Investigating Incidence and Type of Wood Decay Fungi in Almond

Project No.: **15-PATH11-Rizzo**

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Objectives:

The overall goal for this research is to better understand the biology and etiology (cause) of wood decay fungi that lead to windfall and tree mortality in almonds.

1. Identify the main fungi associated with heart rot diseases of almond in California
2. Determine the infection process in orchards

Interpretive Summary:

Wood decay fungi reduce the structural integrity of trees, leading to major limb breakage and wind-driven collapses (windfalls). Basidiomycetes associated with wood decay colonize and digest the heartwood and sapwood of trees. This group of fungi produce enzymes that degrade the cellulose and lignin that comprise the heartwood, reducing the structural integrity of the trees. Basidiomycetes that cause root and butt rot have a distinct biology and etiology from those that cause heart rot. Trees are asymptomatic for some time following infection with individual tree yields remaining relatively unaffected. Often the first sign of infection is when the tree succumbs to windfall. Loss of multiple trees over several years leads to orchard decline and eventual removal. Through this process, these fungi are involved in killing thousands of acres of trees per year. Despite the common occurrence and economic importance of wood decay diseases, limited information on their management is available due to an incomplete understanding of their etiology and biology in orchard settings.

With the exception of *Armillaria mellea*, information about wood decay in almonds is largely limited to identification of species associated with decay. This research aims to expand the understanding of the epidemiology and biology of the specific genera that lead to windfall. Previously, *Oxyporus, Ganoderma, Laetiporus, Phellinus,* and *Trametes* were identified as the main fungal genera associated with wood decay in California almond (Adaskaveg and Ogawa, 1990). Our initial surveys identified *Ganoderma* as the main fungal genus responsible for the

decay in windfall trees. Wood decay pathogens are generally considered to be slow growing and affect older trees, yet there are reports of orchards as young as 8 years suffering severe decay (Krueger, 2004). Additionally, during the preliminary survey, a 10-year-old orchard slated for removal was sampled due to severe decay caused by *G. adspersum*, a pathogen not previously identified on almond.

Materials and Methods:

Windfall trees are destructively sampled as they become known. Twenty-three windfall trees were sampled in 6 different orchards in Butte, Solano, Yolo, San Joaquin, Merced and Kings Counties. On two occasions trees were sampled systematically in the orchard before or during orchard removal. In Colusa County, every tenth tree in two rows were sampled after trees were uprooted. In San Joaquin County, every tenth standing tree in 4 rows were sampled. Sampled trees have ranged in age from 10 to 25 years, and number of trees sampled in each orchard varied from 1 to 16. Varieties sampled include ‘Nonpareil’, ‘Monterey’, ‘Butte’, ‘Padre’, ‘Aldrich’, ‘Price’ and ‘Carmel’; on rootstocks of either ‘Nemaguard’ or ‘Lovell’.

Sampling of trees entails visual inspection for fruiting bodies and assignment of a decay severity rating (0-4) with 0 being no visible decay and 4 being complete decay (**Figure1**) each of four cross-sectional cuts made to the scaffold branches, 18 inches above the graft, directly above the graft, directly below the graft, and to the root mass as a whole (**Figure 2**). Presence or absence of crown gall symptoms is also noted.

Figure 1. Decay rating system (0-4) 0-no decay, 4 complete decay.

Figure 2. Locations on tree of decay evaluation. Scaffold, 18-inches above graft, directly above graft, directly below graft, and root ball.

Efficient protocols for the isolation of wood decay fungi from symptomatic tissue are being developed. Samples, approximately 2mm x 2mm, from decayed areas are plated onto Water Agar (WA) amended with benomyl (4µg a.i./ml) and streptomycin sulfate (100 µg/ml) and sub-cultured onto Potato Dextrose Agar (PDA) after incubation at room temperature for 7-14 days. When possible, fungal species are first identified to the genus level based on morphological characteristics of fruiting bodies and growth in culture. DNA is extracted from pure cultures or directly from fungal tissue using Prepman Ultra Sample Preparation Reagent followed by PCR amplification of the ITS region. Isolates are sequenced and identified using BLASTn searches in GenBank.

