# The Use of Compost as an Inhibitor of *Phytophthora ramorum* in Soil

**Kanesha Pompey**

## ABSTRACT

Oak trees bring in more than $500 million to California alone from timber. Plaguing this million-dollar industry is Sudden Oak Death (SOD), caused by *Phytophthora ramorum*, an oomycete that can survive in leaf tissue and soil. There are currently chemical treatments in use to control SOD but non-chemical methods are being sought. Compost may be a viable solution that can control SOD by introducing microorganisms that compete with *P. ramorum*. Thus, I examined the effectiveness of compost treatment in real forest systems infected with *P. ramorum.* I conducted three rounds of soil collections around infected Bay laurel and Tan oak trees in a San Mateo County, CA watershed: pre-compost, one week after compost application, and one month after compost application. For each soil sample, I quantified *P. ramorum* growth. I conducted a Taqman real time PCR to determine if *P. ramorum* DNA was present in soil but unculturable. I also conducted a microorganism colony count to see if compost introduced new microorganisms into the soil. I found a 55% *P. ramorum* prevalence in round one; a 50% prevalence in round two, and 14% prevalence in round three; rounds one and two did not have a statistically significant difference from each other however, round three had significant difference of *P. ramorum* growth from rounds one and two. These results show that compost may be affective in reducing *P. ramorum* in soil, however length of compost application may be an important factor. More studies should be done on the effectiveness of compost.

## KEYWORDS:

bioremediation, Taqman real time PCR, Biocontrol, Sudden Oak Death, organic solutions

## INTRODUCTION

Oak trees play a vital role in many ecological systems as well as in human markets. Not only are oak trees aesthetically valuable but they are also an important natural resource. As a highly valued source of timber, oak trees bring in more than $500 million to California alone (Kliejunas 2010). Plaguing this million-dollar industry is the epidemic of Sudden Oak Death (SOD). SOD first emerged around 15 years ago (Kliejunas 2010). By the mid 2000s the *Phytophthora ramorum* pathogen affectively spread across North America and Europe (Kliejunas 2010). The Sudden Oak Death problem caused by *P. ramorum* can potentially harm millions of acres of California woodlands that are home to live oaks, tanoaks, and black oak populations. (Garbeletto *et al.* 2001, Kliejunas 2010). In order to effectively control the spread of Sudden Oak Death, understanding the underlying mechanisms of transmission is crucial. (Davidson *et al.* 2002)

Sudden Oak Death is spread via spores on both low level (from tree to tree in the same infected location) and high level transmission (from one infected geographical area to another previously uninfected area). *P. ramorum* is a type of water mold that has both a sexual (oospores) and asexual (chlamydospores) phase which both produce spores (Erwin and Ribeiro 1996, Davidson *et al.* 2002, Grundwald *et al.* 2012). Production and survival of spores is influenced by temperature and weather patterns, such as rain and humidity (Duniway 1983). Sporangia are formed on infected leaves and twigs (Grunwald *et al.* 2012), which can then infect soil as leaf litter. Chlamydospores are produced in infected plant tissue. The chlamydospores allow for *P. ramorum* to survive in infected plants, in plant debris on top of soil and also within the soil. (Grunnwald *et al.* 2012). To aid in preventing the spread SOD chemical treatments have been created to help limit/eliminate the spread of *P. ramorum* sporangia and chlamydospores.
There are many chemical solutions to the problem of Sudden Oak Death but recently more organic methods are being sought after (Kliejunas 2010).

There are several problems with chemical solutions to *P. ramorum*. Firstly, using fungicidal treatment in large forests is not practical (Kliejunas 2010). In addition, trying to prevent the spread of SOD in entire forest that is already infected with *P. ramorum* would be hard to accomplish with chemicals because fungicide is a responsive solution rather than preventative. Fungicides may also mask *P. ramorum* symptoms (Chastagner *et al.* 2010), making

it difficult to detect infected plants. Finally *Phytophthora* species have developed resistance to some chemical treatments. For instance, some *P. ramorum* populations in United Kingdom nurseries have developed resistance to the fungicide metalaxyl-M (Turner *et al.* 2008).

Dry compost is a potentially viable alternative to chemical solutions for treating soils infected with *P. ramorum*. Hoitnik and Boehm (1999) suggest biological mechanisms by which compost provides disease control: competition for nutrients by beneficial microorganisms, parasitism of the pathogen by beneficial microorganisms and antibiotic production by beneficial microorganisms. Hoitnik *et al.*. 1997 noted that bacterium such as *Pseudomonas spp., Bacillus spp.* and some fungi can act as biological control agents in compost-amended substrates. Furthermore, Cohen *et al.* (2006) observed that the bacterium *Pseudomonas fluorescens* strain SS101 caused zoospore lysing and resulted in less *P. ramorum* infection of detached California bay laurel leaves. Elliot and Shamoun (2008) found that antagonistic bacteria such as *Bacillus subtilis* inhibited lesion development in detached Rhododendron leaves. Linderman and Davis (2006) found that both *Baillus brevis* and *Paenibacillus polymyxa* significantly inhibit all Phytophthora species in vitro. The use of compost can introduce these types of beneficial microorganisms and more, into the system and inhibit *P. ramorum* population growth. By inhibiting and possibly even eliminating *P. ramorum* growth, there can be less chance of the pathogen spreading. My study will add to the gap in knowledge about compost effectiveness in real forest systems infected with *P. ramorum*.

