

Pierce's Disease

California is faced with a new threat and it is one that is only getting worse with each passing season. That threat is Pierce's disease, named after Newton B. Pierce, Special Agent to the Secretary of Agriculture, and California's first professionally trained plant pathologist. To understand this new threat that California is facing¹ and how genetic engineering can help, it is important to understand some of the background for this disease, what non-genetic methods are being used to try and stop this threat before it gets out of control, and why genetic intervention seems the most effective approach in the future.

Pierce's disease was first introduced to California in the 1880s when it decimated 30,000 acres of vineyards in the Los Angeles Basin near Pomona and Anaheim. It hit the same area twice more in the 1930s and 1940s with the same ferocity. In 1887 and 1889 Pierce's disease was found in the Napa Valley and northern Sacramento. In 1921 it was found in Tulare County and by 1940 it was identified in mostly all the counties in the San Joaquin Valley. More recently, Pierce's disease has impacted 25% of the 3000 vineyards in the Temecula Valley, which has totaled \$13,000,000 in damage in this county alone. (Wine Institute) Currently, there are 15 counties that have been labeled as "infected" within California and the number is increasing with each passing year. Furthermore, the two areas that have been devastated the most by Pierce's disease are the San Joaquin

¹ Pierce's Disease is a disease that other states than California has had to face (i.e. Florida), but I will chose to only talk about California for this paper.

Valley and the Napa Valley. (Wine Institute) Seeing the trend of rapid and vicious spreading, one might ask how was it possible for this disease to spread so quickly?

The rapid infestation of Pierce's disease throughout California can mostly be attributed to the glassy-winged sharp shooter². The sharp shooter carries the bacterium, *Xylella fastidiosa*, which is the cause of Pierce's disease. When a sharp shooter drinks the sap from one of the vines, it also injects the plant with this bacterium and thus spreads the disease with an amazing quickness. Furthermore, before the growers knew what the disease was or how it spread they would send cuttings from one vineyard to another. Some of these cuttings traveled from the San Joaquin Valley to the Napa Valley and thus the disease was introduced to northern California. The reason that California, and more specifically the San Joaquin and the Napa Valleys, has been affected with this disease is because the sharp shooters and consequently this disease thrive in climates that have mild winters. (Overview of Pierce's disease) Now, to further understand this new threat, it is essential that one understands how the sharp shooter transmits the disease and how the plant is affected from it.

As mentioned, Pierce's disease is the bacterium *Xylella fastidiosa* that thrives in the plant's xylem. The sharp shooter, which feeds on the xylem sap, transmits the bacteria from the diseased plant to the healthy plant every time it feeds on a vine. Once this happens, the bacteria eventually blocks the water conducting system and consequently reduces the flow of water within the plant's cells to the affected leaves. (Overview of Pierce's disease) Therefore, the drying or "scorching" of the leaves on the vine are the first signs of the disease. The leaves then turn slightly yellow along the

² There are other sharp shooters, such as the blue and green, that are spreading the disease as well, but the glassy-winged sharp shooter is the most damaging of the insects.

margins before drying or die rapidly even when they are still fully green. (PICTURE) Furthermore, the bark matures unevenly; mature dark brown bark can be surrounded by immature light green bark. (Overview of Pierce's disease) Since there is a lack of water transport to the plant's cells, the vine prematurely produces small, inedible, raisin-like grapes. Consequently, the vine is now unusable and if left alone will die within two years. However, the growers don't let the plant die on its own because they pull the now useless plant up before it can die naturally. Clearly, this disease is very threatening for growers around California so what are growers doing in order to counter this leafhopping menace?

First and foremost, there have been massive inspections on all transports of nursery stock and bulk grapes throughout California. Considering that the transportation of vines from one area to another is the easiest way for either infected plants or sharp shooters to infect new areas, a lot of effort has been given to ensure that this doesn't happen. In fact, in 2001 alone, 50,000 nursery plant shipments and 100,000 grape shipments were inspected. (Wine Institute) Furthermore, growers are now selectively removing vegetation around the vines that might house the sharp shooters and are conducting heavy pruning of the infected plants. Ground spraying of the infected plants throughout the vineyard with insecticide has also been an alternative. However, it is difficult to find an insecticide that will only target the sharp shooter and not harm the other animals and vegetation in the area. Some insecticides that have shown promise in killing the sharp shooter but they have also been killing healthy vines. Another method, which has shown more promise, is that of antibiotics. Unfortunately, injecting the drugs into the hard grapevines requires countless hours of manual labor and it is difficult for

