INVESTIGATING INCIDENCE AND TYPE OF WOOD DECAY FUNGI IN CALIFORNIA PRUNE ORCHARDS

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Wood decay fungi reduce the structural integrity of trees, leading to wind-driven collapses and scaffold limb breakage, causing tree loss, and reduced production in the prune growing regions of California. Wood decay is caused by a wide array of fungi that colonize and digest the heartwood, and sometimes sapwood, in living trees.

Wood decay surveys in prune orchards throughout the Sacramento Valley began in 2015. A high incidence of *Phellinus tuberculosus* (formerly *P. pomaceus*) was found in every surveyed orchard older than 12 years. Most limb breakage could be attributed to extensive *Phellinus* decay. For this reason, much of our focus this year has been on understanding the epidemiology and biology of *P. tuberculosus* in prune orchards and exploring control and management strategies.

OBJECTIVES

1. Identify the main fungi associated with heart-rot diseases and scaffold limb breakage of *Prunus domestica* in California.

2. Determine the infection process of *Phellinus spp.* in prune orchards.

3. Design and employ taxonomic specific primers for early detection of decay fungi on standing trees.

4. Test indigenous orchard microorganisms as possible bio-control agents.

PROCEDURES

Objective 1 Identify the main fungi associated with heart-rot diseases and scaffold limb breakage of *Prunus domestica* in California.

Surveys throughout the Sacramento Valley are ongoing, and largely based on grower or farm advisor inquiry. These surveys previously indicated that *Phellinus tuberculosus* was the wood decay pathogen of greatest concern. However, as *Phellinus* was not previously considered a significant pathogen of Prune, there is an incomplete understanding of how its incidence varies as a function of orchard age.

We identified 13 orchard blocks ranging in age from 4 to 25+ years in Sutter County. Orchard blocks were in relatively close proximity to each other. All young orchards were adjacent to a known infected block. In each orchard block, four plots of ten trees down a row were examined for evidence of decay (limb breakage) and *Phellinus* fruiting bodies.

Objective 2. Determine the infection process of *Phellinus spp.* in prune orchards.

Pruning wounds remain potentially susceptible to colonization by wood decay fungi if adequate moisture and inoculum is available. Previously, we determined that multiple infections occur on the same tree and even on the same branch.

Brush pile trial

A common question from growers pertains to limbs removed and placed in brush piles serving as a potential source of inoculum. To investigate this, an un-replicated "brush pile trial" was conducted. Broken limbs with evidence of active *Phellinus* decay and/or live *Phellinus* fruiting bodies were collected from infected orchards. Limbs were placed in an un-covered brush pile with various other debris, and observed for a period of 12 months. Development of fruiting bodies and new growth on existing fruiting bodies were assessed every 2 months. After 12 months re-isolation of *Phellinus* was attempted.

Decay trials

To determine the decay rate of *P. tuberculosus* in French prune, branches with a diameter of 1.5 to 2.5 cm after bark removal were cut into 2 cm lengths. Half of the pieces were autoclaved, while the other half were surface sterilized prior to inoculation. Fresh mass was recorded for all pieces. The pieces to be autoclaved were dried and weighed prior to rehydration and autoclaving for 30 minutes twice. Seven prune varieties were included in this trial, and were prepared as previously described. Non-inoculated wood pieces served as control. Each variety and a control were replicated 10 times each for an autoclaved and non-autoclaved treatment.

Each replicate was randomized and wood blocks were placed on a wire mesh inside a moist chamber. A plug of mycelia (3mm x 3mm) from 14 day old *P. tuberculosus* cultures were placed on top of each block. Moist chambers were maintained in the dark at room temperature for ten weeks. After ten weeks, surface mycelia were removed. Wood pieces were dried at 60°C for 48 hr and dry mass recorded. Percent moisture content and initial fresh weight were used to calculate predicted initial dry weights for each wood piece. Analysis of Variance was performed on mass loss using initial dry. Tukey's mean separation of mass loss was performed on prune varieties.

Objective 3. Design and employ taxonomic specific primers for early detection of decay fungi on standing trees.

