Finding Potential Linkages Among Age, Severity, and Water Stress In Pitch Canker, ***Fusarium circinatum***, Infested Monterey Pines, ***Pinus radiata***, In Asilomar State Park, Monterey, California

A Capstone Project

Presented to the Faculty of Earth Systems Science and Policy

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Bachelor of Science

by

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Dear ESSP faculty,

The Monterey Pine in Monterey, California has lost approximately 8919 acres (Jones & Stokes, 1996). A fungus discovered in 1986 known as, ***Fusaruim circinatum***, has been a contributing factor to this problem. This fungus is known to cause pitch canker on native pines, which significantly decreases the tree's overall health. Pitch canker causes the tips of the trees to die back, which can lead to death, if multiple introductions of the virus are made. Spread mainly in California by insects (Storer et al., 1998), pitch canker can spread rapidly through an area. Asilomar State Park is an area that contains the endemic Monterey Pine, ***Pinus radiata***, which is severely affected by this disease.

High pitch canker incidence could entail habitat loss, which would also affect nearby species. Some species of concern that are associated with the Monterey Pine include the black legless lizard, Monterey ornate shrew, pacific grove clover, and Hickman's onion (Jones & Stokes, 1996). However, species wouldn't be the only one affected as, if trees begin to die, it could lead to Asilomar State Park and other Monterey Pine inhabited areas losing revenue. Assuming that the forest is a reason why people come to the park, high tree mortality could detour people who wish to experience "nature".

My main focus of this study is to determine if there's a relationship between age and the severity levels on potential pitch canker infested Monterey Pines corresponding to water stress, which will give insight as to if and how pitch canker affects the xylem of a pine. The study also focuses on how age is affected by pitch canker, as associated by severity level, which will help us understand how the disease impacts the health of the different age classes. Pitch canker is a disease, I feel, can drastically change ecosystems. Areas containing pines could see high mortality rates, which other species are dependent on (Jones & Stokes, 1996). It's my intention to broaden our knowledge of the disease and help researchers identify the potential impacts pitch canker may have on Monterey Pines, in order to slow down, immobilize, and stop the spread of the pathogenic virus.

Lorrie Madison, of Asilomar State Park, has helped me tremendously with this project. She got me permission to collect samples and work within the park, given me her expertise on the feasibility and limitations of my project, and will help me tag some trees for sampling. Such as, I originally intended to sample water stress levels for all age groups (young, mature, and old), however found out that the canopy of the old trees is way above my reach. The other assumption I was corrected on was that once a tree was infected with pitch canker, it spreads throughout the tree, however after extensive literature review I found that the disease doesn't spread far from the point of infection (Gordon et al., 2001). The first area I feel my capstone should be assessed on is Application of Knowledge in the Physical and/or Life Sciences (MLO#3). I'll be using scholarly information to synthesize current facts about known pitch canker effects, transmission, and management issues. The next area I would like to be assessed in is Acquisition, Display, and Analysis of Quantitative Data (MLO#5). My capstone satisfies this MLO, as I must acquire data (branch samples), process it (two-way ANOVA and Chi-Square Contingency Test), and comprehend the results to my research questions.

This report will be submitted to Asilomar State Park, but intended for anyone studying pitch canker.

Sincerely,

Dave Contreras

Abstract

Pitch Canker, ***Fusarium circinatum***, is a disease that affects pines. Particularly of importance in California is the endemic Monterey Pine, ***Pinus radiata***. One aspect of the study was to determine if there is a relationship between age and severity levels on pitch canker infested Monterey Pines corresponding to water stress. The other focus of the study was to see how age is affected by pitch canker, as associated by severity level. Monterey Pine survey data from 2000-2001 was collected by Asilomar State Park Ecologists and used to determine pitch canker presence and water stress samples were obtained. No statistically significant results were formulated from the water stress samples, however age was found to be dependent on severity level (or vice versa). The implications of these results pertaining to water stress, age susceptibility, and future management of pitch canker are discussed within.

