leaf Infection

Random Effect	Variance Ratio	Std Dev
Ind:Fam (Intercept)	2.3653	1.5379
Fam (Intercept)	0.00	0.00

Fixed Effects	Estimate Std.	Std Error	z value	Pr(>|z|)	
Intercept	2.1874	0.354	6.18	6.42E-10	***
InocNum[T.2]	-0.516	0.4137	-1.247	0.212262	
InocNum[T.3]	-1.3008	0.3952	-3.292	0.000993	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Stem Infection

Random Effect	Variance Ratio	Std Dev
Ind:Fam (Intercept)	1.1498	1.0723
Fam (Intercept)	2.93E-12	1.71E-06

Fixed Effects	Estimate Std.	Std Error	z value	Pr(>|z|)	
Intercept	1.1723	0.2666	4.3970	1.10E-05	***
InocNum[T.2]	-0.1236	0.3382	-0.3650	0.7150	
InocNum[T.3]	-0.4172	0.3308	-1.2610	0.2070	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

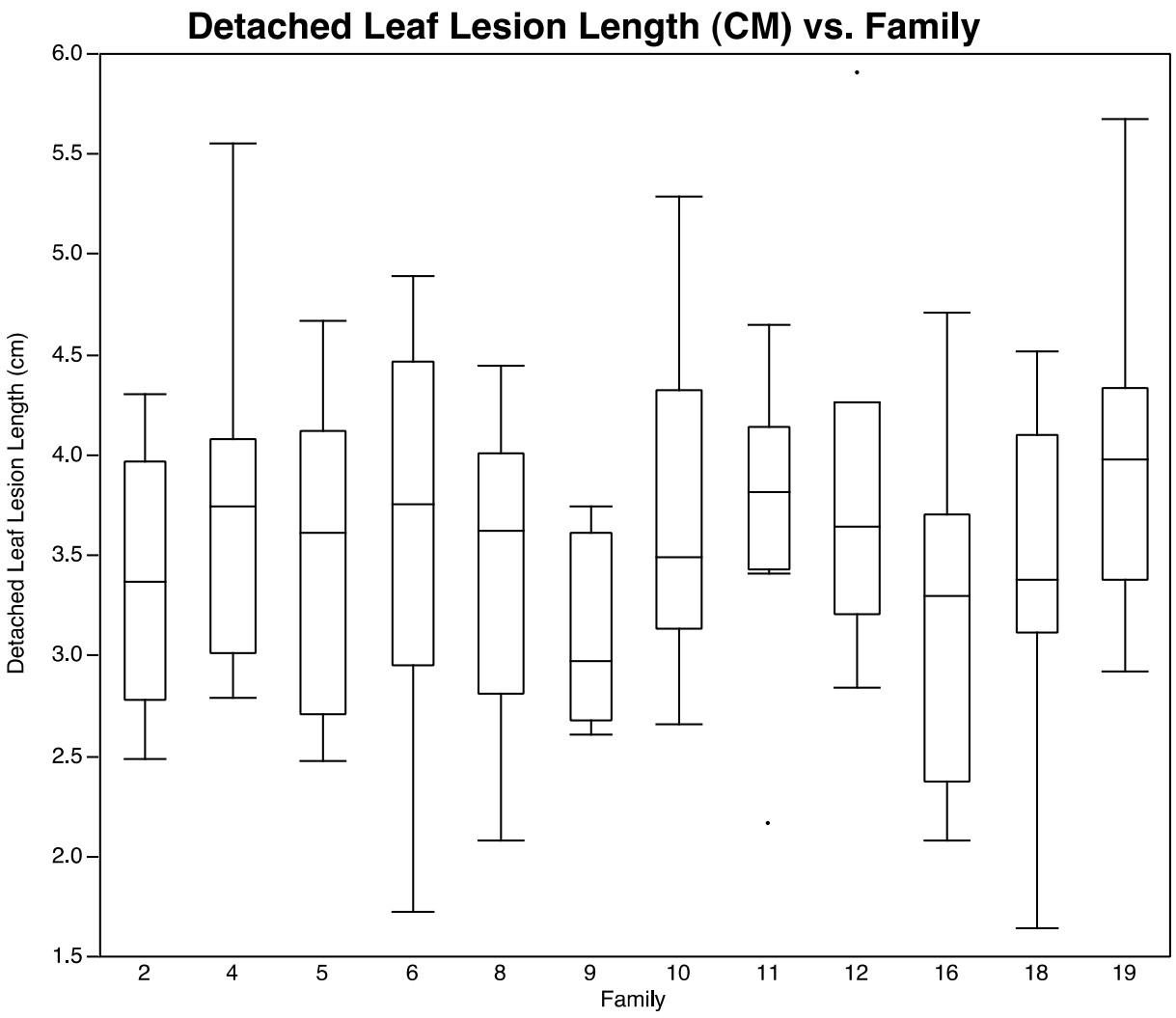

Figure 6: Median Detached Leaf Lesion Length (cm) in each Family

Another inoculation that took place in this study was the detached leaf inoculation. The median values for the detached leaf inoculations ranged from 2.97-3.98cm (Fig. 6). Family 9 has the lowest median value for lesion length, however this is not the case in the sapling leaf lesion lengths. This is also reflected through family 12, which has the lowest medians in the sapling leaf and stem lesions but not in the detached leaf lesion lengths. When the sapling leaf lesion lengths and detached leaf lesion lengths are fit the R^2=0.008, indicating that there is almost no correlation between the two measurements. The pathogen re-isolation results confirm that the lesions in the detached leaves were a result of *P.ramorum* infection (Table 5).

Table 5: Pathogen Re-isolation Results for the Detached Leaf Inoculations: **IG refers to Inner Growth, OG to Outer Growth, and EX is an Extra Sample if any was taken**