**Objectives**

The aim of this study is to determine the effectiveness of compost as an inhibitor of *P. ramorum* in soil surrounding infected Northern Californian oak trees in the San Francisco watershed region. Two main questions will be asked: 1) Can compost successfully control *P. ramorum* populations within soil? 2) Is compost a long-term solution?

## METHODS

**Site description**

Soil infected with *P. ramorum* was collected from a 23,000 acre closed forest area within

a San Mateo County watershed, owned by the San Francisco Public Utilities Commission. As the area is closed of to the public, the chance of disturbance to the sampling site is minimal. The site has been infected with the Phytophthora pathogen for a minimum of 11 years. The probability of obtaining soil with the pathogen was therefore almost certain. We sampled soil from two separate drainage locations within the closed off area (Figure 1). These two sites were chosen because they contained trees that are susceptible to *P. ramorum*. The Pilarcitos drainage site contains mostly *Pseudotsuga menziesii* (Douglas fir) and *Lithocarpus densiflorus* (Tanoak). The second drainage site, San Mateo, contains Coast live oak (*Quercus agrifolia*). *Umbellularia californica* (California bay laurels) are prevalent in both drainage areas. These two sites also consistently tested positive for *P. ramorum* for 2 consecutive years (2009 and 2010).

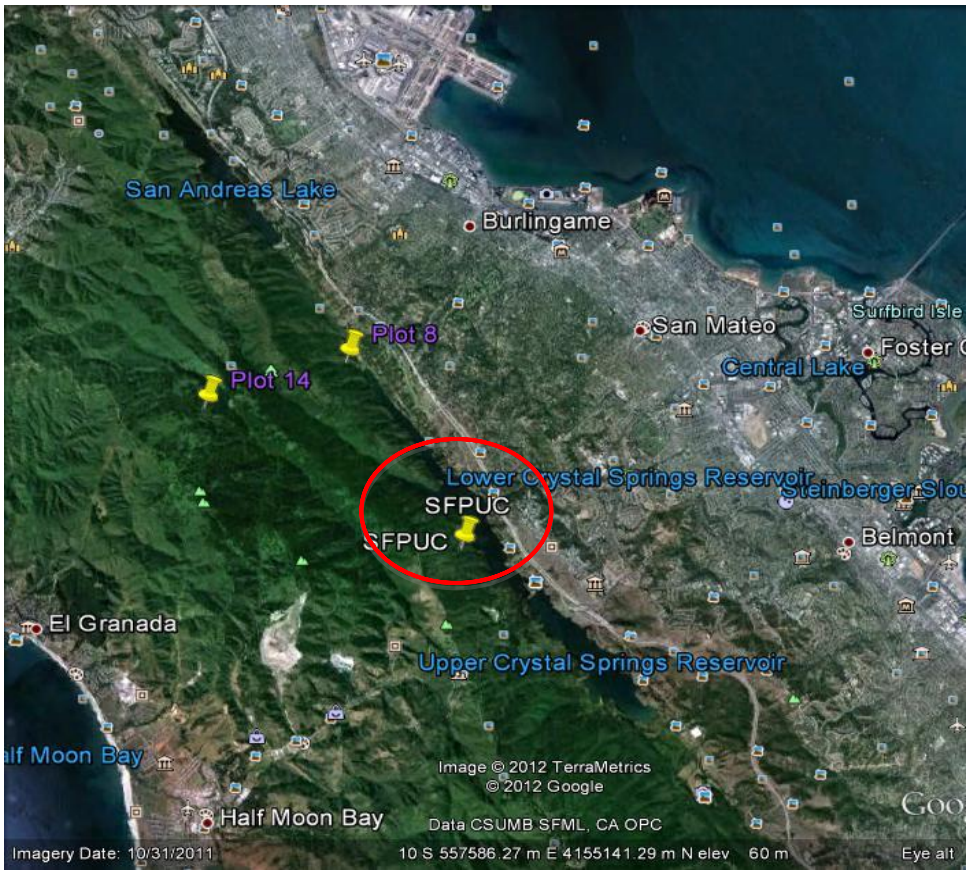


**Figure 1. Map of study Site**. The area is a 23,00 acre forest owned by the San Francisco Public utilities commission. Two drainage sites within site were sampled from.

