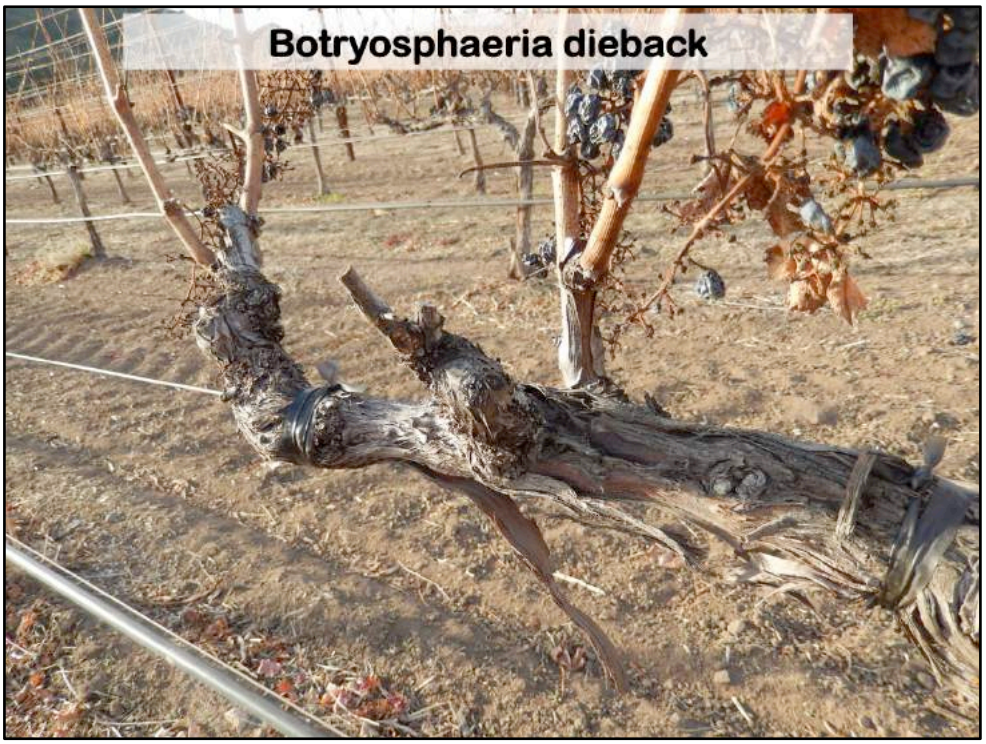
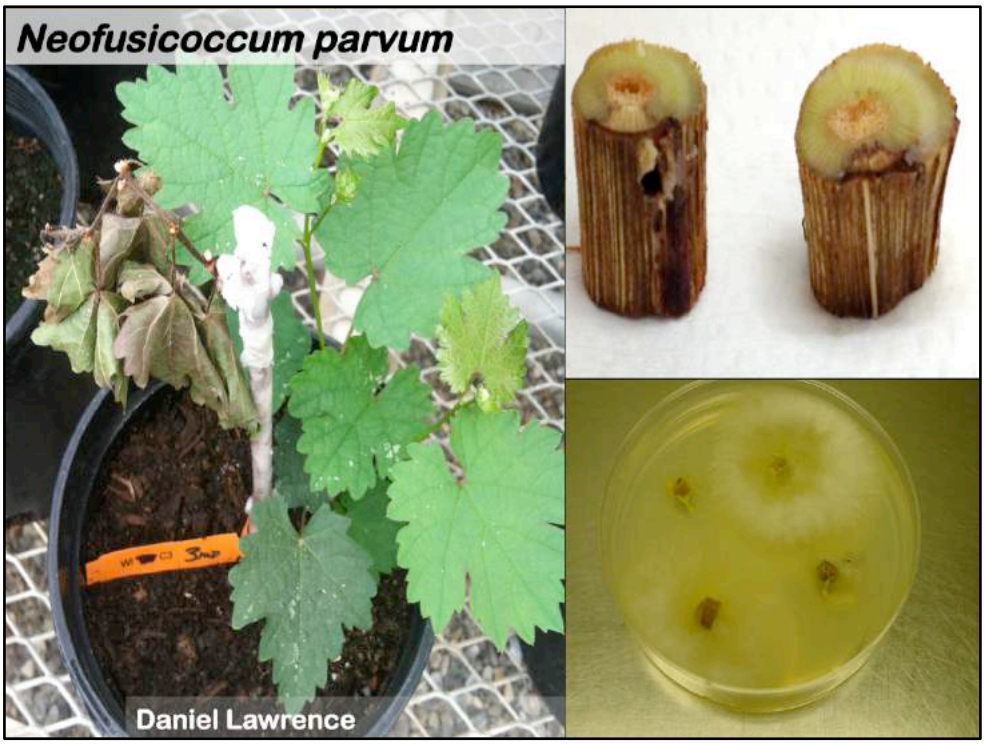
This is a presentation about some of the progress we've made on the detection objective of the SCRI grant. Our goal is to develop a new method to detect trunk diseases by sampling asymptomatic leaves. We demonstrated proof of concept that we can detect grapevine genes expressed in asymptomatic leaves during the latent phase of infection – of the woody stem – by *Neofusicoccum parvum* (causal fungus of Botryosphaeria dieback). These grapevine genes are putative markers of infection. Additional experiments were done in the greenhouse to determine whether these putative markers of infection are specific to *N. parvum*, and do not cross-react with drought or other trunk pathogens.



