Abstract:

Water is essential for proper plant growth. Water stressed plants are found to be plagued by more diseases than those that are not. Diseases that tend to plague water stressed plants are referred to as opportunistic diseases. Botryosphaeria is a fungal disease known to cause disease in nut crops such as almonds. Typically, disease by Botryosphaeria fungi become very severe on trees suffering from water stress. Based on the experiment conducted it was determined that the degree to which the disease affects the almond tree is directly related to the level of water stress the tree is experiencing. We could expect to see larger cankers on trees receiving less water. Statistical analysis done on the canker lengths produced on 1-year-old-almond trees, inoculated with *Neofusicoccum parvum* and *Neofusicoccum nonquaesitum,* resulted in a p value of 0.14. The statistics point towards a negative correlation between the canker and the amount of water received by the tree.

Introduction:

California is considered a desert state that depends on water transportation systems for its water supply. (3) According to U.S drought monitoring systems, more than 80% of California is experiencing some level of drought (4). California has experienced droughts in the past. During the period of 1987 to 1991 the state also experienced a five-year drought that brought changes to the water policies in California (3). The severely dry conditions California is experiencing currently is due to increasing temperatures in addition to the lack of rain fall (6). Drought is a major concern for California because it is one of the most agriculturally important state in the country (3). The largest producing counties within the state are mostly found in arid zones (3). Agricultural lands require large amounts of water just to ensure crops grow and provide the largest yields and the best quality of fruits and vegetables. The scarcity of water in the state has had negative impacts on the agricultural industry (6).

There is a relationship between plants and their environment. If a plant is found in an unfavorable environment it can lead the plant to become stressed (1). Drought and freezing are the two main stressors effecting woody plants (5). These two scenarios create openings in crops to be infected by opportunistic diseases (1). This is also referred to as predisposition (1). Water stressed plants are not as capable of fighting diseases as plants that obtain their required amount of water (1). There is a large number of opportunistic diseases one of which is known as Botryosphaeria canker (5).

Typically, Botryosphaeria does not cause much damage in healthy plants (5). When plants have been stressed for a period of time they are unable to fight the pathogen and it can lead to serious damage to its host (1). Botryosphaeria is a fungal pathogen that affects a large number of woody plants such as almonds, apple, and peaches (5). This fungal pathogen affects its host by targeting the fruit and costing growers an enormous amount of yield loss (5). Botryosphaeria can also cause small lesions on leaves and fruit that expand. (5)

This experiment will focus on the effects of water stress on one-year-old almond trees infected with two strains of *Neofusicoccum* known to cause Botryosphaeria canker and blight. With the drought, we have been experiencing, there has been more restrictions on water use in California. (3) The increase in prices for water has created a demand for finding more water efficient systems that will decrease the amount of water that is put out in a field. The almond industry in California is quite large and is continuing to expand (5). For this reason it is important to study the relationship between Botryosphaeria and water stress to aid growers in finding the best irrigating methods that would result in optimum yield while at the same time avoiding opportunistic diseases.

Materials and Methods:

Isolations of the fungi that would be utilized were first created by transferring a small amount of the fungi into three small petri dishes containing acidified with lactic acid PDA media. Two different fungi species were used that are known to cause Botryosphaeria. *Neofusicoccum parvum* and *Neofusicoccum nonquaesitum* were the two species that were utilized. After three days of incubation the fungi had grown enough to be transferred to larger LA media plates. Four days after the transfer the fungi had expanded and the plates were ready to be used for the tree inoculations. To transfer fungi to the trees 7mm plugs were created on each plate. A total of 12 plates were used five containing *N. parvum,* five containing *N. nonquaesitum,* and the last two contained un-inoculated LA media.

Three treatments were created one being a control and the other two containing the two different species of fungi. A total of 45 trees were used. The trees were separated into three rows containing 15 trees each. Within each row the trees were separated into groups of five, each containing a different treatment. The trees were labeled with flags in order to differentiate between treatments. The trees inoculated with the LA media, the control, were flagged with white flags. The trees inoculated with *N. parvum* were flagged with red flags. Blue flags were used on the trees inoculated with *N. nonquaesitum.*

To determine if water stress has an influence on the susceptibility of the trees to the pathogen each row received a different amount of water. The middle row received 100% of water needed by an almond tree, west row received 50%, and the east row received 25%. To make sure each row was receiving the proper amount of water we used a bottle and funnel at the end of each row to catch the water being put out by that row to each tree. An extra emitter was placed at the end of each row to be able to measure its output. The west row had a 1-gallon emitter, east row had a ½-gallon emitter, and the middle row had a 2-gallon emitter. Measurements were taken of each row after 24 hours had passed. The water output was adjusted based on those measurements.