While several attempts were made to amplify *Phellinus* DNA directly from woody tissue, this protocol demands further development more than the immediate usefulness it would provide to the prune industry. Identification of *Phellinus* and associated decay is straightforward and we have reliable isolation protocols.

Objective 4. Test indigenous orchard microorganisms as possible bio-control agents.

On several instances during our survey work *Trichoderma* spp. were isolated from *Phellinus* decay. *Trichoderma* sp. was also found colonizing a *Phellinus* fruiting body (Figure 1). The presence of *Trichoderma* spp. is intriguing, as it has been tested as a biocontrol in other cropping systems, and is often isolated from decayed tissue when no wood decay fungi can be recovered.

Additionally, in several instances, *Trichoderma* sp. contamination of *Phellinus* spp. and *Ganoderma* spp. cultures in the lab, rendered those cultures unrecoverable. Previously a *Trichoderma* containing product was registered in California tree crops for control of the wood decay pathogen that causes silver leaf disease.

Two isolates of *Trichoderma* (T1 and T2) from prune orchards were identified as *T. atroviride*. Laboratory experiments to test the effects of these *Trichoderma* isolates on *Phellinus* growth and mortality were carried out in plates of 2% malt extract agar (MEA). Plugs from a 14-day old culture of *P. tuberculosus* were placed slightly off center on a fresh MEA plate. After 7 days, *Phellinus* cultures were inoculated with *Trichoderma* strains T1 and T2 by first inserting tip of sterilized scalpel into culture of *Trichoderma* and then into MEA opposite the *Phellinus* plug. Mock inoculations with sterile scalpel served as a control. The diameter of *Phellinus* cultures were measured 7 days after *Trichoderma* inoculation and a subculture from each was plated onto fresh MEA 7 and 14 days after inoculation. This trial was replicated, but with the addition of a combined inoculation of *Trichoderma* T1 and T2.

Similar experiments were carried out on autoclaved disc of prune wood. Wood disc with bark removed 2.5- 3cm in diameter and 1cm thick were autoclaved twice and placed in empty petri dish. Each disc received both *Phellinus* and *Trichoderma* inoculation, applied in one of three treatment ttimings: Both at the same time, *Phellinus* followed by *Trichoderma* at 7 days, or *Trichoderma* followed by *Phellinus* at 7 days. Treatments included strains T1, T2 and a commercial product containing live *Trichoderma atroviride*. *Trichoderma* inoculation was carried out by pipetting 10µl of a spore suspension concentration ~ 2 x 10^7 CFU/ml onto the wood disc. *Phellinus* was inoculated as described above. Recovery of *Phellinus* was attempted 14 days after first inoculation.

Figure 1. Trichoderma colonizing Phellinus fruiting body.

RESULTS AND DISCUSSION

Objective 1.

Phellinus decay was observed in all orchards older than 7 years and was observed as the cause of limb breakage in orchards 9 years old and older. *P. tuberculosus* fruiting bodies were present in orchards as young as 10 years. Percentage of trees containing broken limbs, decay and fruiting bodies increased with orchard age (Figure 2). We found no evidence of *Phellinus* in orchards younger than 5 years, however that does not preclude its presence. This survey suggests that orchards 5 years old and younger should be the focus for possible management interventions.