For Botryosphaeria dieback (as well as all trunk diseases) there is a long delay between when a pruning wound is infected by a spore and the vine shows a symptom of infection. This makes detection of trunk diseases difficult because by the time the spur dies, a permanent wood infection is already established in the cordon.



In the greenhouse, we can create the dieback symptom faster than in the field…in 2 to 3 months for *Neofusicoccum parvum* (causal agent of Botryosphaeria dieback). This characteristic makes it a good study system for examining the infection process, particularly the early stage of infection, when the fungus is in its latent phase. Another reason we focus on this pathogen is because it is widespread among CA grape-growing regions and it is one of the most aggressive trunk pathogens.

If we could detect a latent infection, we could minimize propagation of contaminated nursery stock.

Also, we as researchers would have a tool for evaluating pruning-wound treatments and other management strategies.

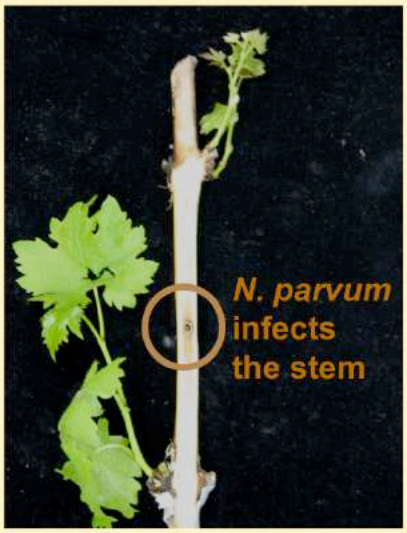
Characterized the latent phase

Latent Phase from 0 to 6 weeks:

- Pathogen stays at inoculation site.
- No change in lesion length.