Pathogen Re-isolation Results

Loc	Fam	Ind	IG	OG	EX	Loc	Fam	Ind	IG	OG	EX	Loc	Fam	Ind	IG	OG	EX
PR	2	307	YES	YES	NO	PR	6	362	YES	YES	N/A	PR	11	362	YES	YES	N/A
PR	2	315	YES	YES	N/A	PR	6	374	YES	YES	NO	PR	12	304	YES	YES	N/A
PR	2	320	YES	YES	N/A	PR	8	307	YES	YES	N/A	PR	12	307	YES	YES	N/A
PR	2	326	YES	YES	N/A	PR	8	331	YES	YES	N/A	PR	12	312	YES	YES	N/A
PR	2	328	YES	YES	N/A	PR	8	339	YES	YES	NO	PR	12	320	YES	YES	N/A
PR	2	330	YES	YES	YES	PR	8	340	YES	YES	NO	PR	12	327	YES	YES	NO
PR	2	342	YES	YES	N/A	PR	8	348	YES	YES	N/A	PR	12	332	YES	YES	N/A
PR	2	352	YES	NO	NO	PR	8	359	YES	YES	N/A	PR	12	342	YES	YES	NO
PR	4	306	YES	YES	N/A	PR	8	361	YES	YES	N/A	PR	12	373	YES	YES	YES
PR	4	314	YES	YES	NO	PR	8	373	YES	NO	YES	PR	16	304	YES	YES	N/A
PR	4	316	YES	YES	NO	PR	9	302	YES	YES	YES	PR	16	309	YES	YES	N/A
PR	4	318	YES	YES	N/A	PR	9	303	YES	YES	NO	PR	16	315	YES	YES	NO
PR	4	319	YES	YES	YES	PR	9	318	YES	YES	YES	PR	16	320	YES	YES	N/A
PR	4	328	YES	YES	NO	PR	9	321	YES	YES	N/A	PR	16	324	YES	YES	N/A
PR	4	332	YES	YES	N/A	PR	9	323	YES	YES	NO	PR	16	325	YES	YES	N/A
PR	4	353	YES	YES	N/A	PR	9	334	YES	YES	YES	PR	16	328	YES	YES	N/A
PR	5	304	YES	YES	N/A	PR	9	341	YES	YES	N/A	PR	16	344	YES	YES	N/A
PR	5	307	YES	YES	NO	PR	9	356	YES	YES	NO	PR	18	304	YES	NO	NO
PR	5	309	YES	YES	YES	PR	10	310	YES	YES	N/A	PR	18	307	YES	YES	NO
PR	5	309	YES	YES	N/A	PR	10	313	YES	YES	N/A	PR	18	311	YES	YES	NO
PR	5	345	YES	YES	N/A	PR	10	322	YES	YES	YES	PR	18	320	YES	YES	N/A
PR	5	345	YES	YES	NO	PR	10	353	YES	YES	N/A	PR	18	331	YES	YES	N/A
PR	5	348	YES	YES	N/A	PR	10	360	YES	YES	N/A	PR	18	333	YES	YES	NO
PR	5	350	YES	YES	N/A	PR	10	367	YES	YES	NO	PR	18	337	YES	YES	YES
PR	5	351	YES	YES	NO	PR	10	370	YES	YES	N/A	PR	18	346	YES	YES	N/A
PR	5	352	YES	YES	N/A	PR	10	371	YES	YES	N/A	PR	19	301	YES	YES	NO
PR	6	332	YES	YES	N/A	PR	11	316	YES	YES	YES	PR	19	304	YES	YES	YES
PR	6	338	YES	YES	N/A	PR	11	317	YES	YES	N/A	PR	19	318	YES	YES	NO
PR	6	342	YES	NO	N/A	PR	11	319	YES	YES	YES	PR	19	324	YES	YES	N/A
PR	6	342	YES	NO	NO	PR	11	329	YES	YES	NO	PR	19	326	YES	YES	N/A
PR	6	348	YES	YES	N/A	PR	11	331	YES	YES	N/A	PR	19	347	YES	YES	NO
PR	6	352	YES	YES	NO	PR	11	341	YES	YES	N/A	PR	19	358	YES	YES	N/A
PR	6	357	YES	YES	NO	PR	11	352	YES	YES	N/A	PR	19	375	YES	YES	N/A

To take another look at the familial responses to the inoculations I used the results from the binary visual evaluations of the stem and lesion infection. A 1 was given to the visibly infected point, and a 0 was given for a visibly uninfected point. Each sapling had three inoculation points and when the evaluation points were totaled the individual scored between 0-3 for leaf or stem results. The following figures are the sums of the individual scores in each value groups 0,1,2, and 3 for each family.