**Sample Collection**

I sampled from within a one-meter radius around the base of four Bay laurel trees from each location. Two trees received the compost treatment while the other two served as untreated controls. Trees for the site were randomly chosen as well as which trees received treatment. The compost I used was dry organic compost derived from yard trimmings and vegetal food waste. The compost came from the Sonoma Compost Company located within Sonoma County, Sonoma, California.

Before treatment application, I used a simple gardening shovel to take twenty random samples from around the one-meter radius surrounding each tree to test for the presence of P. ramorum. Before each sample was collected, the shovel was wiped with 70% ethanol to prevent contamination. I removed surface litter and dug approximately 2 inches for each sample collected. Samples were approximately one foot away from each other. The amount of soil I collected filled half of a 26.8cm by 27.3cm Ziploc freezer bag. Twenty samplers per tree were collected for a total of 160 samples. These samples remained in the Ziploc bags and were stored in a cold room at 8°C for one week in preparation for soil baiting. One day after taking pre-treatment samples, surface litter was removed from soil surrounding designated treatment trees and two inches of compost was applied around the one-meter radius of the tree using a gardening shovel. Nothing was done to the control trees. Post-treatment soil samples were collected a week after treatment application using the same pre-treatment procedure. These samples were placed into a zip lock bag and stored in a cold room at 8°C for one week. One week after collection I retrieved ten samples from each tree from the cold room and performed a soil baiting technique. The rest was stored for a month at 8°C to repeat the baiting analysis in order to determine the efficacy of the soil baiting technique. The pre- treatment period was labeled Round 1, the immediate post treatment period (samples collected and baited 1 week after compost application) was labeled Round 2 and the last treatment period (samples baited after a month in storage) was labeled Round 3.

**Soil Baiting and Plating**

In order to determine the presence and amount of *P. ramorum* in the soil samples, *P.*

*ramorum* needed to be isolated. To do this, I applied a soil baiting technique using the leaves of uninfected *Rhododendron* var Cunningham's White. This type of Rhododendron was chosen because it is particularly susceptible to *P. ramorum* (Swain *et al.* 2006 and Fichtner 2007). I added 500mL of distilled water to Ziploc bags containing the soil samples (Dart *et al.* 2007). Dart *et al.* (2007) used Rhododendron leaves that were wounded by way of slicing the leaves' midribs. However, I hole-punched the Rhododendron leaves to provide a clear precise area that is easy for *P. ramorum* to infect and for analysis. I then placed the punched leaf discs in mesh bags and submerged in the soil/water mixture and left to incubate at 16-18 ºC for 7 days. The leaf disks then were plated onto PARP+H, a growth medium that is highly selective for Phytophthoras (Masago *et al.* 1977, Fichtner 2007), and incubated at the same 16-18 ºC temperatures for an additional week (Dart *et al.* 2007 and Vettraino *et al.* 2009). In order to determine presence of *P. ramorum*, I examined the plated the leaf discs microscopically. Mycelia growth from leaf disks is an indicator of *P. ramorum* presence within the soil in which the leaf was baited. Subcultures were made onto clean PARP plates in order to determine if the mycelium growth was truly *Phytophthora ramorum*.

**Nested PCR**

I used a Taqman real time nested Polymerase Chain Reaction (PCR) procedure in order to check the accuracy of the plating as a detector of *P. ramorum*. For samples with no mycelia growth on plates (our indicator of no *P. ramorum* presence) a nested PCR was used to amplify the DNA of the soil and determine if *P. ramorum* was present but unculturable (Swain *et al.* 2006, Dart *et al.* 2007). A nested PCR was chosen because it is useful in detection of when the amount of pathogen material is very small or when inhibitors are present in host tissue extracts. Also when compared to a single round of PCR a nested PCR is more sensitive (Hayden *et al.* 2006). A soil extraction procedure was used to extract DNA from the soil samples, which had no *P. ramorum* mycelium growth from the plates. This extraction method used to buffers: Buffer A consisted of 100µl of 10M NaOH, 200ml 2% Tween 20 and 9.7 ml water. Buffer B consisted of 40 µl 0.M EDTA, 1 ml 1M Tris HCL and 8.96 ml water. I put 0.25g of soil into 1.5ml eppendorf tubes. I added 1ml of buffer A into the tubes then vortexed them for 5 seconds. I then placed the tubes in 95ºC heat blocks for 10 minutes. I then vortexed and spun the samples for 1 minute at

