Integrated management of Fusarium canker in bare root and container-propagated stone fruit trees

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Project Summary/Abstract

Briefly describe the long-term objectives for achieving the stated goals of the project.

The overall objective of the research is to provide evidence-based guidelines for integrated disease management of the Fusarium canker problem in nursery trees. The specific objectives of this research are to 1.Complete evaluation of other cover crops as alternatives to wheat for their potential to reduce or eliminate soil
borne Fusarium; 2. Complete surveys of the incidence of pathogenic Fusarium species in almond budwood, bare root and container trees at several nurseries and in newly planted and mature orchards; 3. Complete experiments to refine the relationship between relative water content of bark and stem water potential (SWP), and how this relates to host susceptibility to Fusarium spp.; 4. Assess selected fungicides for their penetrate in almond bark; and 5. Prepare manuscripts for publication, and provide disease description and management guidelines for UC website.

Scope of Work

Describe the goals and specific objectives of the proposed project and summarize the expected outcomes. If applicable, describe the overall strategy, methodology, and analyses to be used. Include how the data will be collected, analyzed, and interpreted as well as any resource sharing plans as appropriate. Discuss potential problems, alternative strategies, and benchmarks for success anticipated to achieve the goals and objectives.

IAB Research Priority: Research on other important diseases and pests that significantly affect the quality of nursery stock.

Executive Summary.

California fruit and nut tree nurseries have experienced sporadic losses to a cankere disease caused by opportunistic fungi that attack young trees weakened by stress. The disease occurs in dormant bare root trees maintained in cold storage in refrigerated warehouses, with disease signs and symptoms developing during storage or soon after planting. Affected trees display molds growing on the bark and roots, and necroses of the inner bark, cambium and sapwood, which can girdle and kill the trees. Weak establishment in new plantings also may be associated with the presence of these pathogenic fungi on the roots. Infected but non-symptomatic dormant trees also can develop symptoms later and collapse in the field. With some provided by the IAB, we identified *Fusarium acuminatum*, *Fusarium avenaceum* and *Fusarium brachygibbosum* as the most prevalent and aggressive pathogens associated with the canker disease. Loss of bark turgidity in almond stem segments due to desiccation stress, within a certain range, corresponds with significantly increased susceptibility. In addition, pathogenic Fusarium spp. can be isolated from every aspect of the production system including symptomatic and non-symptomatic almond trees, budwood, wheat rotation cover crops and residues in nursery fields, cold storage facility air and surfaces, and nursery equipment. In two nurseries, genetic testing confirmed that isolates from the various sources were highly genetically similar, suggesting these materials are potential inoculum sources. The overall objective of the research is to provide evidence-based guidelines for integrated disease management. We teamed with Dr. Ken Shackel to determine more precisely the water status of bare root trees during processing and storage and the relationship of this to tree establishment, performance and disease.

Project's Benefit to the Nursery Industry. Healthy, pathogen-free propagation material is crucial fore the success of California fruit and nut tree nurseries and the industries they support. Effective disease management is contingent on understanding the range of causal agents, the mechanisms for entry and spread of the pathogens in healthy trees, and any cultural practices that may compromise trees in storage or during propagation. Fungal pathogens in healthy-appearing trees present a particular challenge because our understanding of what triggers the transition from a cryptic, endophytic phase (i.e., a latent infection) to an aggressive, parasitic phase is limited. However, we know that moderate desiccation of bark is one trigger for disease and symptom development. Weak establishment failure to establish is a recurring problem in the nursery industry, particularly for bare root propagated material. In many cases, weak establishment associated with the presence of pathogenic fungi on the roots, but it is difficult to separate direct pathogen effects from the physiological effects of water stress, which influence tree vigor, and consequently root and stem susceptibility.