Two 10-year-old orchard blocks with high incidence of root and butt rot have been designated as study sites to better understand the etiology and biology of root-rot fungi. Each study site is bordered by two similarly sized and managed younger orchard blocks. The almond variety varies between neighboring blocks, but the rootstock does not. The first, on a sandy loam in Kings County, is 20 acres of Monterey and Nonpareil on Nemaguard rootstock planted in 2005 with double-line drip irrigation. This site was previously in a cotton-alfalfa-corn rotation. The second, on a silty clay loam in Solano County, is 18 acres of Butte and Padre on Lovell rootstock planted in 2006 with microsprikler irrigation. This site was previously in a tomato rotation.

In addition to destructive sampling of windfall trees within each study site and its neighbors, intensive mapping of disease incidence and mortality over time, using historical photographs and orchard surveys, is ongoing. Development of non-destructive sampling protocols is in the early stages.

Results and Discussion:

Destructive sampling revealed that incidence and severity of decay within windfall trees was greatest in the roots and below the graft union and decreased with distance from the roots (**Table 1**). The pattern of decay and discoloration in sampled trees suggest the infection is progressing up from the butt rather than down from the trunk (**Table 1**). Owing to the high incidence and severity of decay in the area below the graft, this will be the target for non-destructive sampling in the next phase of the project.

Table 1. Incidence and severity of decay in windfall and standing trees

	Windfall trees		Standing trees[1]	
	Incidence[2]	Severity[3]	Incidence	Severity
Scaffold	0.05	0.05	0.11	0.14
18" above graft	0.57	0.76	0.39	0.69
1" above graft	0.86	1.67	0.56	1.08
2" below graft	0.90	2.48	0.61	1.53
roots	1.00	3.62	0.64	1.78

[1] A portion of these trees were not standing, but had already been removed.
[2] Incidence refers to the proportion of trees that contained decay at this evaluation point.
[3] Decay rating system (0-4) 0-no decay,
[4] Complete decay.

Although the sample size was small there were no discernable differences in incidence or severity of decay between different varieties. It is worth noting that all of the windfall trees were on a peach rootstock and none on plum or hybrid rootstock.

Table 2. Summary of isolated wood decay fungi

Fungus	**Incidence[1]**	**Tree status[2]**	**Fungi Type[3]**	**Distribution[4]**
Coprinellus micaceus	.02	W	N	1
Ganoderma sp.	.30	W, S	P	2
Ganoderma adspersum	.18	W	P	1
Oxyporus laetimarginates	.02	W	P	1
Peniophorella pubera	.02	W	N	1
Phlebia sp.	.04	W, S	P	2
Psathyrella sp.	.04	W	N	1
Punctularia sp.	.02	W	N	1
Stereum hirsitum	.02	W	N, P	1
Trametes versicolor	.02	S	N, P	1

[1] Incidence refers to the proportion of total sampled trees from which that specific fungus was isolated.
[2] W = windfall, S = standing.
[3] N = nonpathogenic, P= pathogenic.
[4] The total number of orchards from which specific fungi was isolated.

The fungal genera *Ganoderma* was isolated from 82% of all windfall trees sampled and 25% of the standing trees in one orchard. *Oxyporus laetimarginates* and *Phlebia sp*. also caused severe root and butt rot, but were not widespread within the orchards in which they were found (**Figure 4**). The tree from *Oxyporus laetimarginates* was isolated failed about 12" above the soil level at an old shaker wound, but decay was evident and isolations were successful from the crotch down to 18" below the soil line. All other isolates are not known to pathogenic on the roots and butt and are acting as saprophytes coming in on dead or previously decayed wood.