Results:

A total of three measurements were taken to calibrate the emitters. The first calculation was to find out how much water each emitter was putting out before any adjustments were made. The containers to catch the water were set up on July 25 and results were recorded on July 26. The results obtained were 1900mL for the east row, 2680mL for the middle row, and 2200mL for the west row. Next step was to adjust how much water was being put out by each emitter based on the goal to make one row put out 100% ET (high), another 50%ET (medium), and the final one 25% ET (low). The final two measurements were taken to ensure that the emitters were putting out the correct amount of water. Two more recordings were done one on July 27 and another on July 29. The measurements obtained on July 27 were 1100 mL for the east emitter, 5200 mL for the middle emitter, and 2650 mL for the west emitter. The container for the middle emitter was not able to hold all the water being put out by that row. For the last recording made the container was placed within a bucket to collect all the overflowing water. The final recording was on July 29 the east emitter put out 1040 mL of water, the middle emitter was at 9200mL, and the west emitter was putting out 2620 mL. With these final measurements, could confirm that the emitters were set at the desired flow rates. The chart below shows a comparison of the level of water each row was receiving converted to Liters. (Figure 1)

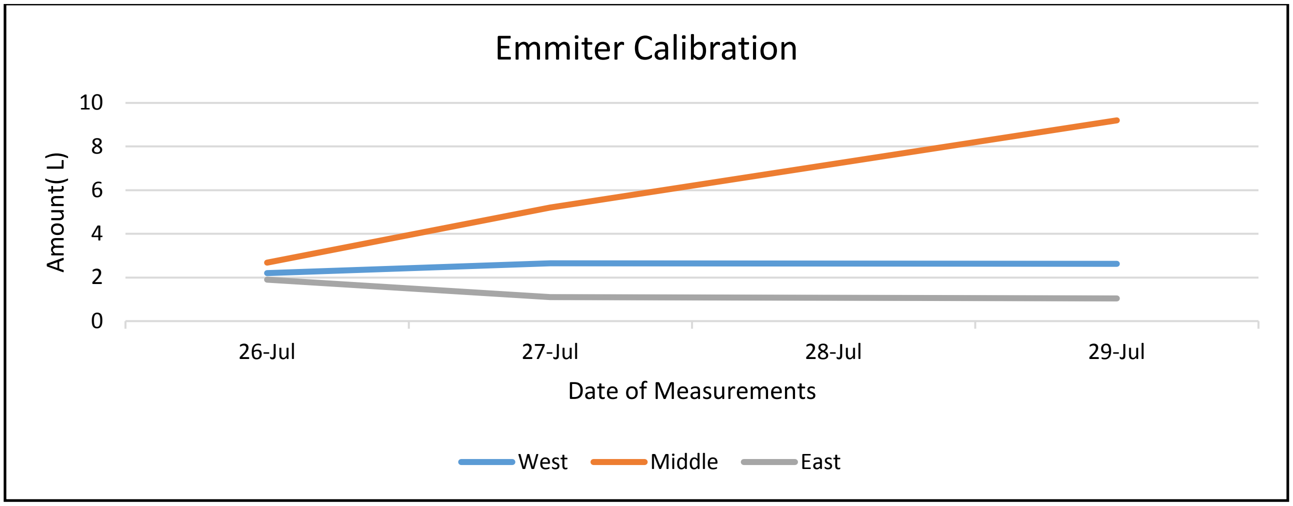


Figure 1: A comparison of the level of water each row was receiving in mL.

The inoculation of the potted almond trees took place on July 26. By August 15th (3 weeks after incubation), trees inoculated with *N. parvum* and *N. nonquaesitum* developed gumming at the site of inoculation. This meant that the fungus was spreading within the trees and the gumming was a sign of a defensive response by the tree. Below is a picture taken on August 2nd of both the *N. parvum* and *N. nonquaesitum* fungi treatments and the gumming that was produced.