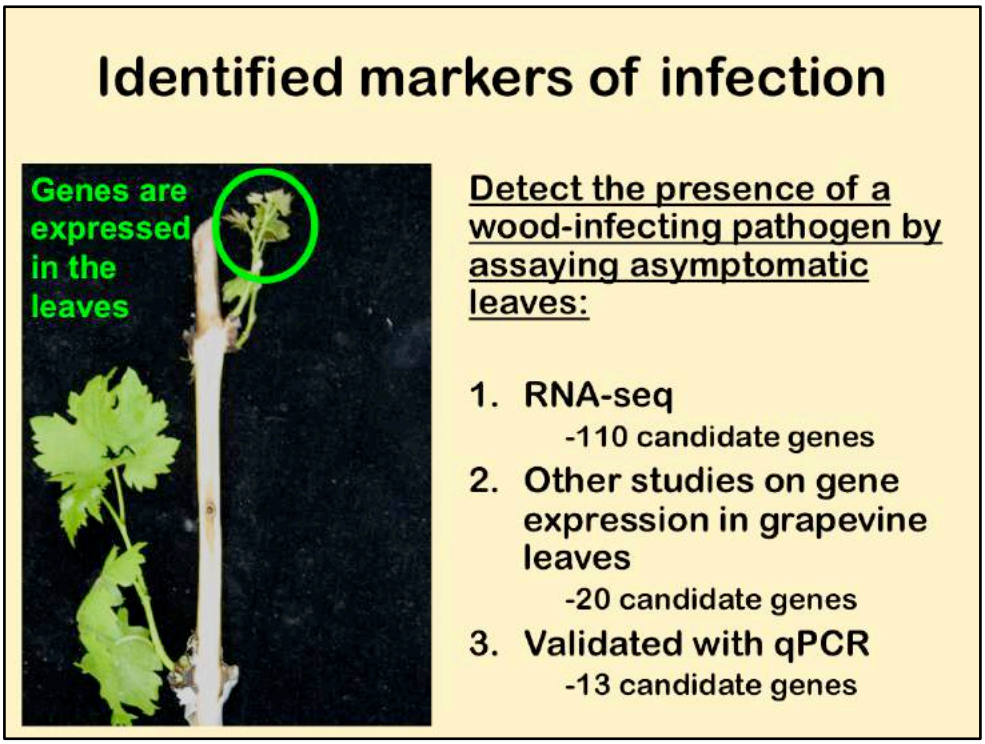
Pathogenic phase from 6 to 8 weeks:

- Pathogen spreads beyond inoculation site.
- Lesion lengths increase.
- Xylem vessels fill with gels.
- Starch is depleted from xylem fibers & rays.

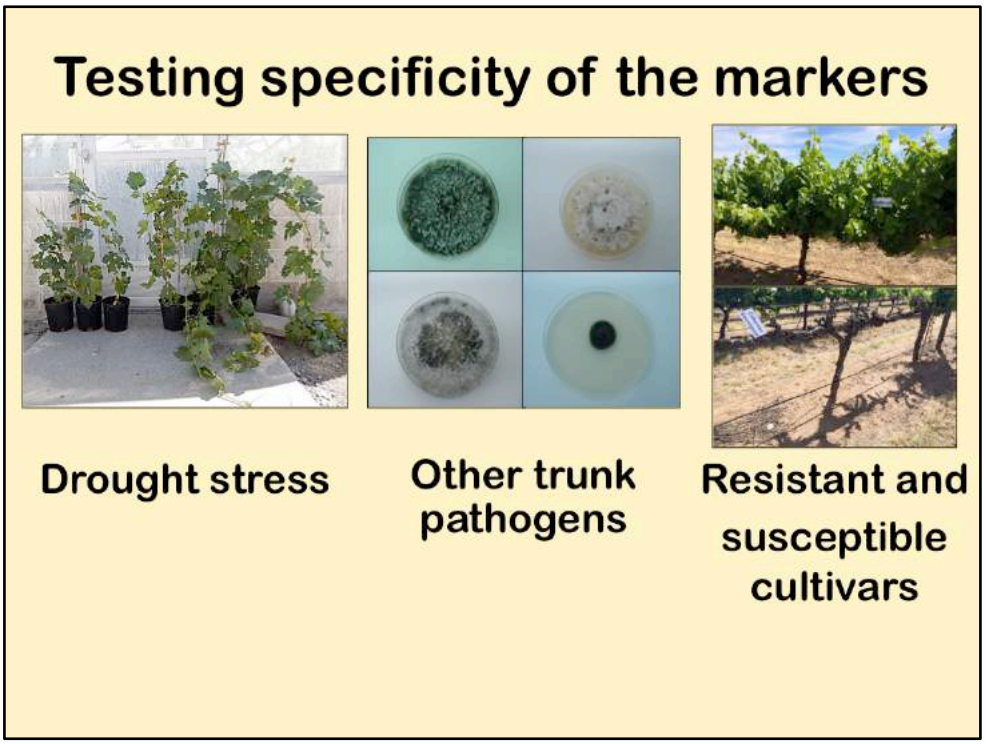


Czemmel S, Galarneau ER, Travadon R, McElrone AJ, Cramer GR, Baumgartner K. 2015. Genes expressed in grapevine leaves reveal latent wood infection by the fungal pathogen *Neofusicoccum parvum*. PLoS ONE 10: e0121828.