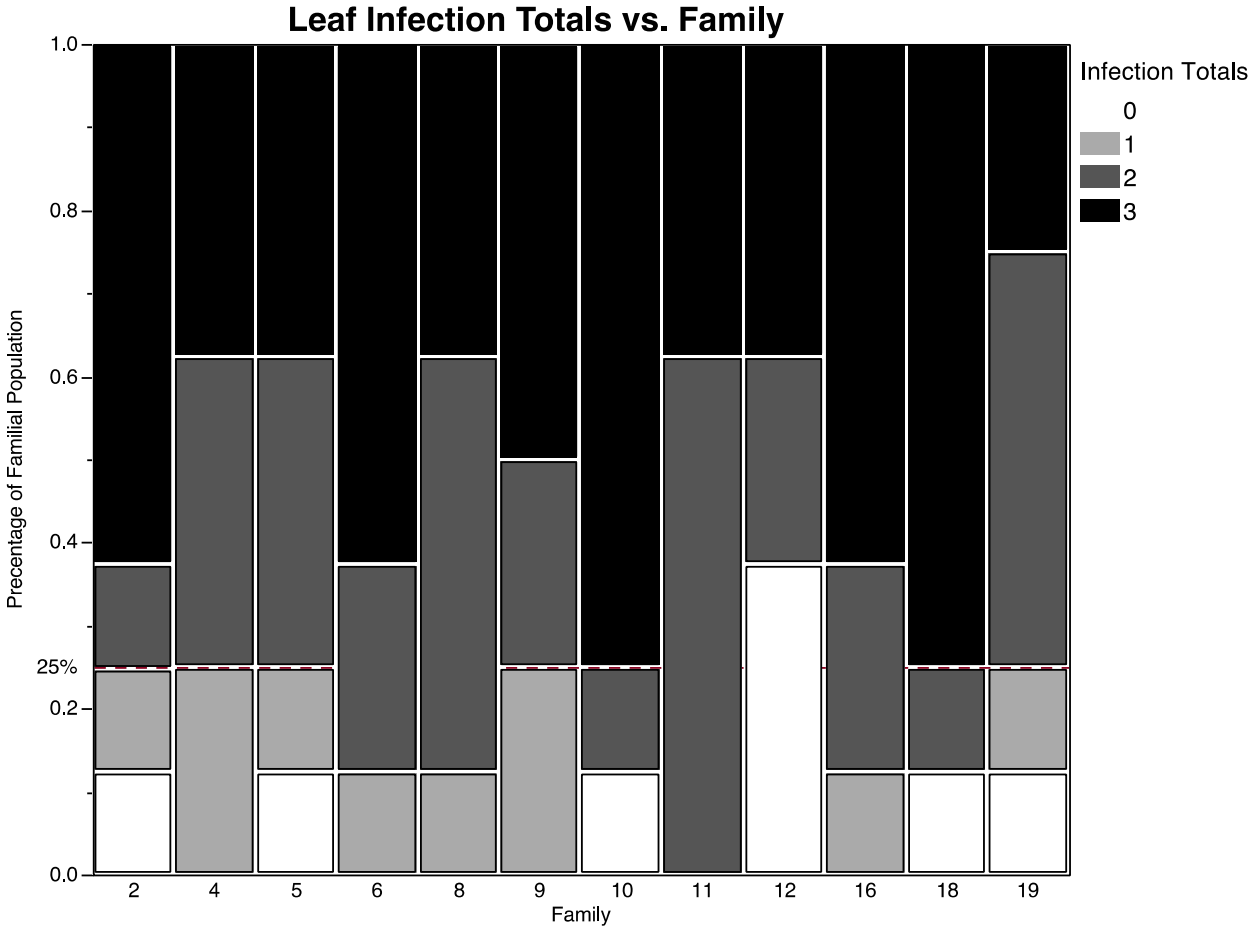

Figure 7: Leaf Infection Totals for each Family: the 25% population amount is indicated by the dashed line.

In the familial total for leaf evaluation family 12 had over 25% of the families population showing no signs of infection (Fig. 7). There are also some families that showed a high impact from the inoculation. Family 11 had over 50% of its offspring showing symptoms of infection on two of their inoculation points; the remainder saplings were showing signs at all three. In the stem evaluation families 5, 10, and 12 had 25% of their individuals showing no symptoms of infection (Fig 8).