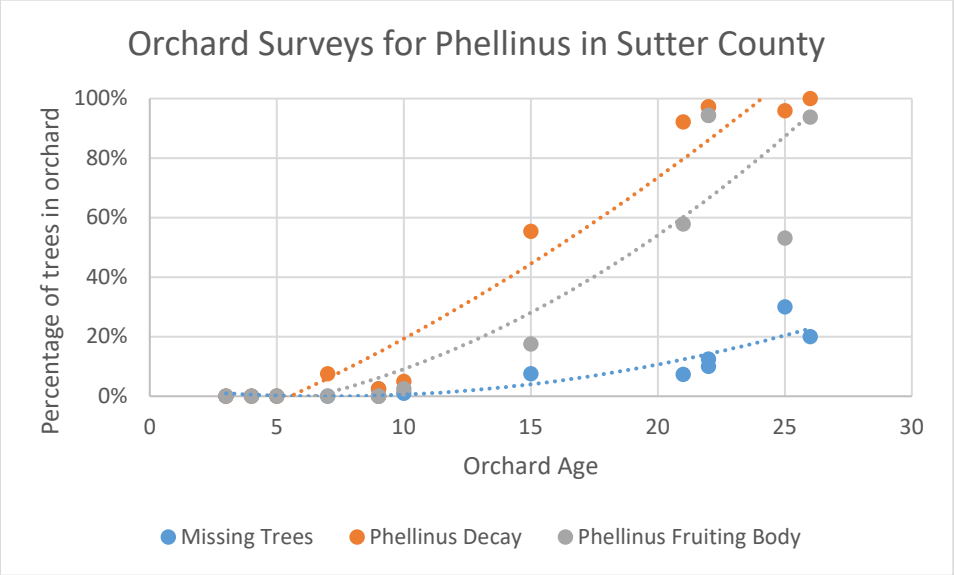


Figure 2. Phellinus survey results from different age orchards in Sutter County

Objective 2.

Brush pile trials

While un-replicated, insights gathered from this trial and field observations may help to alleviate some of the concern regarding orchard waste and brush piles as a possible source of inoculum. After 12 months in a brush pile, no new fruiting bodies were observed, nor was there any new growth on existing fruiting bodies. We were unable to isolate *Phellinus* from any of the limbs placed in the pile. This agrees with our failed attempts to isolate *Phellinus* from broken limbs that have been in contact with the orchard floor for anytime period at all. Although orchard wastes do not appear to be a significant source of *Phellinus* inoculum, they may serve as sources of inoculum for many other orchard diseases.

Decay trials

Phellinus survival on fresh blocks of wood that were only surface sterilized was less than 5% due to contamination. This is in agreement with results from brush pile trial further suggesting

Phellinus is a weak competitor. After 10 weeks of incubation, mass loss of autoclaved wood pieces was significantly greater for those inoculated with *P. tuberculosus* compared to non-inoculated controls (Figure 3). Mass loss was greatest in Improved French variety when compared to the other prune varieties included in the trial. This suggest some degree of adaptation by *P. tuberculosus* to more readily decay the Improved French variety. A shift to more diversity in prune varieties may benefit the industry with regards to problems caused by *Phellinus*. This trial is only preliminary and we will continue to work with breeders to further evaluate susceptibility of different varieties to *Phellinus* decay in the lab and in the field.