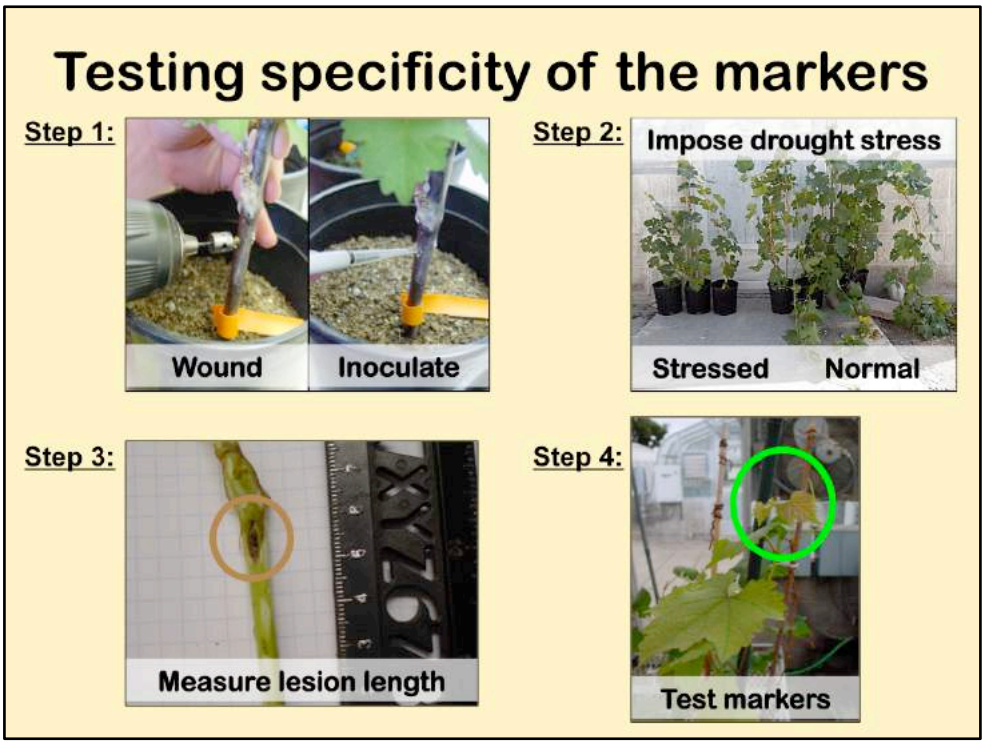
Our first step was to demonstrate proof of concept that we can detect grapevine genes expressed in asymptomatic leaves during the latent phase of infection – of the woody stem – by *Neofusicoccum parvum*. The latent phase of infection was defined as the point before the pathogen begins to spread from the inoculation site and before the host shows anatomical changes (gels form in the xylem vessels, changes in starch content of xylem cells) in response to this colonization. Under our experimental conditions, the latent phase occurred between 0 and 6 weeks post-inoculation (WPI). From 6 to 8 WPI, our findings of significant infection development, coupled with anatomical changes in the wood in response to the infection (but still no canopy symptoms), suggest that this was the start of the pathogenic phase.