Introduction

Pitch canker (***Fusarium circinatum***) is a pathogenic fungus known to cause resins to flow from branches, cones, and the main stem in the infected area of pines (McCain et al., 1987). The Monterey Pine (***Pinus radiata***) is among the greatest affected by pitch canker (Storer & Dallara, 1992), thus making it a species of great concern. Common symptoms of a Monterey Pine infected with pitch canker include tip dieback of the branch, resin formation, and flagging/top kill (Storer et al., 2000). Currently, the disease is found in Florida, Japan, Mexico, Haiti, and South Africa (Storer et al, 1998, references within).

Overseas, “the Monterey Pine is the most widely planted pine in the world and is of great economic importance to large and growing lumber and pulp industries in other counties such as New Zealand and Chile” (CNPS, 1999). If the disease is introduced in New Zealand or Chile, it could result in potential economic hardship, as respectively the Monterey Pine comprise 60% and 93% of the wood production (Newman, 1999). Although pitch canker has yet to be found within these areas, it does not mean the virus cannot be introduced, such as the case in California.

The first account of pitch canker in California was discovered in 1986 (McCain et al., 1987) and currently extends from San Diego to Mendocino County (Gordon et al., 1996). Pitch canker can be carried by wind, but insects also vector the pathogenic virus.

One of California's biggest problems with managing pitch canker seems to be that insects are the primary host for the virus (Storer et al., 1998). Mostly comprised of beetles (Coleoptera), the following have been found to vector the virus: bark beetle, deathwatch beetle (Hoover et al., 1995), cone beetle, and twig beetle (Hoover et al., 1996). Management through chemical, biological, or cultural control to reduce beetle populations is unrealistic over large areas (Storer et al. 1997). The reason why it is impractical is because the beetles are widespread, able to reproduce and reach maturity after several weeks of being born (Koehler et al., 1978).

The effect pitch canker has on its host greatly depends on repeated infections of the virus, as the disease does not advance far past point of infection (Gordon et al., 2001). Therefore, repeated exposure to pitch canker results in a greater health decrease for the Monterey Pine, and could result in mortality. However, the Monterey Pine is not the only species that is affected by the pitch canker pathogen.

As stated above, pitch canker is a "pine" disease, and some fourteen species of pine were susceptible to the disease under greenhouse conditions

(Gordon et al., 2001), as well as from Douglas-fir (***Pseudotsuga menziesii***) (Storer et al., 1997). Although greenhouse efforts were made to artificially inoculate the trees, there is no reason to believe it can not happen, as there seems to be high incidence of the disease among the knobcone (***P. attenuata***) and bishop pine (***P. muricata***) (Gordon et al, 2001). Thus, allowing a “wider” spread of the disease, making it a potential threat to established forest ecosystems. However, we do not know the extent of how pitch canker may effect other trees.

However, not all Monterey Pine seem to be affected by pitch canker the same way. Gordon et al. (1998), artificially inoculated Monterey Pines (n=30) with the disease among five different occasions and found that differing effects among the trees. Thereby, suggesting that some pines may be less susceptible to the disease than others. Further implications of pine resistance was done once again by Storer et al. (2001, reference within), where trees of suffering from a severe case of pitch canker in 1992 were found to be free of disease in 1999, suggesting that repeated exposure to the virus may build a resistance against it. Pitch canker management within California is important as tree mortality could lead to economic problems.

Within California, pitch canker may affect economics in a different manner than the above-mentioned nations, which find economical use of the tree by lumber production. I believe that tree mortality among the Monterey Pine could result in decreases in tourism. Asilomar State Park , in Monterey, California (Fig.1) was confirmed to have pitch canker in 1993 (Storer et al., 1994), which has spread throughout the area since. Asilomar State Park receives over 200,000 registered guests per year, as they host weddings, parties, and lodges/hotel rooms. I assume that the forest is a component contributing to why people come to Asilomar State Park, and for that matter any park. So, if pitch canker causes significant tree mortality, this could lead to significant drops in tourism.