13000rpm. I placed 100µl from the top of the solution into new 1.5 ml tubes and added 900µl of Buffer B. I then stores at -20°C (Xin 2003). I used these soil extractions in the nested PCR, which consists of two rounds. During the first round, I diluted the extractions 1 in 100. I then made a mix of 8µl PCR water, 5µl PCR buffer, 2µl 25mM MgCl2, 2.5µl of 2mM dTNPs, 0.25µl Phyto1 (1microM), 0.25µl Phyto4(1µM), 0.25µl RT1, 0.25µl Lt1 and 0.25µl Taq. I added 18.75µl into 6.25µl of the diluted DNA extractions. I then ran the samples in a PCR machine. During round 2 of the nested PCR, I diluted the amplified samples 1 and 250. I placed 5 µl of the diluted samples into new PCR trays. I made a mix consisting of 0.06µl of 50mMRT Universal, 0.06µl of 50mM Lt Universal, 0.06µl of 50mM Universal probe, 0.06µl of 50mM Pram5, 0.06µl of 50mM Pram6, 0.06µl of 50mM Pram7, 7.5µl Taqman and 2.14µl PCR water. I placed 10 µl of this mix into each sample and then ran it through the real time PCR machine. At the end of this round results were given regarding whether there was or was not Phytophthora ramorum DNA in the soil.

**Microorganism Colony Count**

I conducted a microorganism colony count, in order to determine if my hypothesis (microorganisms introduced by compost out compete the Phytophthora pathogen) was supported. My goal was to see if *P. ramorum* colonies decrease while other microorganism increase after the addition of treatment application. Six soil samples (3 before treatment and 3 after treatment application) were used for the colony count. 200 mg of a soil sample was added to a 2mL tube. Distilled water was then added. 100 ml of the afore tube was added to another tube with 900 ml distilled water making a 1/10 dilution. This was done four more times till five tubes were made (original, 1/10, 1/100. 1/1000, and 1/10,000). One hundred ml of each solution was each spread onto a Malt extract, V8 and PARP medium plates and left to incubate for 2 days. The Malt and V8 mediums are nutrient mediums that promote various types of microorganism growth. The PARP while a selective medium for *Phytophthoras* will give a good indication of *P. ramorum* population growth or decline before and after treatment application. Colonies were then categorized by physical characteristics to determine the number of microorganisms. I looked at size of the organisms as well as color and texture to determine distinctions in microorganisms.

### Data Analysis

I used an ANOVA statistical test to determine if there was any significant difference in *P. ramorum* reductions after compost application. The ANOVA compared counts of *P. ramorum* occurrences to site, round and treatment. I also Used a Tukey-Kramer statistical test to compare the rounds. Round 1 had no compost treatment while two trees in each site received compost for different lengths in round 2 and round 3. The Tukey-Kramer test will show if there is any significant difference and how much difference from the addition of compost.

## RESULTS

### Soil Baiting and PCR

After plating the leaf discs from before compost and after compost treatment onto selective PARP medium I found that there was some decline in the number of successful P*hytophthora ramorum* isolations from the leaf discs used to bait the soil, after treatment application. There was 55% *P. ramorum* prevalence in round one; 50% detection in round two and 14% in round three (Fig.2). I found the difference in no compost and compost treatments to have a p-value of 0.07 meaning there is no statistically significant difference made by compost application on *P. ramorum* populations. The treatment rounds had a p-value of approximately 0.0004 (Table 1). These p-value results mean that compost application had no affect on *P. ramorum* reduction but that the length of time of the rounds did affect *P. ramorum*. I believe the difference in sites may have caused some difference. I assumed that my two drainage sites were the same, however I found that the sites were statistically different having a p value of 0.001 (Table 1., Table 2). I also found that time may possibly be an important factor in *P. ramorum* reduction. Compost left on for a month's time resulted in far greater reduction of the pathogen compared to soils samples that only had compost for one week (Table 4, Figure 3). However, while round three had abundantly less isolations of *P. ramorum* from soil baiting, this may not necessarily be from compost as the soil surrounding the control trees had less *P. ramorum* isolation in this round as well (Figure 2,Table 5). It may also be possible that it takes some time for microorganisms introduced to accumulate a large enough population to outcompete the

pathogen. Overall, there was reduction of *P. ramorum* population within the soil but whether compost application caused this still needs to be determined. The Taqman real time PCR was only conducted on the positives culture isolations and on soil samples that resulted in negative isolations from rounds one and two. The PCR detected no P. ramorum DNA indicating that these negatives were truly negative isolation occurrences. I used these results to assume that the negatives from round three were also truly negative.