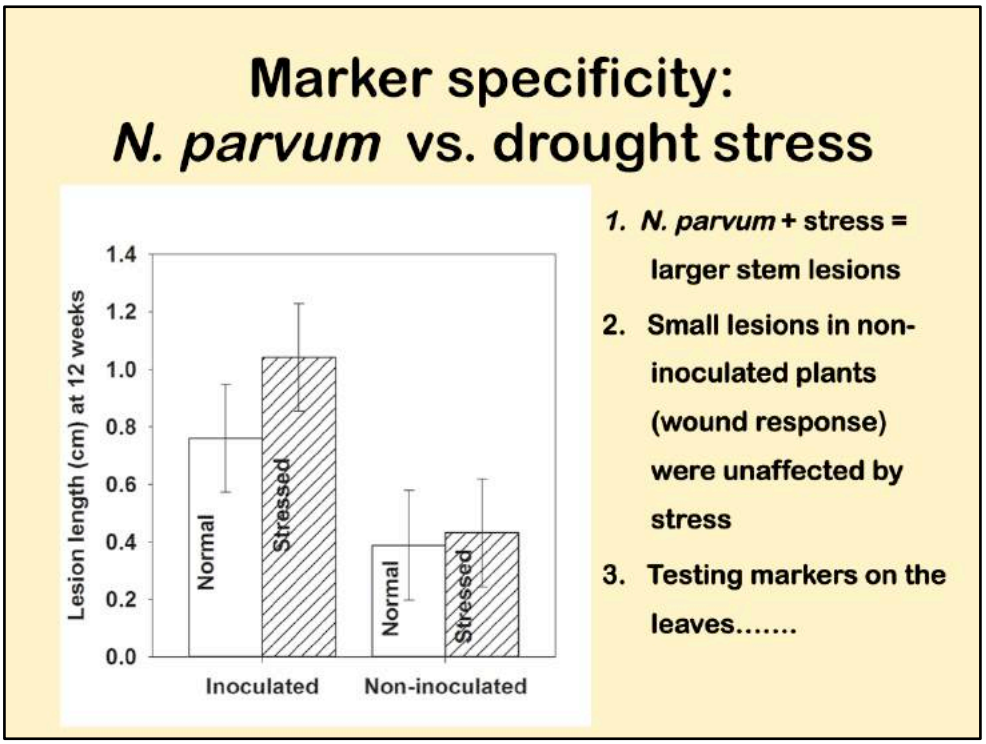
Next we sequenced all the genes expressed by the grapevine in leaves collected throughout our experiment, using RNA-seq. Statistical comparisons between different inoculation treatments and time points revealed 110 candidate genes, which were expressed at higher levels in inoculated plants. We examined the same genes in other published studies, to determine if other researchers found the same genes expressed at higher levels due to other pathogens or to abiotic conditions. This helped us narrow the list to 20 genes. Finally, we went back to our original samples, which we sequenced, and confirmed the sequencing results with a separate technique focused on individual genes –qPCR. This identified 13 genes that were expressed in inoculated plants. These are our putative MARKERS OF INFECTION.



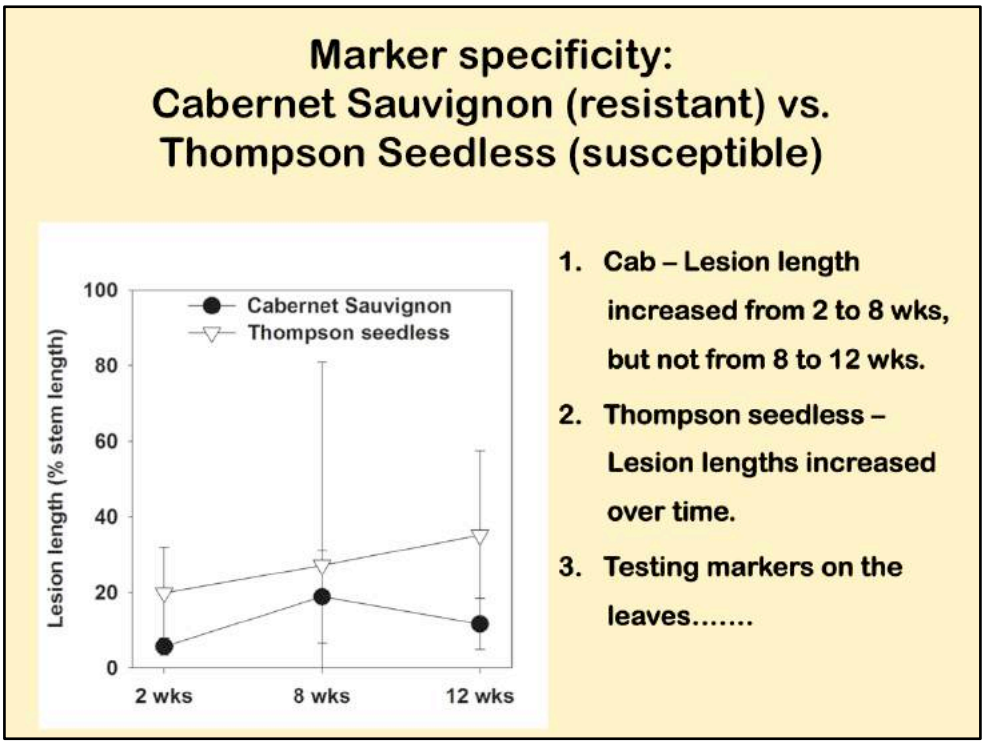
In 2015, we carried out three additional greenhouse experiments to confirm the specificity of the markers of infection for *N. parvum*. We need to ensure that the same genes are not induced also by drought stress or other trunk pathogens. We also need to confirm that leaves of different cultivars, when infected by *N. parvum*, express the same markers.



