Etiology and Management of Trunk and Scaffold Canker Diseases of Almond in California

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Introduction

Trunk and scaffold canker diseases (TSCD) of almond can cause significant yield and tree losses within orchards, while also reducing orchard life spans. TSCD often go unnoticed during the early stages of infection and symptoms appear more visible as trees age. Common symptoms of TSCD include discoloration of vascular tissues, wood necrosis and extensive gumming. Dieback of scaffold branches can occur and eventually the whole tree may die. In California, several pathogens are known to cause cankers in trunks and scaffold branches of almond. Among them *Ceratocystis fimbriata* has been widely studied as the causal agent of Ceratocystis canker. While this disease is generally associated with shaker damage and bark injuries at harvest, *C. fimbriata* is also capable of infecting branches from fresh pruning wound made during late fall and winter (Teviotdale and Harper, 1996). In recent years, several botryosphaeriaceous fungi have been associated with band canker, an unusual, non-chronic canker disease affecting the trunk of rather young trees. The same pathogens can also produce perennial cankers that can sometimes kill trees (Inderbitzin et al., 2010). *Eutypa lata* the causal agent of Eutypa dieback of stone fruit has also been recovered sporadically in California from cankers in trunk and scaffold branches, but the extent and significance of this disease in almond orchards is currently unknown. Additional pathogens causing perennial cankers in trunks and scaffolds have included *Phytophthora* spp., a group of soilborne pathogens of almond. Additional report of TSCD in almond outside of the United States have included Leucostoma (Cytospora) canker caused by *Leucostoma persoonii* as well as Collophora canker caused by *Collophora hispanica* (Arzanlou and Dokhanchi, 2013; Gramaje et al., 2012; Arzanlou et al., 2016).

Field diagnosis of TSCD is done commonly by pest control advisers and farm advisers based on symptoms observations. Previous reports have indeed suggested distinctive symptoms among some of the TSC diseases. These include differences in canker morphology as well as in the outer distribution of resin exudate or gumballs. Nevertheless, the main causes of TSCD and almond tree death in California is still uncertain and field diagnosis of TSCD remains challenging as symptoms delineation among these diseases is not clear. The relative importance of these different infectious diseases and their respective distribution in California are unknown. Management strategies against TSCD rely for the most part on remedial surgery, cultural and prophylactic practices including removal of trees. Accurate disease diagnosis is therefore essential to the implementation of appropriate control methods against TSCD. The aim of the proposed

research is to improve diagnosis and management strategies of TSCD by gaining new knowledge on the etiology, biology and symptomology of TSCD diseases in California.

Material and methods

Survey and fungal isolation. During 2015 and 2016, samples were obtained by visiting symptomatic almond orchards or by submission to the laboratory for diagnostic services. Symptoms included dieback, gummosis, girdling, resinosis and vascular discoloration on almond branches, scaffolds or trunks. Symptom differences were annotated for the different causal agents. Trunks and scaffolds displaying symptoms were sampled by removing bark and branches to reveal the inner wood revealing the canker. In the laboratory, wood samples were surface disinfected in of 2% sodium hyphochlorite solution for 2-3 minutes and rinsed twice with sterile distilled water. Samples were processed using several methods to assay for a wide range of potential causal agents. This included plating necrotic wood pieces on acidified potato dextrose agar (APDA) for the isolation of true fungi, on PARP medium for the isolation of *Phytophthora* spp. and the use of humid crispers for the isolation of *Ceratocystis fimbriata.* Pure cultures were obtained by transferring colonies onto APDA or PARP medium. In the case of *Ceratocystis fimbriata*, ascospore masses produced on perithecia growing from incubated wood pieces were transferred onto APDA.

Molecular identification. Fungal mycelium in pure cultures on PDA was used for DNA extraction with the FastDNA™ SPIN KIT (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer instructions. Amplification and sequencing were first performed with the internal transcribed spacer region (ITS) of the rDNA to determine our isolates to species. In addition to the ITS region, additional loci were sequenced to enhance phylogenetic resolution and included: translation elongation factor (TEF1-α), beta-tubulin (BT), and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). All loci were amplified using PCR conditions previously described and DNA was sequenced using Sanger sequencing technologies. Sequence identities were confirmed using GenBank BLASTn searches.

Pathogenicity trials. To determine pathogenicity, a trial on almond was set up in August 2016 to test the aggressiveness of 21 isolates representing the various TSCD associated fungi isolated during the survey. One-year-old potted 'Nonpareil' almond saplings were inoculated at the Kearney Agricultural Research and Extension (KARE) Center and maintained in a lath house. The central portion of the stem was inoculated by placing a 5-mm-diameter mycelium plug from a 7- to 10-day-old PDA culture in a wound made by a 5-mm-diameter cork borer. Wounds were sealed with petroleum jelly to maintain moisture during the incubation period and protected with Parafilm. Fungal treatments were compared to a control treatment inoculated with non-inoculated agar plugs. The experiment was set up in a randomized complete block design with seven replicates. Trees were examined after 6-weeks post-inoculation to assess length of vascular discoloration (lesions) and percent fungal recovery from disease tissues.