From our previous work, we have shown that bud wood contamination occurs and that *Fusarium* species reside endophytically in trees without apparent effect one the plant until a stress event or, possibly, other unknown factor(s) triggers disease expression. However, information about the frequency of contamination in nursery stock is incomplete. With nurseries shifting towards more containerized propagation, we believe that the latter is an important question to address. We have sampled at various nurseries and locations to better assess the prevalence of *Fusarium* contamination, and this work is ongoing. While bare root production of trees continues on a large scale, it will be importance to raise awareness and mitigate potential sources of inoculum and environmental factors, to the extent possible, to reduce risk of disease. Almond *(Prunus dulcis)* is our focus because we have extensive experience in this species with this disease; however, the information is relevant for other Prunus and Malus species.

To conclude this project, we propose the following objectives that we put forward in our previous award, modified as follows:

1. Complete evaluation of other cover crops as alternatives to wheat for their potential to reduce or eliminate soilborne Fusarium.
2. Complete surveys of the incidence of pathogenic Fusarium species in almond budwood, bare root and container trees at several nurseries and in newly planted and mature orchards.
3. Complete experiments to refine the relationship between relative water content of bark and stem water potential (SWP), and how this relates to host susceptibility to Fusarium spp. (in conjunction with Dr. Ken Shackel).
4. Assess selected fungicides for their penetrate in almond bark.
5. Prepare manuscripts for publication, and provide disease description and management guidelines for UC Davis website.

Work Plans and Methods:

Objective 1. Complete evaluation of other cover crops as alternatives to wheat for their potential to reduce or eliminate soilborne *Fusarium.*

1.a. Task: We are comparing mustard species with wheat to determine their influence on levels of soilbome *Fusarium.*

1.b. Activities and methods description. After consulting with cover crop specialists and members of the Sierra Gold Nursery team, we selected two potential cover rotation crops - *Sinapsis alba* (white mustard) and *Brassica juncea* (brown mustard). These were chosen with consideration of how they might fit into a nursery management regime and their known benefits as cover crops in other cropping situations. We are conducting greenhouse scale experiments to determine the effect of cover crop species on soilbome propagules of *Fusarium acuminatum.* Prior research indicates that wheat is a conducive host for *Fusarium.* We are interested to know whether soilbome populations of the pathogen are supp01ied or decline in the presence of mustards in comparison with wheat and a soil-only control. If we see benefit with the mustards, then we will recommend these for small replicated plot experiments in nursery production fields having a history of soilbome pathogenic *Fusarium* species. We conduct pre- and post-plant soil sampling using an appropriate design, and estimate fungal inoculum density on the basis of colony forming units per gram of soil using a selective medium e.g., Komada's medium (5). After solving initial logistical challenges, we conducted the experiment once, and will repeat it several more times.

1.c. Task products/deliverables and estimated completion dates. These experiments are underway. If successful, we anticipate that we will identify an alternative rotation crop that either reduces or does not support the build-up of soilborne *Fusarium* inoculum. We anticipate completing this objective by fall 2018.

Objective 2. Complete surveys of the incidence of pathogenic Fusarium species in almond budwood, bare root and container trees at several nurseries and in newly planted and mature orchards.

2.a. Task: Budwood from infected but non-symptomatic trees is a potential primary source of the inoculum and point of entry for the causal agents. We have sampled at a number of California nurseries, and will continue this effort during 2018 to increase our sample size to obtain more accurateee incidence data. We will work closely and confidentially with selected commercial nurseries to assessee the incidence of symptomatic and non-symptomatic infections by F acuminatum, F avenaceum, andee other pathogenic Fusarium species. Using selective media and other methods (see below), we willee characterize the extent and frequency of contamination in almond budwood, and young and mature trees.

2.b. Activities and methods description.
Isolations from host tissue: In previous research, we collected samples at three major nurseries from healthy-appearing budwood and stems and roots of bare root trees with signs and symptoms of canker disease, and sampled trees and other sources as describe above. From these samples, we isolated one or more of the following Fusarium species: F acuminatum, F avenaceum and three additionalee Fusarium species, two of which (Fusarium brachygibbosum and a new Fusarium sp.) proved to be pathogenic on almond in our branch assays.