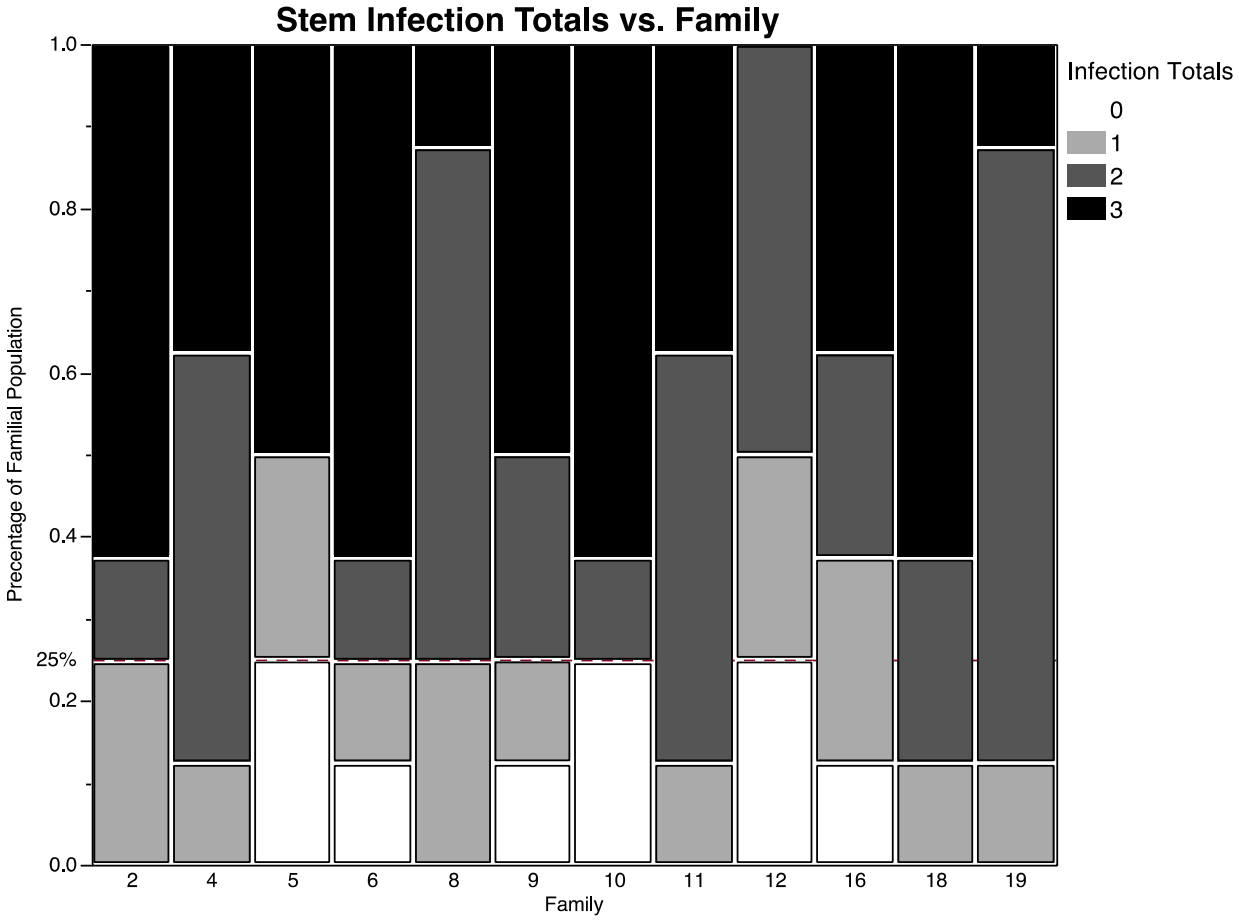

Figure 8: Stem Infection Totals for each Family; 25% of the population is indicated with the dashed line

DISCUSSION

This multi-part experiment took a close look at the familial and individual responses to cloning and *P.ramorum* inoculations. The results of this study showed familial resistance, occurring in a different way than predicted. Previous results found tanoak resistance to SOD was done through a polygenetic model of additive loci, but these results indicate that resistance may be occurring in terms of a major gene model. The future directions of this study could have major impacts on forest health and composition. Continued research on these findings could benefit the future of tanoaks in the western coast of the United States.