Results and discussion

During 2015 and 2016, we visited and sampled approximately 70 almond orchards throughout the Central Valley, spanning 15 counties with symptoms of almond TSCD. Approximately 300 isolates were isolated from cankers and were characterized using morphological and molecular methods. To date, Botryosphaeriaceae cankers and Ceratocystis

canker were the most commonly encountered canker diseases of almond. Other prevalent canker diseases found in almond included Phytophthora canker, Eutypa canker, and Cytospora canker. Cankers caused by *Diaporthe* (*Phomopsis*) spp. and *Collophora* spp. were also identified in this survey, but did not appear to be as widespread. Ceratocystis cankers were found in all the counties surveyed and in both young and mature trees. Ceratocystis canker of almond has not been investigated since the emergence of modern techniques in molecular biology and the genetic diversity and population structure of *C. fimbriatra* is currently being reexamined. Questions such as the origin of the inoculum of Ceratocystis canker and the role played by mechanized harvesting equipment in spreading the pathogen remain elusive. Botryosphaeriaceae cankers were associated with pruning wounds and prevalent in 3-4th leaf orchards in the Sacramento Valley. *Neoscytalidium dimidiatum*, also in the Botryosphaeriaceae, was associated with pruning wounds and tree crotch infections in 3-4th leaf almond orchards in Madera, Merced, Fresno and Kern Counties. This fungus was recently reported in California on walnut and is found on table grape, citrus and figs in the state. It has emerged within the last few years as the state has experienced a severe drought, suggesting that drought conditions and the rising of temperatures may be conducive to the pathogens fitness in tree crops. Cytospora cankers were also detected near pruning wounds and found in 3-4th leaf orchards and older in Merced, Fresno and Stanislaus counties. To date we have recognized at least six different species in almond. Little work has been conducted on Cytospora cankers in almond but studies in sweet cherry in California have shown that *Cytospora* species constitute some of the most aggressive canker pathogens in this host (Trouillas et al. 2012). *Eutypa lata* was associated with pruning wounds and infections at the tree crotch in 3-4th leaf orchards. Infections at the crotch of the tree may be indicative of early infections during scaffold selection in an orchard. *Collophora hispanica* and *C. paarla* were associated with reddish-colored, circular branch cankers. Additionally, *Phytophthora cinnamomi* was isolated from second leaf almond trees in an orchard in Kern Co.

The pathogenicity test revealed that *Neofusicoccum arbuti* and *Neoscytalidium dimidiatum* were the most aggressive on almond. *Neofusicoccum arbuti* killed 20-60% of inoculated saplings. From the broad diversity of Botryosphaeriaceae species tested in this trial, we observed significant variation in their capacities to cause lesions in almond. *Cytospora* sp. K226 was aggressive causing large lesions and gumming at the point of inoculation. The other *Cytospora* species varied greatly in virulence. Other species (*C. fimbriata*, *E. lata*, *Phomopsis/Diaporthe* spp., and *Collophora* spp.) proved to be pathogenic on almond causing vascular discoloration, but not as aggressive compared to cankers caused by Botryosphaeriaceae species. The results of the pathogenicity test suggest that Botryosphaeriaceae cankers are the most devastating, especially on young trees.

Management of canker diseases rely for the most part on prevention as no chemical treatments can cure these diseases. Overall, canker diseases may be managed by avoiding bark injuries from mechanical shakers and avoiding pruning trees before or during rain events. Management of canker diseases may rely on remedial surgery (removal of the cankered area), however, cankers are generally difficult to remove and may require multiple surgeries over several years before all the infected tissue is removed. Removal of infected tree parts and dead trees will reduce inoculum in the orchard. When whole, diseased trees are removed, the stumps should also be removed as the bark of stumps can be covered with the pathogen fruiting structures and thus serve as sources of inoculum. Adjusting sprinkler irrigation so that tree trunks and tree crotches are not wetted helps reduce the incidence of canker diseases. Proper primary scaffold selection and

avoiding stress are the most effective preventive measure to reduce risks of infection by canker pathogens.

Future work will target management of almond canker diseases using pruning wound protectants as TSCD pathogens are primarily associated with pruning wounds, which serve as the main infection court. Pruning wound protection should prevent fungal entry and infection. Several pruning wound protection products to be tested include chemical fungicides, biological control agents (*Trichoderma* species) and wound sealants (paint and wax). Protection of pruning wounds combined with prophylactic measures including cultural practices should help mitigate the impact of canker diseases of almond in California.

Literature Cited

Arzanlou, M. and Dokhanchi, H. (2013). Calosphaeria canker of almond caused by *Calosphaeria pulchella* in Iran. Arch Phytopathol Plant Protect *46 (2)*, 215–226.

Arzanlou, M., Ghasemi, S. and Baradaran Bagheri, M. (2016). *Collophora hispanica*, a new pathogen and potential threat to the almond industry in Iran. J Phytopathol *164 (10)*, 833-839.

Gramaje, D., Agustí-Brisach, C., Pérez-Sierra, A., Moralejo, E., Olmo, D., Mostert, L., Damm, U., Armengol, J. (2012). Fungal trunk pathogens associated with decay symptoms on almond trees in Mallorca (Spain). Persoonia *28*, 1–13. http://dx.doi.org/10.3767/003158512X626155.

Inderbitzin, P., Bostock, R.M., Trouillas, F.P., Michailides, T.J. (2010). A six-locus phylogeny reveals high levels of species diversity in Botryosphaeriaceae from California almond. Mycologia *102 (6)*, 1350–68.

Teviotdale, B.L. and Harper, D.M. (1996). Almond pruning wounds, bark abrasions susceptible to Ceratocystis. *Calif Agr 50 (3)*, 29–33.

Trouillas, F.P., Peduto, F., Lorber, J.D., Sosnowski, M.R., Grant, J., Coates, W.W., Anderson, K.K., Caprile, J. and Gubler, W.D. (2012). Calosphaeria canker of sweet cherry caused by *Calosphaeria pulchella* in California and Australia. Plant Dis *96 (5)*, 648–658.