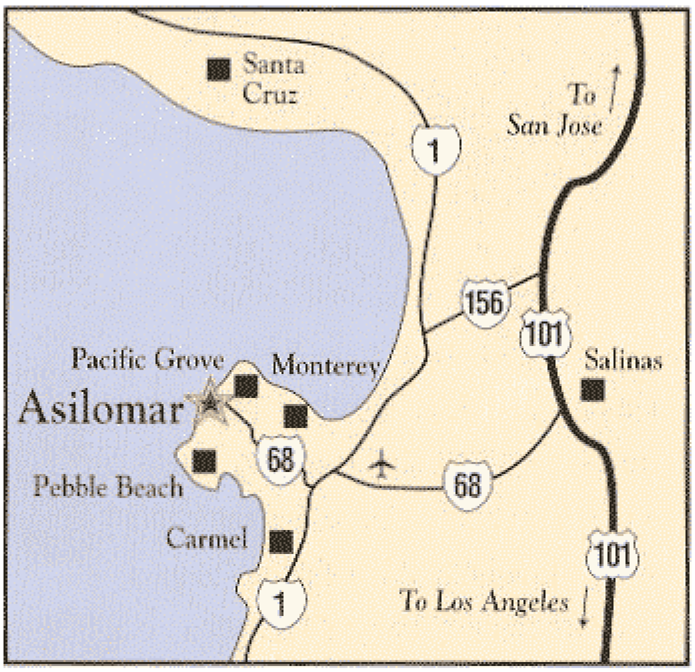

Fig.1. Location of Asilomar State Park.

Water stress occurs when transpiration from the leaf exceeds the rate it can be replaced (Taiz and Zeiger, 1998). During water stress, stomata close, which reduce the plants ability to photosynthesize. Mechanisms that may have an affect on water stress are atmospheric moisture, slope, aspect, soil type, and soil depth (Aber and Mellilo, 1991). However, they will not be introduced factors within this experiment due to time. Furthermore, studies have shown that slope and aspect respectively show minimal and no relationship to the pitch canker disease (Newman, 1999). Studies have shown that fungi block the water vascular system (Koehler et al., 1978; Swiecki and Bernhardt, 1990), which would increase water stress.

One aspect this study focuses on is to see if there is a relationship between age (young and mature) and the severity levels on pitch canker infested Monterey Pines corresponding to water stress. This will allow us to see if, and how pitch canker affects the xylem of the Monterey Pine, as I believe we will see increases in water stress as the severity increases. Furthermore, I also believe that the younger tress will experience a higher amount of water stress. If pitch canker is found to cause significant increases in water stress as the severity level increases, then new methodologies could be looked into, as how to increase xylem water flow.

The other component of this study is to determine how age is affected by pitch canker, as associated by severity level. The reason why this is being studied is to determine how pitch canker impacts the young, mature, and old trees, which could lead to more specific management policies according to age group, as I believe that the young will be most susceptible to the disease.

Methods

<u>Pertinent Past Data</u>

Past pitch canker Information was collected in 2000-2001 into two data sets that were used for this study. The first data set contains information for the trees' health in Asilomar State Park, where over 3,000 trees were surveyed. Data sets were broken up by severity level (initial, moderate, and severe), where each data set contained information on the tree's age, ID number, and observational data. Severity classification was based on the number of instances of tip dieback, branch top-kill (flagging), and bole cankers occurring on each tree. The second data set contained GPS[1] (global positioning system) points of the Monterey Pines in Asilomar State Park. These two data sets were joined, using the "Tree ID" as the common field. This produced six shapefiles in ArcView, a GIS[2] program, containing different severity levels.