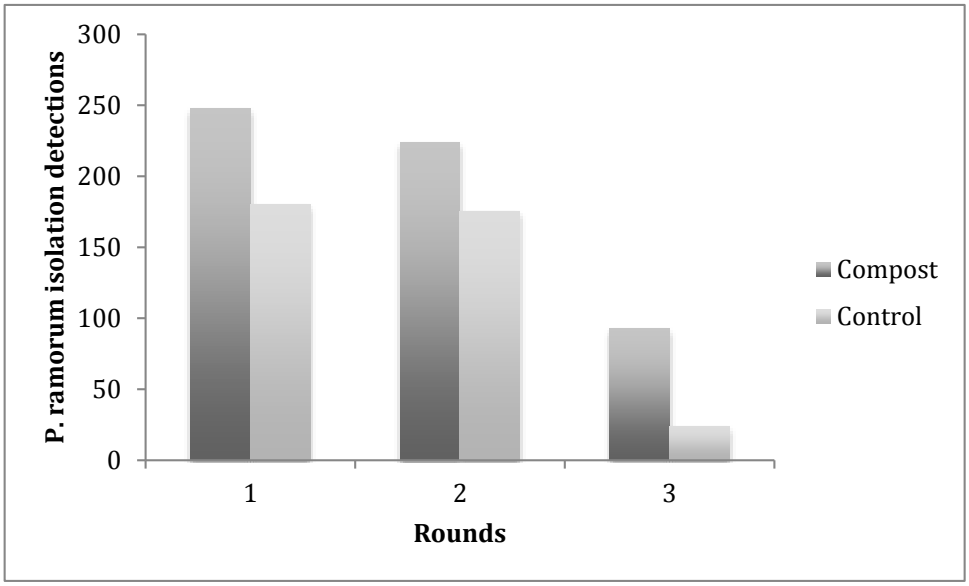


**Figure 2.** *P. ramorum* detection. The graph shows the amount of *P. ramorum* isolations made from leaf discs baited with soil that had received compost application and compares it with *P. ramorum* isolations made from non-compost control soils. Rounds one and 2 had similar amounts of *P. ramorum* isolation but round 3 had significantly lower occurrences of *P. ramorum* isolations. All rounds had less isolations made from the control soil.

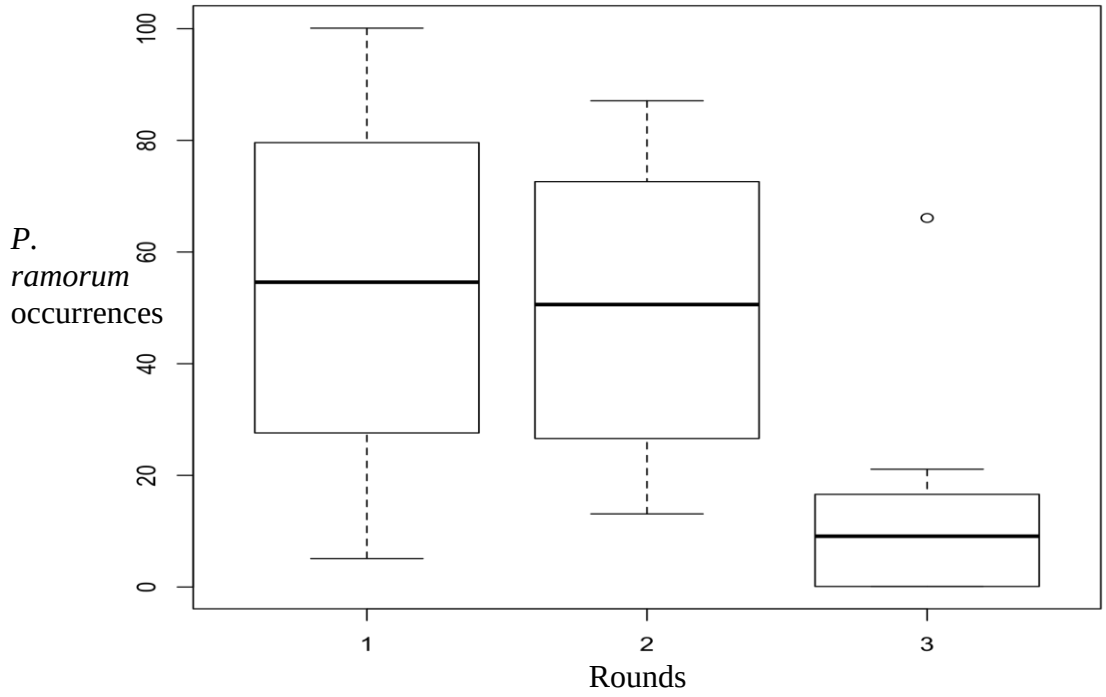


**Figure 3. Boxplot of total *P. ramorum* occurrences per round.** The box indicates the middle 50% (between the 1st and 3rd quartile) of the data, the top whisker indicates the upper 25% of the data, and the bottom whisker indicates the lowest 25% of the data. Rounds one and two were somewhat similar but round three showed a big difference.