Clones

There were slight familial differences of survivorship observed within the tanoak clones. Families numbered 52 and 31 had the highest survivorship percentage of 59% and 58% respectively. Compared to the family 53 that had the lowest survivorship of 45%, there is a 14% difference. This value could indicate that there are some families that have a better chance of survivorship in these rooting techniques. The techniques that could have determined the survivorship of the clones were; application of rooting hormone (Hormex), cuttings were kept in an enclosed misting chamber within the greenhouse, and heating pads were placed underneath the perlite filled trays. More in depth studies were able to find 53% rooting success using a similar enclosed misting technique in sweet chestnut cuttings (Jinks 1995). For future tanoak cuttings removing the heating mats after a month or two might increase the overall survivorship. This would increase the drainage and possibly increase root establishment.

Sapling Inoculation

After analysis of the sapling inoculations some interesting results of variance among individuals and families were found. The attached leaf lesion and stem lesion length analysis both resulted in zero variance among families. In the quantitative genetic model, heritability (h^2) is a function of the proportion of total phenotypic variance that is due to family (Lynch and Walsh 1998). When zero variance among families, the h^2 result is zero. This shows that there is

no heritable resistance among families under the polygenetic model. Previous studies of tanoak resistance in the same nursery and with similar methods, but using a different isolate of *P.ramorum*, have found h^2 =0.15 in detached leaf lesions (Hayden et al. 2009) and h^2 =0.10 in stem lesions (Hayden unpublished data).

While there is no value for heritable resistance under a polygenetic model there seems to be evidence that resistance is occurring. When observing the median leaf lesion lengths by family (Fig. 4) there are families with significantly lower medians than others. Family 12 has the lowest median leaf lesion length, followed by family 5. Family 12 also has the lowest median values in the stem lesion lengths (Fig. 5). The decreased lesion medians in both stem and leaf indicate that the family had decreased affects from the inoculation. This result is not reflected in the analysis of the variance because the family lesion lengths have uneven distributions. This would account for the zero variance among families. However there is variance among the individuals within the family, which is reflective of major gene effects of heritable resistance.

The possibility of resistance is also seen in the leaf and stem binary infection totals (Fig. 7 and Fig. 8). In these binary totals some families have 25% or more individuals in the family showing no symptoms of infection from the inoculation. This proportion is large enough to future indicate that some other form resistance is taking place among the individuals and families, such as a major gene effect.

The differences in results from this study and a similar study preformed by Haden et al (2009) could be explained by a few experimental differences. This study used saplings from Point Reyes; this population was not included in the Hayden et al samples. The Haden et al study also utilized multiple populations of parents in a variety of west coast forests, while I used one population of parents in Point Reyes. Another item that could explain the difference is the choice of *P.ramorum* used. In this study isolate number 1461 was used, while isolate PR52 was used in the Hayden et al study. These factors could all result in the differences, but future experimentation should be done to further confirm resistance is a result of major gene effects.

Limitations

While this project was able to find interesting results there were some limitations to the study that should be considered. The data from the inoculated saplings might have benefitted

from a larger sample size. The size of 12 Families and 8 Individuals within each family has the ability to yield some beneficial data but a larger size might have made the findings more substantial. A larger sample size could also be beneficial for a future direction of the study, making a concrete determination of a major gene interaction requires a large sample sizes (Fain 1978). A larger sample size would have increased the validity of the binary infection totals. With a larger sample size there would be increased accuracy of the percentage of uninfected individuals in the family. Another limitation that should be considered is one isolate was used for the inoculations, and one population of parent trees which could have limited the amount of variation in the response.

Future Directions

This project could have major impacts on forest management and understanding tanoak resistance to SOD. If this project is done on a larger scale it could be possible to confirm that a major gene for resistance is present in some families of tanoaks. Including many more individuals within the families, and possibly more families, could confirm the major gene model of resistance. Knowing these factors could increase the understanding of the interactions that are taking place, and maybe lead to the future discovery of a possible gene for resistance to SOD.