A new field was added to each severity data set, where the observational data were used to determine if pitch canker was thought to be present. Four different variables were made for the new field, which were Y = Yes (pitch canker is present), N = No (pitch canker is not present), M = Maybe (perhaps pitch canker is present), and Un = Unknown (not enough data to determine if pitch canker is present). The criteria used to determine if pitch canker was present in the initial severity dataset were if the tree showed three characteristics (i.e. flagging, tip dieback, and resins), for the presence of pitch canker. The moderate and severe levels were done the same way, except the presence of pitch canker was deduced by two characteristics showing symptoms of pitch canker. Once the new field was completed, a new join was made with the previous data through ArcView, by once again using the "Tree ID" as the common field.

[1] GPS is a feature (point, line, or area) processed through a GPS receiver, which computes location of the feature through satellite positioning.
[2] GIS is a program to process and display features (also called geospatial data).

Pressure Bomb[3]

Five trees were randomly selected for each severity class (initial, moderate, severe) and two age groups (young and mature) thought to contain pitch canker. However, four trees were not found or had high canopy, in which case they were replaced. A GIS map was used to locate the trees in the field, where they were marked prior to sampling (Fig. 2). Sampling occurred pre-dawn (3:00a.m. – 7:00a.m.), as this is the time a tree experiences its lowest water stress. Three leaf samples were taken from the lower canopy of each tree and averaged together. Each leaf was held in place by a seal on the pressure bomb, where the cut part of the leaf extended out of the pressure chamber, while the other portion of the leaf was put into the pressure chamber (Fig. 3). Pressure was then exerted on the leaf via pressurized NO_2 gas, until when the cut portion of the leaf began to fizzle. This was done for the young and mature trees for the initial (n = 5), moderate (n = 5), and severe (n = 5) severity stages. No old tress or mid to upper canopy were sampled, as the canopy extended out of reach. Healthy trees were not sampled as requested by Asilomar State Park, as they represent less than 1% of the total population. Blades were wiped with “all purpose cleaner” containing 1 % sodium hypochlorite between each leaf cut to ensure no further spreading of the disease.

[3] A pressure bomb is a device to measure water stress, where it is assumed that the amount of pressure exerted to return water to the cut portion of the leaf equals water column tension before the leaf was cut (Aber and Mellilo, 1991, reference within).

<u>Legend Of Samples</u>

Blue = Initial
Yellow = Moderate
Red = Severe

Fig. 2. Asilomar State Park water stress samples.

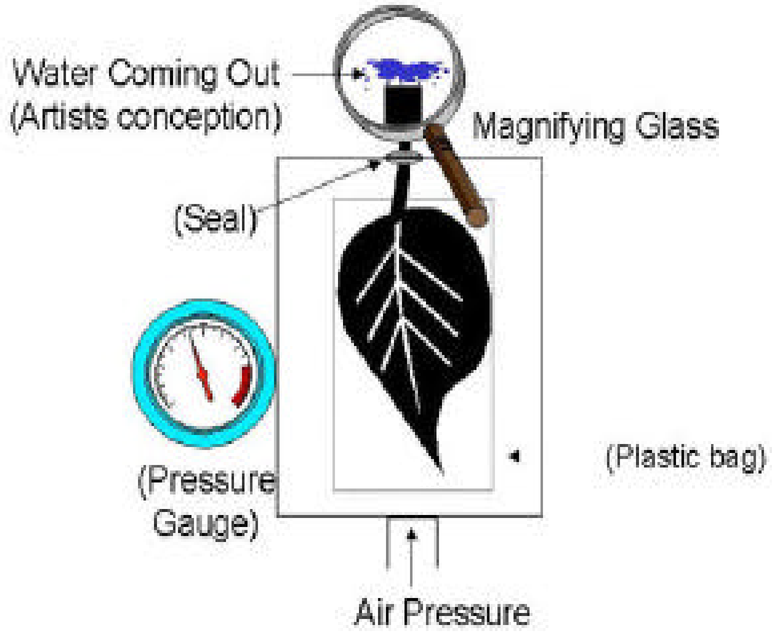

Figure 3. Pressure bomb illustration.
Taken from: http://fruitsandnuts.ucdavis.edu/bomb-fig.1.html

Analysis

To determine if there's a relationship between age (young and mature) and severity level (initial, moderate, severe) on pitch canker infested Monterey Pines corresponding to water stress, a two-way ANOVA was used. The two-way ANOVA allows us to test for statistical differences between age groups and severity levels (Ott, 1998).