An important finding from this early survey was the high incidence of decay in young orchards. Severe decay has been described before in younger orchards (Krueger, 2004). *Ganoderma* sp*.* was isolated from 100% of the windfall trees in the 10-year-old orchard in Solano County (**Figure 4**). This site was missing approximately 4% of the trees in 2016. Many *Ganoderma* species are not aggressive and require some form of stress in order to effectively colonize (Schwarze and Ferner, 2003). While *Ganoderma* was responsible for the decay that led to the windfall, this orchard is severely stressed due to J-rooting and extensive crown gall. Not only does the stress predispose a tree to colonization, but the exposed roots, girdling and galls can all serve as effective infection sites.

The other 10-year-old orchard in Kings County is experiencing exponential tree loss and is scheduled to be removed at the end of 2016 with more than 13% of the trees failing over a 2-year period (**Figure 3**). *Ganoderma adspersum* was isolated from 67% of the windfall trees at this site, however decay characteristics from all windfall trees and old stumps removed in previous years were consistent with *G. adspersum*. *G. adspersum* was shown to be highly aggressive in comparison to other *Ganoderma* species and is able to overcome the reaction zone of the tree that generally confines wood decay fungi to the heartwood (Schwarze and Ferner, 2003). Initial surveys of the adjacent 8-year-old and 7-year-old blocks suggest that they may be experiencing the early stages of this exponential tree loss, and will be more extensively studied.

These early findings are guiding the next steps in understanding and addressing this problem, including investigating dissemination pathways, determining the survival time of infectious debris in the soil, and developing non-destructive diagnostic protocols.

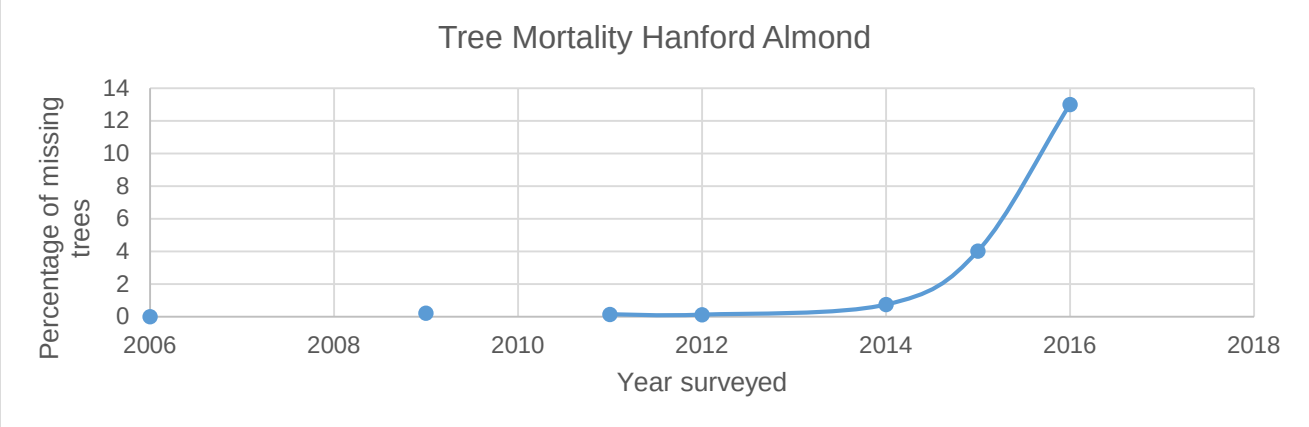

Figure 3. Tree mortality caused by *G. adspersum* at a site in Kings County, planted in 2006.

Figure 4. Clockwise from top left: Butt rot caused by *Ganoderma* sp., root and trunk rot caused by *Oxyporus laetimarginates*, butt rot caused by *Phlebia* sp., decay and discoloration caused by *G. adspersum*.

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