growers continually inject their numerous vineyards. (Alright Seed) There has also been a method of biological control that introduces a parasitic wasp that lays eggs in the eggs of the sharp shooter. This method has been shown to be an inexpensive, long-lasting and environmentally friendly alternative. Consequently, it can be seen in Temecula that because of the implementation of this method, the levels of sharp shooters have slowly declined. (Wine Institute) However, using this method also means that there will be many more wasps to deal with in the vineyards. Consequently, researchers are trying to breed a stingerless wasp to deal with this immediate problem. Though these methods have had success in stopping the sharp shooter, each method is very time consuming because the growers would have to do all of this work manually and check the plants often to ensure that further outbreaks don't occur. Furthermore, Pierce's disease is spreading faster than these present preventative measures can slow the disease down. Consequently, the wine industry in California needs to find a way to eliminate the threat of Pierce's disease, not slow it down. To do so growers must turn to scientists to fight their battle.

Brazil was threatened with a disaster to their citrus trees, much like California is facing with their vineyards today. The culprit was, indeed, *Xylella fastidiosa*. Consequently, the Brazilian government commissioned the Ludwig Institute for Cancer Research in Sao Paulo, Brazil to decipher the genetic sequence of *Xylella fastidiosa*. (Wine Institute) Finally, after two years, a team of 200 scientists, working in 34 laboratories sequenced *Xylella fastidiosa*. (*Xylella fastidiosa*) With this new knowledge scientists are now working on a disease resistant vine. The resistance is based on the fact that once a vine becomes infected with the disease it is more resistant to that disease in the future. Therefore, the genes for the resistance come from the virus itself.

Consequently, a non-infectious viral gene is inserted into the vine to make a protective vaccine. The process of injection needs six things: 1) recipient cells that are capable of growing into whole plants, 2) a method to transfer the genes into the cells, 3) proper expression of the genes by the transformed plant cells, 4) a method to select the transformed cells from the non-transformed cells, 5) regeneration of whole plants, and 6) evaluation of disease resistance. (Genetic Engineering) Furthermore, successful results only came when researchers used embryogenic cultures. These cultures, consisting of embryo-germinating clumps of plant cells, were grown under sterile conditions in an artificial growth medium in the lab. Furthermore, the plant cells used originate from the somatic cells instead of the egg or sperm cells. This is so that the new cells can be exact clones of the original plant. Normally, to insert genes into these embryogenic cultures, researchers rely on modified strains of *Agrobacterium*, which transfer genes into plants as part of its normal life cycle. (Genetic Engineering)

Within the Geneva laboratory at Cornell University, another method has been found. They rely on a biological ballistics method where DNA-coated particles are used to carry foreign genes into grapevine cells. The DNA coding for the genes of interest are coated onto a tungsten-microprojectile and are accelerated at a very high speed into the cultured cells using a biolistic device called a gene gun. Normally, the microprojectile transfers several genes into each cell because in addition to the gene of interest, there is another gene that is used to help separate the normal cells from the transformed ones. This is important because less than 5% of the cells receive and maintain the genes long-term. (Genetic Engineering) Now, the Geneva laboratory uses a gene that confers resistance to the antibiotic, kanamycin, as a transformant. The transformant is selected

within a medium containing kanamycin, and is now able to develop into embryos. Normal cells that do not contain the newly inserted gene will die on the medium with kanamycin. Consequently, the only growth within the plant should be from the cells with the newly inserted genes. (Genetic Engineering) The result will be a vine that is immune to Pierce's disease. Researchers are close to creating this disease resistant vine, and expect to find it within the next several years. However, it is feared that in the future the product of a single gene will be more readily overcome by a pathogen. Therefore, it is likely that there will be insertions of multiple disease resistant genes in the hopes that the resistance will be longer lasting and more effective. Until then, work is being done to try and isolate important genes within the vines in hopes of finding a vine that isn't susceptible to Pierce's disease.

It is clear that present non-genetic methods of combating Pierce's disease are working, but nothing is working on the level that is needed to completely halt the threat of Pierce's disease for the future. Consequently, it is obvious that only scientists will be able to win this battle and see that the threat of Pierce's disease no longer exists. However, once finished it is going to be necessary to convince a skeptical public that genetically engineered grapes are necessary for the future of the California wine industry. I strongly feel however that once consumers find higher prices and less selection of their favorite Californian wines, due to Pierce's disease, most if not all skepticism will be a thing of the past...

Work Cited

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