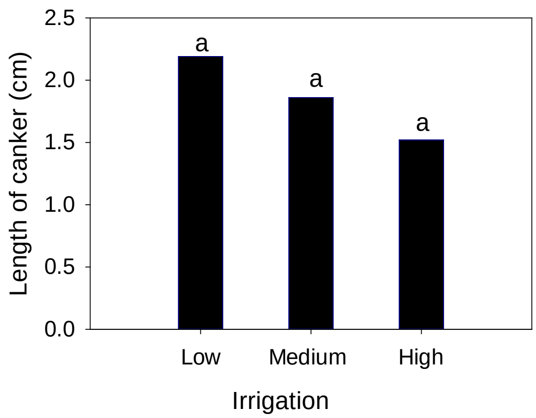
 

Figure 2a. Left photo shows gumming produced by almond tree inoculated with *Neofusicoccum parvum.*
Figure 2b. Right photo shows gumming produced by almond tree inoculated with *Neofusicoccum nonquaesitum.*

On August 19, the trees began showing a single canker on the site of inoculation. None of the cankers had extended past the parafilm at that point. It took the fungi about another month before the cankers had expanded enough to take proper measurements. (Figure 2c) The cankers were closely observed each week to determine if their growth was large enough to measure. The final measurements were taken in October. The cankers on the control groups found in the medium and low irrigation rows had gumming at the site of inoculation. While the cankers on the control group in the high irrigation row had no gumming, and the inoculation site was completely healed. Measurements were only obtained from the trees inoculated with *N. parvum* and *N. nonquaesitum* in each row. The control group developed no canker therefore no measurements were obtained from these groups of trees in any of the rows.

Figure 2c. Photo above shows a canker that developed in the west row on a tree inoculated with *N. nonquaesitum.* The canker began very small and was limited to the area around the site of inoculation but it quickly spread until it could be seen above and below the parafilm.

Figure 3, 4, and 5 show us how the combined canker lengths of all the trees treated with *N. parvum* and *N. nonquaesitum.* This included 30 trees in total. Five trees were inoculated with *N. parvum* and another five were inoculated with *N. nonquaesitum* per row so the combined total for the three rows was 30 trees. Figure 5 shows us an inverse regression with negative correlation between the irrigation and length of cankers. Figure 4 contains a linear regression. The R^2 value for Figure 4 is 0.33 and for Figure 5 it is 0.95. The higher R^2 value indicates that the inverse regression is a better match for the values present. In Figure 3, we can directly observe what each canker length average is for the given irrigation amount.



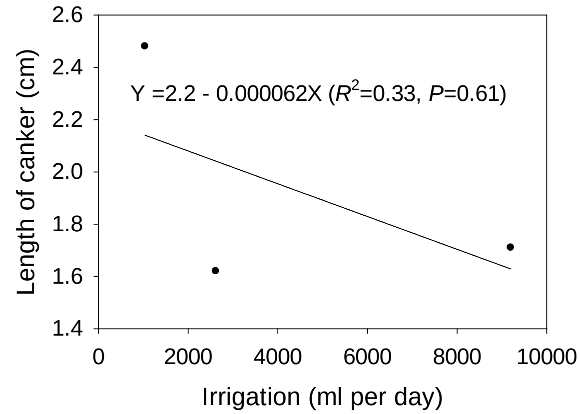
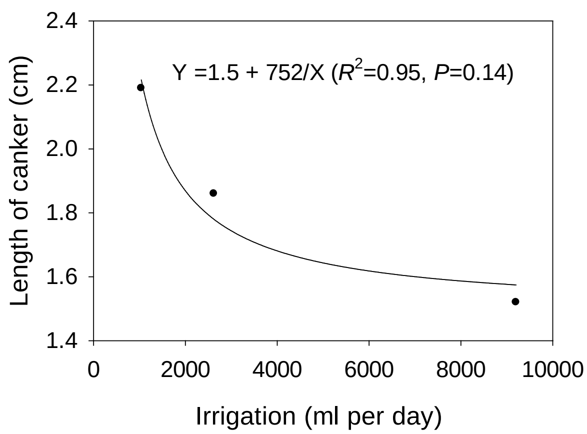


Figure 3. On the left, canker length by both fungi combined, after subtracting the diameter of original 7-mm plug.
Figure 4. On the right, canker length by both fungi combined, after subtracting diameter of original 7-mm plug (Linear regression).