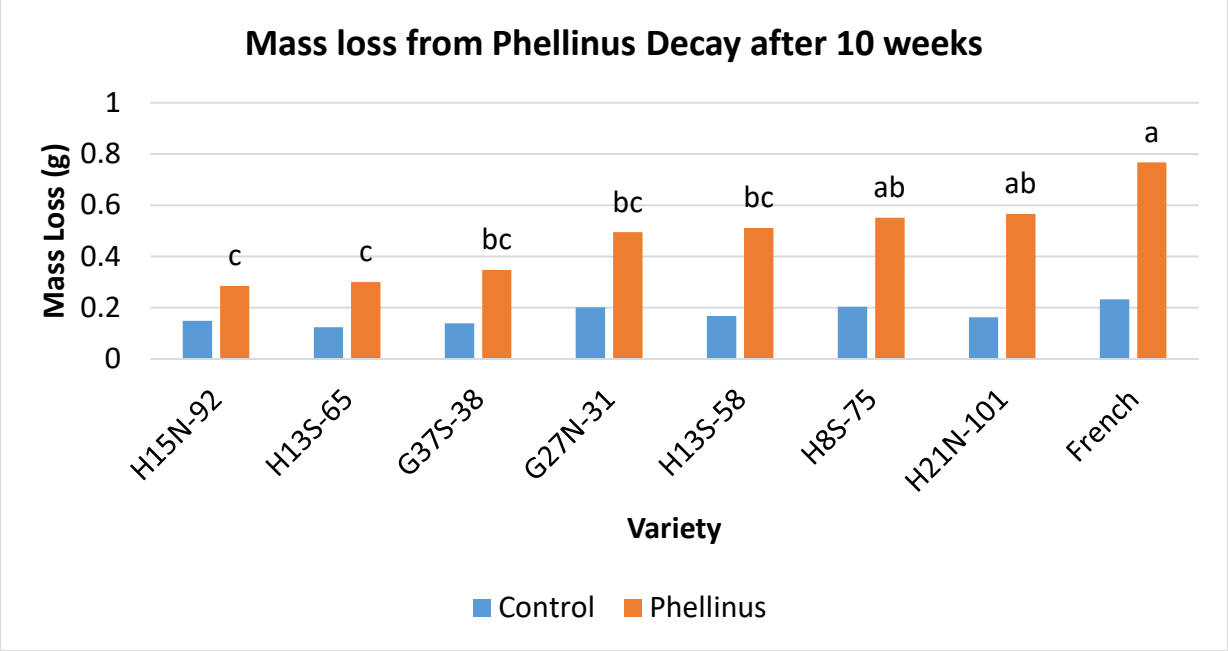


Figure 3. Mass loss caused by P. tuberculosus on various prune varieties after ten weeks of incubation. Tukey's mean separation groupings are presented above bars, different letters indicate significant difference at p<.05.

Objective 3.

There are no results to report for this objective.

Objective 4.

The positive results from our laboratory experiments coupled with previous success in other crops suggest that applications of *Trichoderma* spp. may serve as an effective control for *P. tuberculosus* in prunes.

For both trials on MEA, *Phellinus* cultures inoculated with either T1, T2, or a combination had significantly smaller diameters than non-inoculated controls (Table 1). Seven days after inoculation all but one *Trichoderma* treatment resulted in complete *Phellinus* mortality, while mortality was complete for all treatments after 14 days.

In co-inoculation trials on wood disc comparing *Trichoderma* strains as well as a *Trichoderma* containing product (Vintec), inoculation at the same time resulted in 100% *Phellinus* mortality (Figure 4). The *Trichoderma* product generally performed better in all treatment timings. *Trichoderma* appears to be more affective when applied prior to *Phellinus* inoculation. This trial is currently being replicated, albeit on larger wood pieces and the rate of decay is being determined.

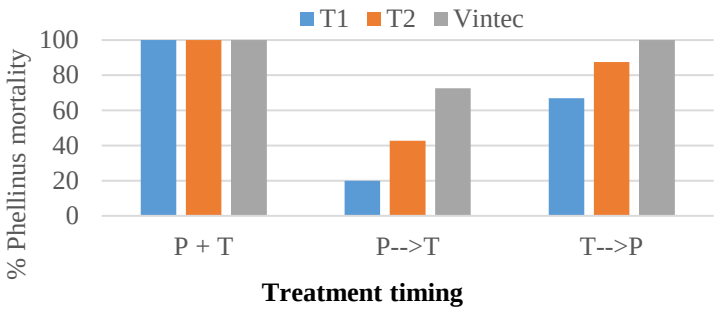
Field evaluation of *Trichoderma* products will be planned and carried out in 2018.

Table 1. Colony growth and mortality of *Phellinus* co-inoculated with *Trichoderma* sp.

	Trial 1		Trial 2	
Treatment[1]	**Colony diameter (mm)[2]**	***Phellinus* mortality[3] % 7dai/14dai**	**Colony diameter (mm)**	***Phellinus* mortality % 7dai/14dai**
Control	38.8 A	0/0	38.9 A	0/0
T1	29.0 C	50/100	28.8 C	100/100
T2	31.0 B	100/100	34.3 B	100/100
T1+T2	--	--	28.6 C	100/100

[1]*Trichoderma* treatment was applied 7 days after *Phellinus*. [2]Colony diameter was measured 7 days after *Trichoderma* treatment. [3]*Phellinus* mortality was assessed 7 days after inoculation and 14 days after *Trichoderma* inoculation.

Figure 4. Phellinus mortality in the presence of Trichoderma 14 days after first inoculation



BUDGET SUMMARY

Grant support from the almond board is being used concurrently to cover salary and other expenses. Remaining funds will be used to continue this project through spring of 2018 and will be fully expended by April, 2018.

	17-18 available	expended	remaining
Employee salary and benefits	**13928**	**6069**	**7859**
Supplies and Equipment	**5228**	**2565**	**2663**
Total	**19156**	**8634**	**10522**