Water stress data were entered by age and severity and analyzed within Excel (Sincich, 2002).

A Chi-Square Contingency test was chosen to test if pitch canker severity depends on tree age. Chi-Square is calculated by comparing the expected value for each field to the observed value, where differences between the two indicate one variable is dependent on another (Watt, 1998). The following severity level datasets were used for analysis: initial, moderate, and severe. The number of pitch canker incidences and non-incidences for each severity dataset was counted for each age group (young, mature, and old). A Chi-Square test was run separately for all three severity levels within Excel. Percentages of pitch canker incidences were found by dividing each age's pitch canker occurrence in each severity level by the total number of trees within that age group to infer which age class.

Results

From the Chi-Square tests we find that initial severity level trees seem to be equally effected among all age classes (df = 2, p = .94). However, the proportion of pitch canker occurrences for the moderate and severe level, varies depending on age (df = 2, p<.006). From Figure 4, moderately affected young and old trees have a higher pitch canker incidence, than the mature trees. Severe young trees also have a higher pitch canker incidence over mature and old trees.

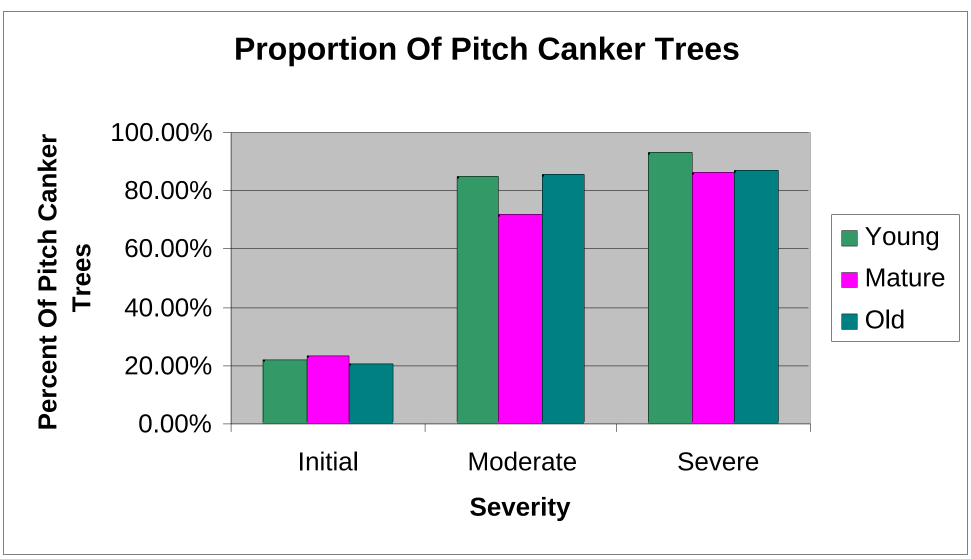

Fig 4. Pitch Canker Incidence.

Both age and severity statistically found no relationship with water stress (df = 1, 2, $F < .82$, $p > .22$). Water stress averages were relatively equal among age and severity levels, however contained a huge amount of variation (Fig. 5). This variation within each treatment gave way to a high sum of squares value, which made it difficult of obtain a high F value.