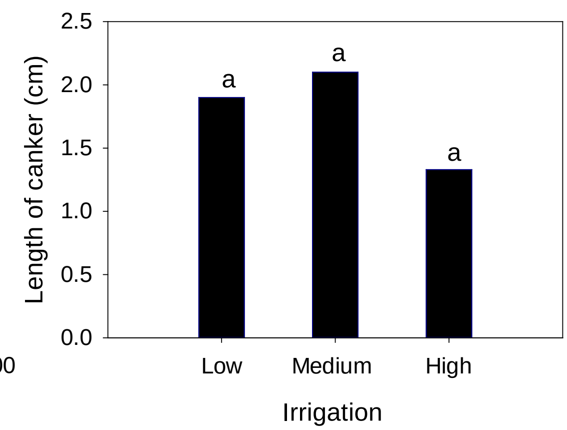
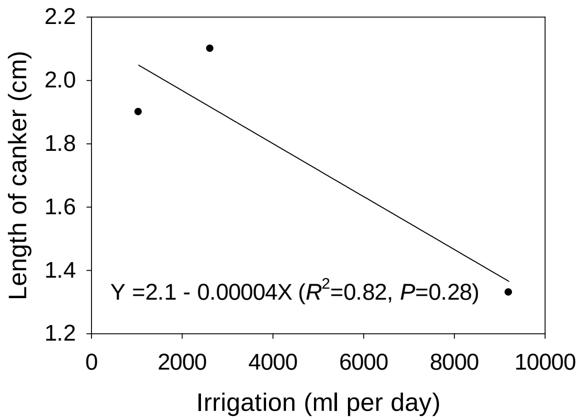


Figure 5. On the left, length of canker after subtracting length of original 7-mm plug (Inverse first order regression).
Figure 6. On the right, *Neofusicoccum nonquaesitum*. Subtracted length of original 7-mm plug.

Figures 6, 7, and 8 show us results for trees inoculated with *N. nonquaesitum.* This data involved less trees. Charts only used half as many measurements as the first three figures. Each of the rows contained five trees inoculated with *N. nonquaesitum* this yielded five measurements per row giving us 15 data points. Figure 6 shows the average length for each irrigation amount. When compared, figures 7 and 8, a linear regression fits the data points better than an inverse regression.



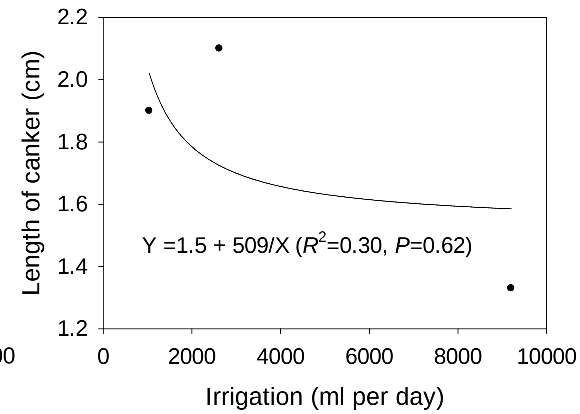
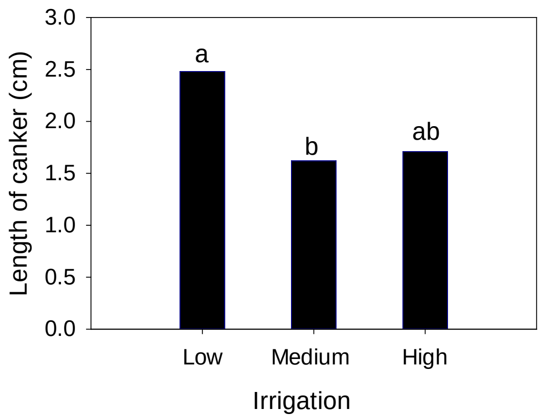


Figure 7. On the left, *Neofusicoccum nonquaesitum*. Subtracted length of original 7 mm plug (Linear regression).
Figure 8. On the right, *Neofusicoccum nonquaesitum.* Subtracted length of original 7 mm plug (Inverse first order regression).

Figures 9, 10, and 11 include results for trees inoculated with *N. parvum.* These three charts use up the other half of the total trees measured. A total of 15 trees were inoculated with *N. parvum.* In figure 9 we can see how the averages for each irrigation compared to one another. Some of the averages seemed to be significantly different from one another. In the linear regression found in figure 10 the R^2 value is 0.33. In the inverse regression in figure 11 the R^2 value is 38. Since the R^2 value for the inverse regression is larger than it fits better with our data points.