These experiments use a similar approach: plants are propagated from dormant cuttings in the greenhouse. The plants are then subjected to wounding and inoculation and, in the case of the drought stress experiment, subjected additionally to drought stress (at 8 WPI). After 12 WPI, we measured the length of the lesion in the woody stem. 'Lesion' is synonymous with the term 'canker'. These lesion lengths are a measure of the extent of the infection. We also harvest leaves to test for the markers of infection, after extracting RNA from them.

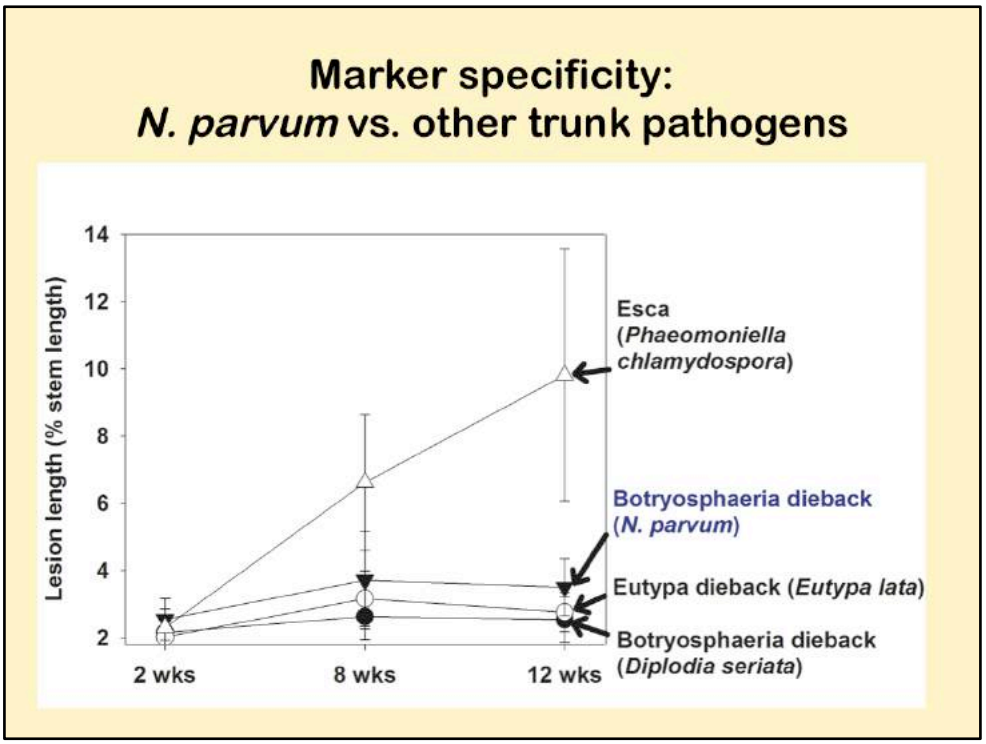


1. Between the two groups of inoculated plants, drought stressed plants had larger lesions. This suggests that drought stress exacerbates the infection.
2. Between the two groups of non-inoculated plants, we found no differences in the small lesions, which are more of a wound response than a measurement of the infection.
3. Now we are testing the leaves for our markers of infection. The markers were previously shown to not be expressed in non-inoculated plants. We want to make sure this holds true, even for stressed plants that are not inoculated.