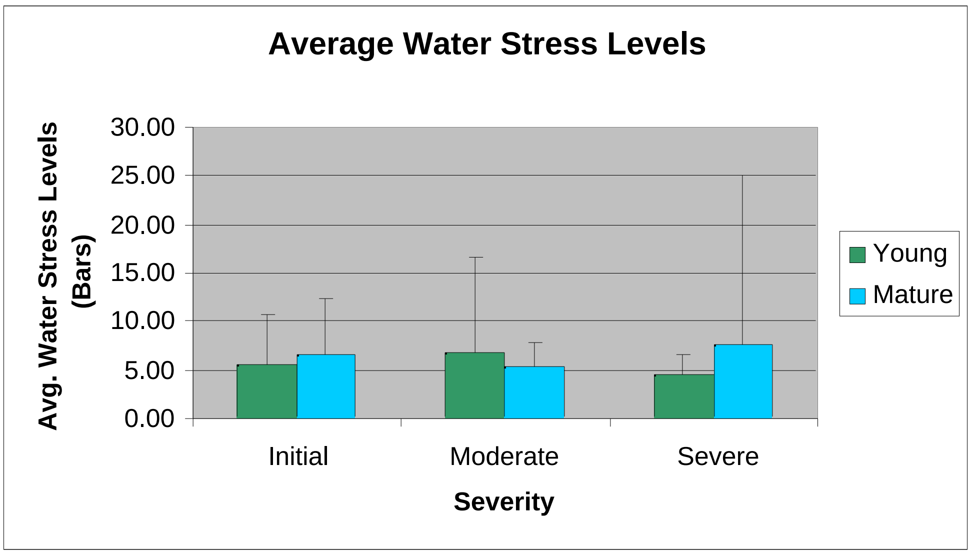

Fig 5. Average water stress levels.

Discussion

Water stress does not seem to be a factor on pitch canker infested trees, as its distribution does not show any patterns among age or severity levels. Therefore suggesting, the xylem does not seem to be affected by the pitch canker fungus. This is contrary to many studies that say that the fungi block the water vascular system (Koehler et al., 1978; Swiecki and Bernhardt, 1990).

However, effects of water stress correlation should not be entirely discredited when dealing with pitch canker severity levels. All samples were taken from the lower canopy of the tree, where perhaps the upper canopy would show greater water stress effects. Furthermore, the branches chosen had to contain green needles, as dead branches would not give a water stress level. Therefore, branches that may have been killed off by the disease could not be sampled.

Age was found to be dependent on severity (or vice versa). From Fig. 5 we can infer that both moderate and severe young and moderate old trees are being most affected by the disease. This can perhaps be attributed to mature trees developing resistance to the disease through repeated exposure of the virus (Storer et al., 1999). However, it can also be attributed to water stress. As the younger trees experience pitch canker from beetle attack, pheromones are emitted from the tree and beetle attract more beetles (Koehler et al., 1978). This leads to the younger tree experiencing higher potential pitch canker exposure. Since, the tree is so young, they may experience higher water stress as they may still be developing effective defense mechanisms, however defense mechanism development among the Monterey Pine have yet to be shown.

The most successful way of battling the pitch canker pathogen seems to be through the planting of pitch canker resistant pines. Although it's not truly known if this trait can be transferred to a forest setting (Gordon et al. 1998). However, if the trait is transferable and can be genetically passed off to offspring, then measures should be taken to introduce these species into pitch canker infested forests.

Other ways we can reduce pitch canker spread are through the inspection of lumber when importing or exporting trees from high-risk areas. Careful attention should be placed to areas where the Monterey Pine hasn't been affected by pitch canker and is important economically.

To take my study a step further one could factor in soil type, a control, and obtain water stress measurements on older trees. Measurements of water stress in the mid to upper canopy regions would provide a better representation as to how pitch canker is affecting the whole tree. Further studies on water stress and pitch canker infested pines may provide information the perpetuation of disease development.

In summary, particular care should be under taken for younger trees, as they seem to be most susceptible to the disease. Therefore, high mortality rates among young trees may impact ecological components, as certain species, such as the "eleven special-status species and fourteen special-status plant species are associated with Monterey pine forest at Monterey" (Jones & Stokes, 1996). Greater protection of Asilomar's younger trees is detrimental for future establishments of Monterey Pines.

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