**Table1. ANOVA.** The *P. ramorum* counts were analyzed in regards to site, treatment and round. The rounds and site showed statistically significant difference but not compost treatment.

| | **Df** | **Sum Sq** | **Mean Sq** | **F Value** | **Pr(>F)** |
|---|---|---|---|---|---|
| Site | 1 | 49.32 | 49.32 | 13.948 | 0.001404 |
| Treatment | 1 | 12.86 | 12.86 | 3.637 | 0.071728 |
| Rounds | 2 | 83.72 | 41.86 | 11.837 | 0.000459 |
| Residuals | 19 | 67.19 | 3.54 | | |

**Table 2. Tukey-Kramer comparison for sites.** The study sites were statistically different from each other.

| | **diff** | **lwr** | **upr** | **p-value** |
|---|---|---|---|---|
| Pla-Bay | -2.867096 | -4.473887 | -1.260305 | 0.0014041 |

**Table 3. Tukey-Kramer comparison for Treatment.** Compost application was not statistically different from no compost treatment indicating that compost may not have had an affect on *P. ramorum* populations.

| | **diff** | **lwr** | **upr** | **p-value** |
|---|---|---|---|---|
| Compost-treatment | -1.464141 | -3.070932 | 0.1426499 | 0.0717279 |

**Table 4. Tukey-Kramer comparison for rounds.** Rounds one and two were not statically different from each other but round three was different from both round one and round two.

| | **diff** | **lwr** | **upr** | **p-value** |
|---|---|---|---|---|
| Round2-round1 | -0.1282443 | 2.516833 | 2.260345 | 0.9898012 |
| Round3-round1 | -4.0244830 | -6.413072 | -1.635894 | 0.0011229 |
| Round3-round2 | -3.8962386 | -6.284828 | -1.507649 | 0.0015263 |

**Table 5. Counts of *P. ramorum* isolates from soil bait.** The table shows the amount of P. ramorum isolations from each tree per round. It also notes whether the tree received compost treatment.

| TreeID | Site | Round | Treatment | Counts |
|---|---|---|---|---|
| 1342 | Bay | 1 | Yes | 100 |
| 1342 | Bay | 2 | Yes | 69 |
| 1342 | Bay | 3 | Yes | 6 |
| 2342 | Bay | 1 | Yes | 72 |
| 2342 | Bay | 2 | Yes | 87 |
| 2342 | Bay | 3 | Yes | 66 |
| 1001 | Pla | 1 | Yes | 45 |
| 1001 | Pla | 2 | Yes | 15 |
| 1001 | Pla | 3 | Yes | 0 |
| 2262 | Pla | 1 | Yes | 31 |
| 2262 | Pla | 2 | Yes | 53 |
| 2262 | Pla | 3 | Yes | 21 |
| 2385 | Bay | 1 | No | 87 |
| 2385 | Bay | 2 | No | 48 |
| 2385 | Bay | 3 | No | 0 |
| 2386 | Bay | 1 | No | 64 |
| 2386 | Bay | 2 | No | 76 |
| 2386 | Bay | 3 | No | 12 |
| 2167 | Pla | 1 | No | 5 |
| 2167 | Pla | 2 | No | 38 |
| 2167 | Pla | 3 | No | 12 |
| 2169 | Pla | 1 | No | 24 |
| 2169 | Pla | 2 | No | 13 |
| 2169 | Pla | 3 | No | 0 |

## Microorganism Colony Count

I detected two different microorganism colonies within our soil samples. I was certain these two microorganisms were not *P. ramorum* because they did not have the characteristic growth morphology of the *P. ramorum* pathogen. The physical characteristics of these colonies (Table 6) and the length of time in which the soil samples were stored before doing the colony count indicated that these were different organisms, although their genus and species was not specifically identified. Additionally *P.ramorum* does not survive for over 6 months in extreme cold conditions and these colonies were found from soil samples stored for 8 months in a cold room.