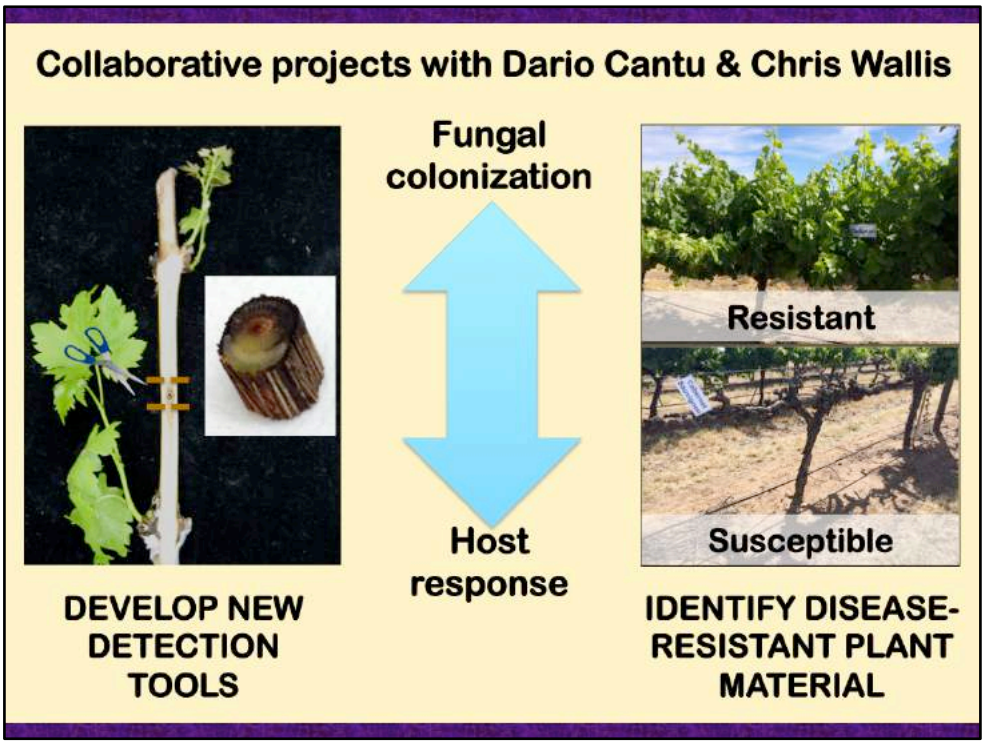
Cabernet Sauvignon is typically more resistant to *N. parvum* than is Thompson seedless. Here we see the lesion lengths on plants collected at 2, 8 and 12 weeks after inoculation. Lesion length is shown as a percentage of the total length of the stem.

1. For Cabernet Sauvignon, we found that the lesion lengths were slightly lower at all time points. From 2 to 8 wks, they increased in both cultivars. From 8 to 12 weeks they continued to increase in Thompson seedless, but kind of leveled off in Cabernet Sauvignon.
2. Now we are testing the leaves for our markers of infection. We want to make sure the markers are expressed at the early stage of infection in both cultivars.

