*Research Project Progress Report*

# Understanding 'foamy bark rot' of Fukumoto navel

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Editor's Note: The research reported on in this article is from one of several studies under the research project titled "Investigating important diseases of citrus in California."

'Fukumoto' navel orange budwood was introduced into California from Japan by Glenn Dale of the United States Department of Agriculture (USDA) in 1983 (www.citrusvariety.ucr.edu). Fukumoto has many desirable attributes. It matures early (late October), typically three to four weeks earlier than Washington navel, it has deep reddish rind color that makes it highly desirable for packinghouse processing. However, "foamy bark rot" disorder of Fukumoto causes serious and understandable concerns that may exceed the positive attributes of the variety.

Many Fukumoto groves in California are experiencing a decline of trees leading to their death in some instances. Declining trees usually show stunting and aberrant growth at the graft union with growth of several suckers (shoots) near the graft union (Figure 1A). Other growth abnormalities associated with foamy bark rot include off-type fruit, excessive thorniness, and delayed fruit maturity.

Splitting may occur on the bark of the trunk and branches, and gum exudates (Figure 1B) and foamy substances are released, which attract ants. Especially during hot summer days, the splits could also release whitish foamy exudates that have a smell similar to when beer is brewed and a cross section of the stem at that stage is shown in Figure 1C, though the release of exudates does not occur frequently. Later in the year when the exudates subside and dry out, the tree is left with cankers (Figure 1D), which makes the trees more vulnerable to many pathogens. These symptoms are rarely seen in other varieties, but almost all Fukumoto orchards planted on trifoliate or trifoliate hybrid rootstocks are affected.

Initially, stresses such as soil pH and irrigation were suspected to initiate the decline. However, irrigation experiments did not support that hypothesis; there was no significant difference in decline symptoms on trees with or without irrigation stress.

Genetic abnormality of the Fukumoto budwood source tree(s) as well as copper deficiency has been suspected to play roles in the bark rot. An association between trunk gumming and ants has also been suggested. It has been suggested that incompatibility between scion and rootstock might be partly responsible for the aberrant growth pattern of Fukumoto trees, which probably prevented carbohydrates produced in the leaves from being transported back to the roots.

Preliminary microorganism isolations from symptomatic trees revealed that several biological agents are associated with foamy bark rot of Fukumoto. Therefore, we hypothesized that foamy bark rot is initiated


**Fig. 1. Symptoms of foamy bark rot on Fukumoto tree.**

by scion-rootstock incompatibility, resulting in nutritional and/or physiological abnormalities, which provide opportunity for infection by certain pathogen(s). The objective of this study is to isolate fungal and bacterial pathogens associated with foamy bark rot; identify the isolates using morphological and molecular techniques; and determine which of these cause or aggravate the condition.

**Sample collection, analyses, and results**

Shoot, root, and soil samples were collected from Fukumoto orchards in Tulare, Fresno, and Kern counties, where the variety is commonly grown. The samples were transported on ice to the laboratory at the University of California Riverside (UCR). Grower records were consulted for information on the age and rootstock of the trees, irrigation management, cultural and fertilization practices, and disease and pest histories of the orchards. Soil samples were analyzed at the Agriculture and Natural Resources Analytical Laboratory, University of California Davis to determine any correlation of soil characteristics with the symptomatic trees.

Small pieces of tissue from symptomatic root and branch samples were plated onto nutrient agar and potato dextrose agar (PDA) amended with 0.01% tetracycline (PDA-tet) to isolate bacteria and fungi respectively. Root samples were also plated onto a growth medium named PARPH to screen for *Phytophthora* species. PARPH is a *Phytophthora*-specific medium, and the major constituent is cleared V8 juice to which five antibiotics (Pimaricin, Ampicillin, Rifampicin, PCNB, and Hymexazole) were added.

Pure cultures of fungal and *Phytophthora* isolates were obtained by excising a hyphal tip from colony margins emerging from the tissue pieces and transferring them onto fresh media plates while single colonies of bacterial isolates were spread over fresh media with an inoculating loop. Morphology was used to screen out fungal saprophytes and Oomycetes other than *Phytophthora* species. About 200 isolates were collected in the first sampling conducted in the spring/early summer of 2010.

Selected isolates were further analyzed using molecular methods. Genomic DNA was extracted and used in a polymerase chain reaction (PCR) test. Multiple primers were used to ensure correct molecular identification of all isolates.

**Fig 2. Rootstocks (A, Carrizo and B, Volkameriana) growing at UCR for propagation with Fukumoto.**

For the bacteria, two pairs of universal molecular markers for all bacteria (U1/U2 or 8F/1492R) were used. Additionally, markers specific to *Xanthomonas* (ALA4/L1) and *Erwinia/Pantoe* (27F/L1rc) species were used during screening.

For fungi and *Phytophthora*, three different molecular markers (ITS4/5, Bt2a/2b, and EF1-728F/EF1-986R) were used for total identification. In some cases two primers were enough to fully resolve fungal identity; however, isolates with *Botryosphaeria*-like morphology were screened with all three primers because many *Botryosphaeria* isolates are similar in morphology and genetic composition.