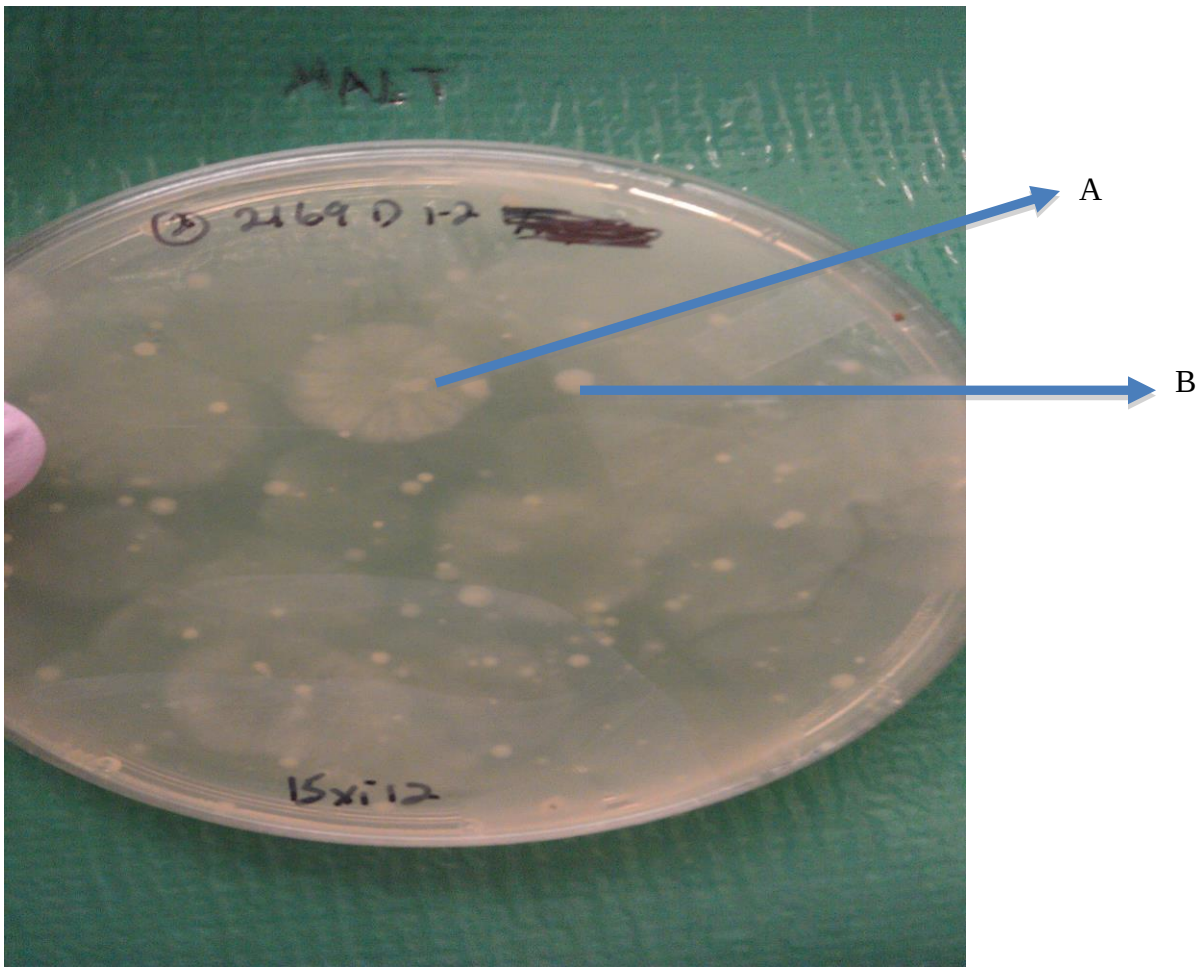


**Figure 5. Picture of microorganisms found.** A and B are assumed to be different microorganism added to the soil from compost. A has a cloudy spherical appearance while B is smaller and a solid sphere.

**Table 6. Physical Characteristics of microorganisms found.** The different physical characteristics of A and B indicate that they are different organisms from each other and are not the *P. ramorum* pathogen.

| Microorganism | Description |
|---|---|
| A | This organism has a clear cloudy spherical appearance and is large in shape |
| B | This organism is white, small and round |

## DISCUSSION

The goal of my study was to determine if using compost on *Phytophthora ramorum* infected soil could inhibit the growth and spread of the pathogen. I found that there was reduction in the amount of *P. ramorum* in soil but it is not clear if this was a direct result from compost treatment. There were also other microorganisms added to the soil by way of compost application that potentially outcompeted *the P ramorum*. After being on soil for a month, the compost worked in reducing populations of *P. ramorum* in the soil. These results agree with other findings from the use of compost on *P. ramorum* infected soil (Noble 2005, Linderman 2006, Swain *et al.* 2006). My study addresses the gap in knowledge about the use of compost in real forest systems infected with *P. ramorum* while highlighting the importance of time needed for compost to successfully introduce organisms that will inhibit *P. ramorum*.

### *P. ramorum* growth

Other studies found the amount of *P. ramorum* to be lower after compost application (Linderman 2006, Swain *et al.* 2006) however; I found that *P. ramorum* population was slightly reduced immediately after compost application (Figure 1). This detection percentage can be used as an assessment of the to *P. ramorum* population in soil. A lower percentage of isolations from leaf discs used for baiting would indicate that the population of *P. ramorum* had declined. In contrast to round one and two, I observed a much lower population presence from soil that had compost applied for a month. The detection rate for round three was around 15 percent indicating a decrease in *P. ramorum* population within the soil.

Other studies had experimental designs that recognized heat of compost piles as a factor of *P. ramorum* suppression (Hoitnik 1997, Swain *et al*. 2006). Hoitnik notes that there are three phases to compost that affect potential Biocontrol agents. Temperatures begin to rise during the first phase, during the second phase thermophilic microorganisms are abundant and curing occurs during phases three (the most important phase). During the third phase, temperature of the compost falls (below 40) and bio-agents re-colonize the compost. It is important that compost piles be turned so all parts are exposed to the temperatures (Hoitnik 1997). Temperature and intensive management of compost (i.e. scheduled turning/mixing) is therefore an important

factor in pathogen suppression (Yuen and Raabe 1984, Ryckeboer*et et al.* 2002, Swain *et al.* 2006).