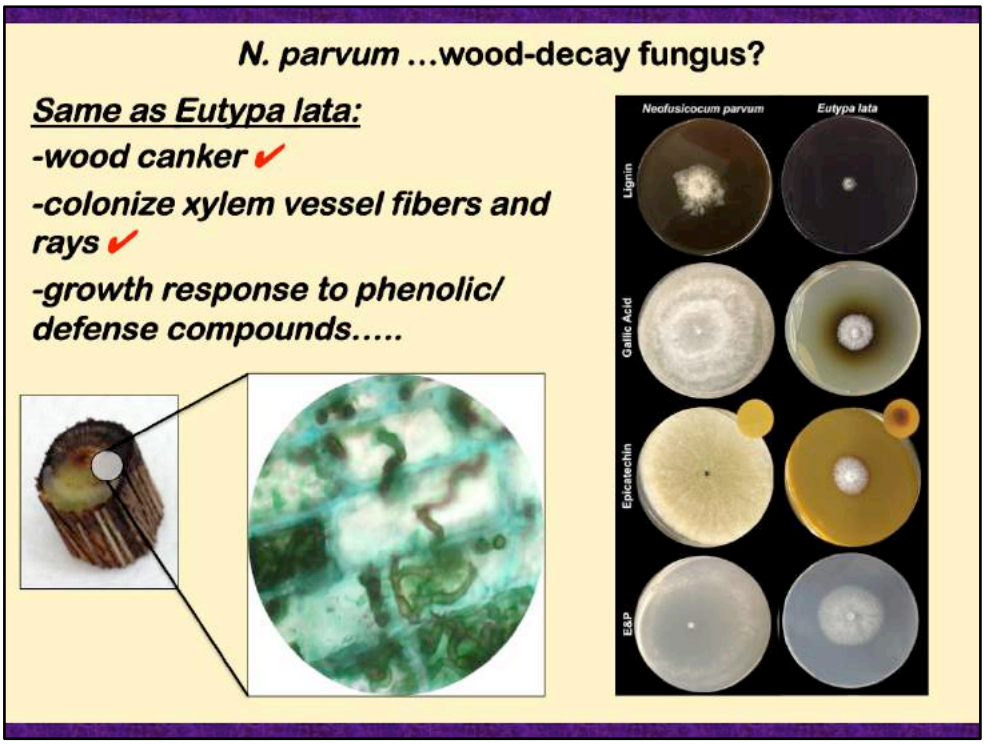


Here we inoculated different plants with four different trunk pathogens:

1. *N. parvum* showed a similar pattern as in the other experiments, where lesion lengths leveled off at 12 weeks.
2. We had smaller lesions for *Eutypa*, which is in part due to the slow growth of this trunk pathogen….it can take a year to get decent lesion lengths….after only 12 weeks. (However, since we are interested in markers of **early** detection, we didn't carry out the exp. for a whole year).
3. The smallest lesions were from *Diplodia*, which is related to *N. parvum*, but is much less aggressive. Our surveys and spore trapping studies suggest that *Diplodia* is much more common in the field than *N. parvum*. We do not want our markers to detect a very weak pathogen.
4. The largest lesions were from the Esca pathogen *Phaeomoniella*.
5. Now we are testing the leaves for our markers of infection. We want to make sure the markers are specific to the plants inoculated with *N. parvum*.



From the same greenhouse experiments completed in 2015, we can examine colonization by the pathogen, the host response to this invasion, and whether the host response, in turn, affects further colonization. These projects are possible because Dario has sequenced the genomes of the trunk pathogens and because Chris has characterized the grapevine's response, in biochemical terms, to infection of the woody stem. Characterizing different patterns of fungal colonization and different host responses between resistant and susceptible cultivars can help us understand mechanisms of resistance, a research question that connects our TWO RESEARCH OBJECTIVES of developing early detection tools and identifying sources of resistance in the germplasm.



One of the questions we are interested in answering is whether *N. parvum* is a wood-decay fungus, like *Eutypa*. After all, they both a cause similar-looking wood canker. When we examine a very thin section of a canker under the microscope, we can see that…like *Eutypa*…*N. parvum* colonizes the same cell types in the wood. Now we are trying to determine if the two fungi respond similarly to phenolic compounds and other defense compounds that are made by the grapevine to stop the spread of the fungus. Some of the discoloration we see in the wood canker is due to these defense compounds. When we amend fungal growth media with these compounds, we can see if they inhibit or encourage growth of the two fungi. In the photo at right, we can compare growth of N. parvum and Eutypa on plain medium (labeled 'E & P') and then when that medium is amended with defense compounds the grapevine makes in response to infection. When we add lignin (a component of woody cell walls), we can see that both fungi have much smaller colonies. In contrast, with gallic acid (a phenolic acid) or epicatechin (a flavan-3-ol), they grow better. Therefore, it appears these trunk pathogens can feed on these two compounds that the grapevine makes to try to stop the fungi. D'oh!