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

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OBJECTIVES

1. Identify the main fungi associated with heart-rot diseases of dried plum in California

2. Determine the infection process in orchards.

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

INTRODUCTION

Wood decay fungi reduce the structural integrity of trees, leading to wind-driven collapses and scaffold limb breakage, causing tree loss and lost 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. Heartwood, being composed of dead xylem cells, serves as an area of "storage" for plant byproducts and provides structural support for the tree; sapwood conducts water and tends to be more resistant to decay. This group of fungi is able to colonize the heartwood; cellulose and lignin are degraded leading to a reduction of the structural integrity of the trees. Limb breakage as a result of decay can have significant impacts on yield, and loss of multiple trees over several years leads to orchard decline and eventual removal.

The last survey of heart-rot diseases in California nut and fruit crops was conducted in 1988 by Adaskaveg and Ogawa, who determined species incidences and suggested possible entry points for infection. This survey used orchards aged approximately 15 years and older, and identification of heart-rot associated fungi was done using mainly fruiting bodies and culture methods. Dominant fungal genera identified on *Prunus* spp. were *Oxyporus, Ganoderma, Laetiporus, Trametes, Fomitopsis, Armillaria, Phellinus,* and *Perenniporia*. While this survey provided information on the identity of the major fungal species associated with heart-rot, it had limited impact on disease control.

Adaskaveg and Ogawa found *Fomitopsis* and *Phellinus* were the most prevalent fungal genera responsible for wood decay in California plum and prune. Both *Fomitopsis* and *Phellinus* produce a conk like fruiting body on the trunk of the tree. When these fruiting structures appear, the wood decay fungi are already well established in the interior of the tree and significant loss of structural tissues has already occurred. Trees often appear healthy and remain productive until they fall over during wind as a result of loss of structural integrity. 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. Currently there are no effective management strategies against heart-rot diseases of prune, UC Integrated Pest Management guidelines simply states to avoid mechanical damage and maintain tree vigor. Infection most likely takes place in the early stages of tree development, many years before the appearance of visible symptoms and damage in orchards.

For many decay fungi, there is not a clear understanding of the early stages of infection. This includes the source of inoculum, timing of infection, possible latent period, and specific virulence. However, this information is needed to develop and implement effective management strategies against heart-rot diseases.

PROCEDURES

Preliminary disease surveys and sampling began in September 2015. Three prune orchards in Yolo County with substantial limb breakage were surveyed and three or four samples per tree from approximately four trees per orchard were collected. Using a chain saw, 1.5 inch cross sectional wafers were cut from scaffold limbs or in the case of uprooted trees, from decayed areas on the trunk and root system.

Orchard wide, whole tree disease evaluations are being carried out during the orchard removal process after trees are uprooted, but still in windrows. In an almond orchard in Colusa County we evaluated every tenth tree in every fifth row and assigned a disease severity rating from 0 (no evidence) to 4 (severe rot) to the root mass and at each of 4 cross sectional cuts: directly below the graft union, directly above the graft union, 24 inches above the graft, and in a scaffold branch. Evidence of crown gall was noted and representative samples of decay and any fungal fruiting bodies were collected.

To isolate fungi from samples, five or six small shavings from decayed and decaying tissue in each sample were inserted in two different culture media, potato dextrose agar and water agar both amended with the antibiotics benomyl and streptomycin. Wood decay fungi are slow growing and are often out competed in culture media, so the less nutrient dense water agar media resulted in a higher proportion of uncontaminated plates and has been adopted for future isolations. After 2 to 6 weeks fungi were sub-cultured onto potato dextrose agar. We extracted DNA from pure cultures using Prepman Ultra DNA extraction kit and used PCR with Basidiomycete specific primers to amplify the internal transcribed spacer (ITS) regions. ITS positive samples were sequenced and BLAST was used for identification.

RESULTS AND DISCUSSION

While whole orchard disease assessments have yet to be carried out in prune orchards, previous observation and sectioning of several individual prune trees indicates presence of wood decay in prunes is found throughout the entire tree, including: roots, trunk and scaffold and was often

associated with pruning wounds. This is in contrast to preliminary results from whole orchard disease assessment in almond, where decay tended to be more severe in the roots and butt of the tree and very limited in the trunk and scaffold branches (figure 1).

Although isolations and identification have thus far been limited; fruiting bodies from the wood decay fungi, *Phellinus sp*., were common place throughout all three orchards and were collected from more than half of the sampled trees. *Phellinus sp*. was also isolated from decaying tissue from several samples. This finding is consistent with earlier studies of wood decay fungi in prunes. *Umbelopsis sp*., and an unknown Ascomycete were also identified, but are most likely secondary pathogens and not the primary cause of the wood decay.

This project is in the preliminary phases and to this point substantial time has been devoted to development of appropriate protocols for isolation and identification of wood decay fungi. In 2016 orchard surveys will continue and more cooperating growers will be identified and we will employ an additional protocol to extract fungal DNA directly from infected tissue for identification. Young prune trees will be inoculated with fungi identified in preliminary surveys. Trees will be observed for outward signs of the disease over the course of several years and destructively sampled to determine virulence and rate of spread with in the tree.

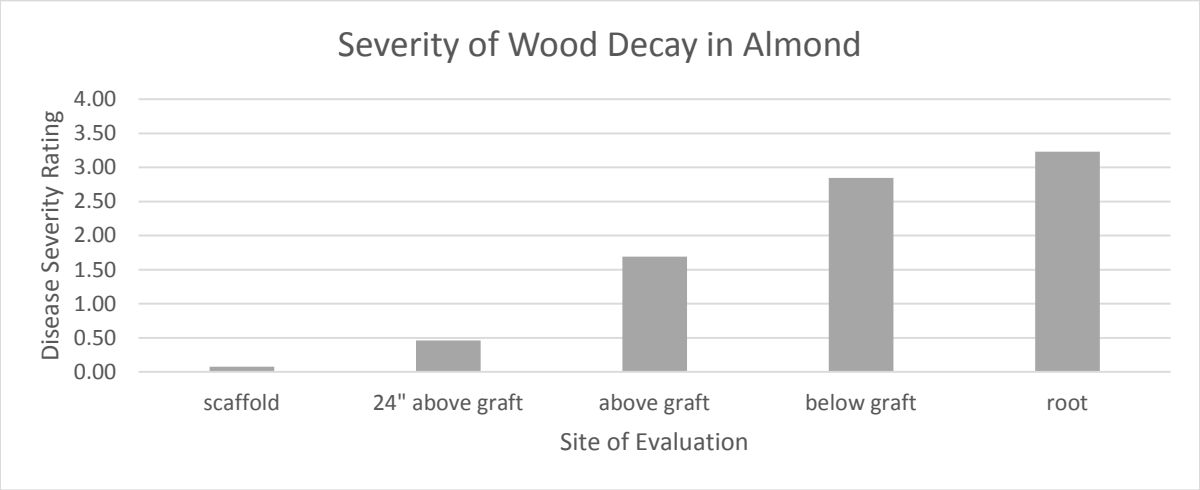

Figure 1. Severity wood decay fungi in almond trees at time of orchard removal. Disease severity rating from 0 (no evidence) to 4 (severe rot). N=23.