In my study I applied compost on top of soil then left it for a week before collecting samples. The small amount of decline in *P. ramorum* population in round two of my study supports the aforementioned studies findings that soil should be mixed and fully incorporated into applied compost. The reduction in *P. ramorum* during round two and three might have been greater if I had mixed soil and compost sufficiently. Intensive management of the compost is therefore, just as important if not more so than the application of compost treatment. In addition compost type is important in the inhibition of *P. ramorum*. Compost moisture is an important factor in allowing beneficial microorganism to colonize soil. Hoitnik (1997) notes that compost needs to have a moisture level of at least 40% in order for re-colonization during phase three to be effective. I did not measure moisture level of my compost. It is possible the compost I used had too little moisture. I used vegetal compost but perhaps manure-based compost as used by Linderman (2006) or compost tea may have been more effective. I believe that it is the temperatures induced during composting process, the bio-control agents' introduced and frequent turning and monitoring that all contribute to suppression of the *P. ramorum* pathogen.

It is important to recognize the influence the sites may have had in *P. ramorum* reduction. It is possible that the compost did not introduce microorganism that outcompeted the pathogen, or that maybe these organisms were not solely responsible for the reduction in *P. ramorum*. Organic matter and pH of soil contributes to the ability of the *P. ramorum* pathogen to survive. Certain trees are associated with organic matter and pH levels of their surrounding soil (Fitchner *et al.* 2007). For example, Bay laurels have been found to have 13.7% organic matter and an average soil pH of 5.93 while Tanoaks had 9.5% organic matter and a 5.62 average pH (Fitchner *et al.* 2007). The first drainage site in my study was dominated by Bay laurels while the second drainage sites were mixed with coast live oaks and tanoaks. The effect of the trees to the soil of the sites my have worked together, against, or with the microorganism of the compost.

**Microorganism Colony Count**

I hypothesized that compost would introduce microorganisms that could compete with *P. ramorum* for resources or even be parasitic to the pathogen thus inhibiting *P. ramorum*

population (Hoitnik and Boehm 1999). It has been noted that microorganism species such as *Pseudomonas spp., Bacillus spp.* can act as effective bio-control agents against *P. ramorum* (Hoitnik *et al.* 1997, Elliot 2009). I was limited in finding the exact species of microorganisms observed from my colony count. The high population of these two organisms and the reduction of *P. ramorum* observed in rounds two and three support my hypothesis that compost introduced, organisms out competed the *P. ramorum* for energy resources and space ultimately resulting in a decline in the pathogen. However, these microorganisms may need time to accumulate a high enough population that will effectively outcompete the *P. ramorum* population as round three (one month of compost on soil) had a statistically significant reduction of the pathogen.

**Limitations and Future Direction**

My study assumed that the two drainages in my sight were relatively the same and would produce the same *P. ramorum* detection rates. However, I found that the two sites were statistically different. This might have impacted the accuracy of our results. Lower *P. ramorum* population may not have been the application of compost alone but compost in combination with specific traits of a site.

The SOD epidemic is still prevalent and negatively affecting the timber industry (Kliejunas 2010) and continual application of chemical solutions can cause *P. ramorum* to become resistant (Turner *et al.* 2008). As such, compost treatment should continue to be studied. This study should be repeated but include proper mixing of compost and soil. There should be a plot of tress with non-mixed soil/compost, a plot that is only mixed once and a plot that is mixed frequently. One quarter of the trees should be applied with vegetative compost another with compost infused with *Pseudomonas spp. and Bacillus spp,* another quarter with manure-based compost and the final quarter with compost tea. This will inform us if compost in a real forest system works and of any necessary amendments and/or management is needed for successful *P. ramorum* suppression. In addition careful attention should be paid to the characteristic of the sites to determine if soil type, surrounding tree populations and/or microclimate play a part in *P. ramorum* suppression. These future studies will still be worthwhile. It will help determine the best type of compost to use in infected soils of particular forest ecosystems.

**Broader Implications and Conclusion**

My study addresses the gap in knowledge about the effectiveness non-chemical solutions to SOD. We found that simple vegetative compost applied on top of infected soil, did not significantly inhibit *P. ramorum* population after one week. There was significant inhibition of *P. ramorum* after one month of compost treatment. I believe it is not enough for just microorganisms to be introduced to infected soil. Compost may still be a suitable solution (Noble 2005, Linderman 2006, Swain *et al.* 2006) but specific microorganisms that can hinder *P. ramorum* should be added. Additionally the compost should be mixed frequently as the heat cycles of the composting process are important to hindering pathogen survival. Further studies using compost infused with these specific species and using rigorous management should be done While compost needs management, it still offers a non-chemically harmful solution that is easy to apply and relatively easy to manage.

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