The general strategy is the following. Fungal isolations are made from sections of bark and cambium (5-10 cm2) excised from both diseased and healthy-looking tissues. Tissue pieces are surface-sterilized (15 min in 1 % NaOCl + 10% ethanol followed by two washes in sterile deionized water) and placed on selective (Nash-Snyder & PDA + 100 ppm tetracycline) and non-selective (water agat·) media (Nelson et al. 1983). Fungal isolates are identified and/or described, grown and stored at -80°C in 15% glycerol.

To isolate from non-symptomatic budwood and other samples, freezing surface-sterilized stem and root segments for 48 hr at -20°C (ONFIT) is a convenient method to assay for the presence of latent infections. Using this technique, the endophytic microflora of the samples is determined and evaluated for the presence of the *Fusarium* species of concern. Budwood cuttings obtained at commercial nurseries were found to be free of infection or to have a very low incidence.

Confirmation ofspecies identity: The fungal isolates are typed to species by characteristics of their growth in culture andespore morphology and by PCR and partial sequencing of their internal transcribed spacer *(ITS)* elongation factor 1-a *(Efl-a.)* and RNA polymerase II large subunit *(RPBJ)* genes.

2.c. Task products/deliverables and estimated completion dates. Contamination by endophytic, oree cryptic, pathogens could be minimized by providing clean plant source material through screening andee indexing. If *Fusarium* "endophyte-free" tissue can be provided through screening, we could helpee assess the costs andbenefits and the technical and economic feasibility for incorporation into nursery operations. We will continue our survey and assessment of nursery material at several nurseries and complete these studies by fall 2018.

Objective 3. Complete experiments to refine the relationship between relative water content of bark and stem water potential (SWP), and how this relates to host susceptibility to Fusarium spp. (in conjunction with Dr. Ken Shackel).

3.a. Task: Continue laboratory assays of susceptibility on stems from plants indicative of the range in SWP found in the analyses we have conducted with Dr. Shackel. An important goal here is to refine the relationship between SWP as determined with a pressure bomb and bark relative water content (RWC), an older, traditional method that was used in previous studies of tree canker disease.

3**.b. Activities and methods description:** Our results this past year indicate that the relationship between bark RWC and SWP is more complex than initially thought, and that there is a degree of independence between the measures, which varies seasonally. Thus, the relationship of tree moisture status to disease susceptibility needs to take into account how moisture status is determined. The disease assays used in this task will be those published (6) and modified by Seidel et al. (8) and Kuffel et al. (this project). Virulent isolates of two Fusarium species will be used in these disease assays. Fusarium acuminatum, which we have found to be the most common species detected in the almond production system, and Fusarium avenaceum, which is highly virulent. Stem segments (~ l O cm) of dormant trees are surface-sterilized and two needle stabs made at about 4 cm apart One stab wound is inoculated with a filter paper piece (~4 mm2) colonized by the test fungus, which is affixed onto the stem with Buddy-Tape (Riken USA Corp., NJ). The other stab wound is also covered with Buddy-Tape and used as a wound control. Stem segments inoculated with isolates of the pathogenic fungus Monilinia fructicola are included with each assay as a positive control. Inoculated stem segments are then placed in sterile test tubes fitted in foam plugs and incubated at l 5°C at ambient relative humidity (25-35%) for 14 days. After 14 days, the bark from around inoculation points is removed and lesion length and width measured. These experiments are underway and will be completed during 2018.

3.c. Task products/deliverables and estimated completion dates. The key product from this task will be a statistical test (either correlation analysis or an analysis of covariance) to determine whether the susceptibility of any given cultivar is a function of the level of SWP that may be expected in commercial practice. The incubation time for this assay is 14 days, and experiments will be completed in 2018.

Objective 4. Assess selected fungicides for their penetrance in almond bark.