Conclusion

This study has been able to conclude that the genetics of tanoak resistance may be acting in different a way than what was previously believed. Tanoak resistance to SOD may not be acting in through a polygenetic model of additive genes. The high possibility of resistance acting through major gene effects could impact the future understanding of the host and pathogen interaction, management strategies, and modeling of the future spread of disease. These findings could lead to a change in the future of tanoaks in the western forests that have been severely impacted by the continuous spread of *P.ramorum.* The continuation of tanoaks in western forests will allow them to continue to feed, shelter, and provide for a variety of forest species (Ramage et al. 2011, Barrett et al. 2006, The California Department of Fish and Game 2002). The fate of this valuable keystone species could be taking a change in the positive direction.

ACKNOWLEDGMENTS

I would like to give tremendous thanks to everyone who was able to make this project possible. Most importantly I would like to thank **Katherine Haden** for all of her support and continuous help throughout my project. I am so thankful for everything that she has done with me this past year and a half.

I would also like to thank:
ES196 Instructors:
Patina K Mendez
Kurt Spreyer
Melissa Eitzel
Seth Shonkoff

My Parents
Kevin Grump and Michelle Montjoy

My wonderful roommate and friend
Emma Lantos

And last but in no way least
All of the amazing people in my ES class
Especially the Hooligans Corner
For keeping me and each other sane through this amazing journey.

Phytophthora ramorum is subject to state and federal regulation and quarantine. For that reason, all pathogen propagation and plant inoculations were completed under permit and according to conditions set by the United States Department of Agriculture Animal and Plant Health Inspection Service and the California Department of Food & Agriculture, including sanitation protocols to prevent pathogen escape.

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APPENDIX

R Coding

```
> levels(LeafInf)
[1] "I" "U"
> LInfN<-ifelse(LeafInf=="U",0,1)
> Inf.glm<- glm(LInfN~InocNum, family="binomial")
> Inf1<-glmer(LInfN~InocNum + (1|Fam/Ind), family="binomial")
> Inf1
Generalized linear mixed model fit by the Laplace approximation
Formula: LInfN ~ InocNum + (1 | Fam/Ind)
   AIC   BIC logLik deviance
 312.5 330.8 -151.2    302.5
Random effects:
 Groups  Name        Variance Std.Dev.
 Ind:Fam (Intercept) 2.3653   1.5379
 Fam     (Intercept) 0.0000   0.0000
Number of obs: 288, groups: Ind:Fam, 96; Fam, 12

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)    2.1874     0.3540   6.180 6.42e-10 ***
InocNum[T.2]  -0.5160     0.4137  -1.247 0.212262
InocNum[T.3]  -1.3008     0.3951  -3.292 0.000993 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
          (Intr) IN[T.2
InocNm[T.2] -0.662
InocNm[T.3] -0.710  0.593
```

```
> SInfN<-ifelse(StemInf=="U",0,1)
> Snf1<-glmer(SInfN~InocNum + (1|Fam/Ind), family="binomial")
> Snf1
Generalized linear mixed model fit by the Laplace approximation
Formula: SInfN ~ InocNum + (1 | Fam/Ind)
   AIC   BIC logLik deviance
 357.4 375.7 -173.7    347.4
Random effects:
 Groups  Name        Variance   Std.Dev.
 Ind:Fam (Intercept) 1.1498e+00 1.0723e+00
 Fam     (Intercept) 2.9298e-12 1.7117e-06
Number of obs: 288, groups: Ind:Fam, 96; Fam, 12
```

```
Fixed effects:
             Estimate Std. Error z value Pr(>|z|)
(Intercept)    1.1723     0.2666   4.397 1.10e-05 ***
InocNum[T.2]  -0.1236     0.3382  -0.365    0.715
InocNum[T.3]  -0.4172     0.3308  -1.261    0.207
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
            (Intr) IN[T.2
InocNm[T.2] -0.650
InocNm[T.3] -0.666  0.524
```