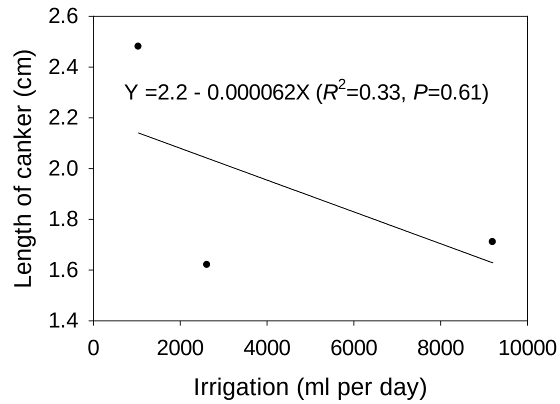
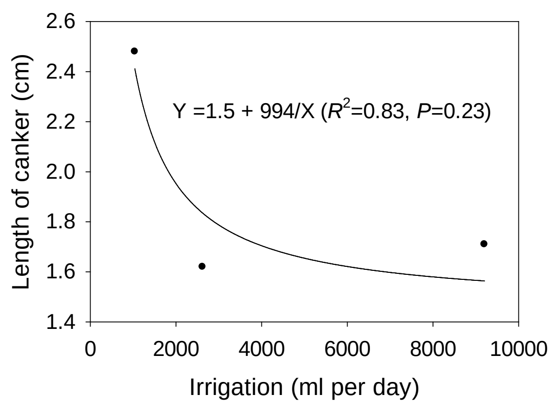


Figure 9. Top left, length of cankers by *Neofusicoccum parvum* after subtracting the diameter of original the 7-mm plug.
Figure 10. Top right, length of cankers by *Neofusicoccum parvum* after subtracting the diameter of original 7-mm plug (Linear regression).
Figure 11. Bottom, length of cankers by *Neofusicoccum parvum* after subtracting the diameter of the original 7-mm plug (Inverse first order regression).



Discussion:

While observing the trees, it was apparent that the trees found on the low and medium irrigation rows were showing symptoms of water stress. The trees on the low irrigation row quickly began to show symptoms of water stress after the water supply was reduced. The trees on this row appeared far less vigorous than the trees on the high and medium irrigation rows.

The foliage of the trees in the low irrigation row also had a lot of chlorotic tissue as well as large regions of necrotic tissue.

The control trees inoculated with the plain agar pugs without the pathogen were another good indicator of water stress in the low and medium irrigation rows. Gumming was found on the site of inoculation for these trees when the cankers were measured. However, the control trees on the high irrigation row showed no indication of gumming at the site of wounding/inoculation with the agar plug. That indicates that some other opportunistic disease could have entered the trees due to their water stress. Gumming was produced later, but the gumming was clear and not amber in color. The amber color indicates that the infection is active and so it was only present in the trees that were inoculated with the pathogens. Although there was clear gumming in the control trees, there was no canker development.

Based on the canker length charts (Figures 3 through 11), it is determined that there is a negative correlation between the irrigation and the length of the canker. This means that the less irrigation applied the larger the canker will develop. The average of the canker lengths (Figure 3) shows that the low irrigation row contained the largest cankers and the high irrigation row contained the smallest cankers. The pattern of growth can also be seen in figure 5. This chart provides the *P* value of 0.14. Since there are only 3 points on the chart the value is higher than .05, it would be expected to have a far lower number if more points were available.

If the measurements are split based on what fungus was used during inoculations, it appears that the averages of the canker lengths varied. (Figure 6 and 9) The fungi *N. nonquaesitum* was more aggressive than N. parvum at infecting the almond trees. This fungus produced larger cankers on the trees that received low to medium irrigation compared to those receiving higher volumes of irrigation. (Figure 6) *N. nonquaesitum* can spread farther in trees experiencing high levels of water stress. *N. parvum* on the other hand did not seem to be as effective as *N. nonquaesitum. N. parvum* did produce large cankers in the low irrigation row, however, it did not spread as much in the medium irrigation group. (Figure 9) The medium and low irrigation groups were significantly different from one another. The high irrigation group was not significantly different from both the medium and low groups. This can be attributed to the few measurement points being used for the charts. When measurements for both fungi are combined, there is no significant difference between any of the three irrigation amounts (Figure 3). Based on this we can conclude that the data is not due to random chance, but that there is a trend showing direct relationship between the water stress and susceptibility to *Botryosphaeria* pathogens.

Acknowledgements:

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Appendix

Moisture experiment was conducted using fruits and shoots of walnut trees. The result from this short experiment showed that walnut fruits had more moisture content than walnut shoots did. Experiments used 20 shoots and 20 fruits. Initial weights were received for all fruits and shoots. They were placed in a dry oven in groups of five. Weights were taken of the fruits and shoots regularly and final weights were obtained once a stable weight was achieved. Charts below show average weights obtained for both fruits and shoots.