4.a. Task: Following our initial studies from 1998-2002, some nurseries have adopted a fungicide program where trees are sprayed at or near the time of lifting and prior to cold storage. This practice may have contributed to the anecdotal decline in incidence of canker in the past decade. However, to our knowledge there are no controlled studies to assess the efficacy of this practice. We found several fungicides currently registered for use on almond to be particularly effective *in vitro* and *in vivo* against the *Fusarium* pathogens of concern, at least under small-scale, highly controlled laboratory conditions. These are fludioxonil (Scholar), fluopyram/trifloxystrobin (Luna Sensation), and fluxapyroxad/ pyraclostrobin (Merivon).ee

4.b. Activities and methods description. In highly controlled laboratory experiments with stem sections that were artificially-inoculated, we previously found that fungicides can protect stem pieces, and may have limited curative action. However, under real-world production practices, fungicide may not provide sufficient protection. A nursery trial this past year compared three bare root almond scion/rootstock varieties that had been sprayed with a fungicide cocktail or left unsprayed prior to cold storage. After several weeks in storage, stem sections from these trees were collected and subjected to the ONFIT. We found that pathogenic Fusarium species emerged in both treatments with approximately equal frequency. This could indicate that the trees were already infected in the field and prior to treatment and storage, and/or that the fungicides may not have penetrated sufficiently into the bark to prevent or restrict infections. To assess penetrance of the fungicides into almond bark, stems of young trees will be treated and then sampled over a time course. The bark will be carefully dissected to separate the outer bark from the underlying tissue (cork cambium and wood). The tissues will be biodisected to assess if sufficient residues are present to inhibit fungal growth in culture. We will also explore the feasibility of conducting chemical analyses to test for residues.

4.c. Task products/deliverables and estimated completion dates. Laboratory screening of fungicides against Fusarium canker pathogens has been completed. The penetrate assays will at least indicate whether sufficient fungicide enters the bark to inhibit Fusarium. These experiments will provide the industry with empirical information to base decisions on whether fungicides merit further investigation for efficacy and optimization in mold and canker disease management.

Objective 5. Prepare manuscripts for publication, and provide disease description and management guidelines for UC Davis website.

5.a. Task: Prepare manuscripts for publication in peer-reviewed journals. Prepare write-up for the UC IPM website.
5.b. Activities and methods description: Analyze data and prepare technical publications.
5.c. Task products/deliverables and estimated completion dates. Two papers from the master degree thesis of Abigail Stack are in preparation, and we anticipate one or two papers from Mr. Kuffel's research. These will be written and submitted during 2018, along with a disease description and management guidelines for the UC Davis site.

Personnel and budget justification: The proposed budget will provide two-months of salary and benefits for Randy Kuffel, who has worked on this project for two years and will be leaving for graduate school at the end of August, 2018. We are also seeking support for an undergraduate student assistant (~100 hours) to assist Mr. Kuffel and to help complete any unfinished experiments after he departs. Co-PI Bostock is requesting a small portion of salary support. Additional monies for supplies and publication costs are also requested. Supplies include standard labware (petri dishes, plasticware, etc.), reagents and growth media, and DNA sequencing, and for any facilities charges.