The fungal and bacterial PCR products obtained were sequenced at the UCR Genomics Core Sequence Facility, and the sequences were further analyzed. The isolates that have been fully identified are presented in Table 1.

| Organism | Number isolated |
|---|---|
| ***Bacteria*** | |
| *Acinetobacter* | 3 |
| *Bacillus* spp. | 6 |
| *Pantoea* sp. | 1 |
| *Pseudomonas* spp. | 5 |
| **Total bacteria species:** | **15** |
| ***Fungi*** | |
| *Aternaria citri* | 1 |
| *Alternaria* spp. | 2 |
| *Botryosphaeria* | 3 |
| *Fusarium solani* | 12 |
| *Fusarium oxysporum* | 4 |
| *Fusarium* sp. | 2 |
| *Fusarium equiseti* | 3 |
| **Total fungi species:** | **27** |
| ***Oomycete*** | |
| ***Phytophthora citrophthora*** | **1** |

**Table 1: List of isolates from first set of Fukumoto roots and shoots samples.**

The preliminary information presented here supports our original hypothesis that a scion/rootstock incompatibility is creating the physiological disorder, which then opens the tree to infection by certain pathogen(s), and foam production is possibly due to yeast activities (thus the beer brewing smell) while the foam and gum act as an attractant to ants.

**The study continues**

An alternative Fukumoto budwood source was introduced into California (released from quarantine in summer of 2009) from Spain, where no Fukumoto abnormalities have been observed for approximately the prior 10 years. In a different study, field trials with trees produced with this "new" Fukumoto source are currently underway in collaboration with Craig Kallsen, UC Cooperative Extension citrus farm advisor in Kern County.

The analysis of the first set of

soil samples indicated no significant correlation between foamy bark rot and any of the parameters measured – pH, total N, C, Olsen-P, K, Na, Ca, Mg, and lime. However, analysis of additional soil samples will have to be completed before any final conclusions can be drawn. A new set of soil, root, and branch samples, collected during winter 2010-11 are currently going through microorganism isolation and analysis in order to identify any variation in results from the spring 2010 set of samples. Also, some yeasts of interest isolated in the first sample set have to be fully identified.

**Koch's postulates** are four steps designed to prove that a microorganism is the causal agent of a disease:

1. The pathogen must be found associated with the disease in all the diseased plants examined.

2. The pathogen must be isolated and grown in pure culture on nutrient media and its characteristics described (nonobligate parasites), or it must be grown on a susceptible host plant (obligate parasites) and its appearance and effects recorded.

3. The pathogen from pure culture must be inoculated on healthy plants of the same species or variety on which the disease appears, and it must produce the same disease on the inoculated plants.

4. The pathogen must be isolated in pure culture again, and its characteristics must be exactly like those observed in step 2.

If all the steps have been followed and proved true, then the isolated pathogen is identified as the organism responsible for the disease.

*Source: Agrios, George. Plant Pathology (textbook), Fourth Edition.*

It has been reported that Fukumoto trees on sour orange or Volkameriana rootstocks were not affected by the bark rot syndrome while trees on Carrizo, C-35 or Swingle Citrumelo were more susceptible. However, this past year Fukumoto on Volkameriana rootstocks was found affected in Tulare County.

To further investigate these observations, Carrizo and Volkameriana seedlings (Figure 2) obtained from the California Citrus Clonal Protection Program (CCPP) are currently growing in a greenhouse at UCR. The rootstocks are almost ready for propagation with Fukumoto scion and will be used for pathogenicity tests with the microorganisms presented in Table 1. These experiments will help us to understand which of the isolated organisms are contributing to the symptoms observed in the trees with foamy bark rot.

More specifically, fresh shoots of one-year-old Fukumoto navel trees will be inoculated with the identified microorganisms in a randomized complete block design. One representative of each species will be chosen if strains were the same genetically and morphologically.

For bacterial inoculation, a $10^8$ cfu/ml bacterial suspension (cfu = colony forming units) will be made, and a 20 µl drop of suspension will be placed onto the stem of each of the ten replicate plants per isolate. An 18-gauge syringe needle will then be inserted through the drop until it is sucked into the phloem and xylem tissues. This procedure will be repeated on the other side of the stem to ensure a cross-sectional inoculation.

For fungal inoculations, the primary stem will be wound-inoculated with an agar plug of each isolate and wrapped in parafilm. Ten replicate seedlings per isolate will be utilized. The negative control plants will be inoculated with water or blank agar.

All inoculated plants will be incubated at a greenhouse at UCR and will be monitored for symptom development over a period of three to six months. Isolations will be performed on symptomatic tissues to confirm the pathogenicity of any of the previously inoculated microorganisms. This line of experimentation will fulfill the basic phytopathological principle of Koch's postulates (see text box) and will help us to determine the pathogenicity and aggressiveness of the isolated microorganisms on Fukumoto navel. Findings will provide a baseline for the management of foamy bark rot.

***Project Leader Dr. Akif Eskalen is a UC Cooperative Extension Specialist and Plant Pathologist; Dr. Anthony Adesemoye is a Postdoctoral Scholar; Dr. Georgios Vidalakis is the Director of the California Citrus Clonal Protection Program and a Cooperative Extension Specialist; Dr. Caroline Roper is Bacteriologist and Plant Pathologist; Joey Mayorquin and Peng Wang are graduates students at University of California Riverside; Neil O'Connell and Craig Kallsen are Farm Advisors, UC Cooperative Extension in Tulare and Kern counties, respectively.***

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## CALENDAR

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| Jan 26 | Citrus Day, Citrus Variety Collection, University of California, Riverside, CA |
| Feb 14-16 | World Ag Expo Tulare, CA |
| Mar 8 | CCM Citrus Showcase Visalia, CA |
| Apr 19 | CRB-UCCE Postharvest Seminar Exeter, CA |