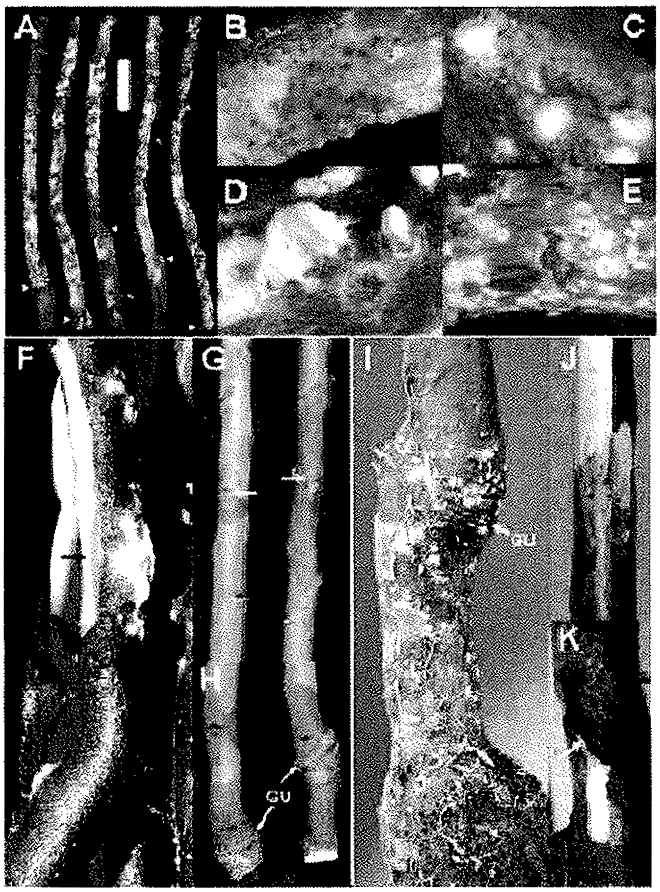

Figure 1. Symptoms and signs of cold storage canker on almond and apple trees. Almond trees are from two nurseries (A-H) and apple trees are from a third nursery (I-K). Most symptoms and signs were localized around the graft union ("GU", arrowheads A, arrows in H & I). However, some lesions appeared to start around the nodes of the scion (arrows, G). Punctate sporulation appeared to emerge through the lenticels over colonized necrotic tissues (F & I). In advanced stages, sporulation coalesced into beige, brown and/or maroon sporodochia-like structures (B-F). Necrosis of the inner bark, cambium and sapwood primarily occurred on the scion (arrows, F-H, J), but occasionally spread to the rootstock (arrow, K). (*from Marek et al.,2013. Plant Dis. 97:259-270*)

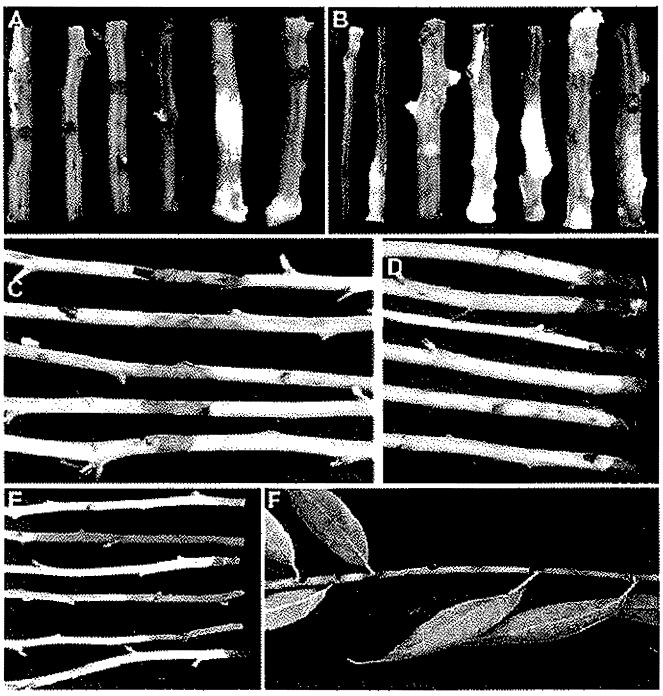

Figure 2. Activation of endophytic non-symptomatic infections and potential sources of primary inocula. A, Non-symptomatic infections harbored by surface-sterilized, healthy-appearing almond (cv. Padre) stem segments were activated after 32 days desiccation at ambient humidity at 15ºC, and B, after an overnight freeze at -20ºC followed by 32 days at 100% RH and 22°C (room temperature) to promote sporulation. *F. acuminatum* and *F. avenaceum* were frequently recovered from activated infections of stem segments. Non-symptomatic infections of almond scion budwood (cv. Sonora, C and D, cv. Padre, E) were activated after cold storage (4-10ºC) for 30 days. Fungi recovered from budwood infections included *Botrytis cinerea*, *Cylindrocarpon* sp., and *F. acuminatum*. F, Peach "Nemaguard" rootstock seedlings developed nodal lesions with raised margins after 60 days in the greenhouse; however only saprobic fungi were isolated (*from Marek et al.,2013. Plant Dis. 97:259-270*)