Research Project Progress Report

Current knowledge on *Fusarium* dry rot of citrus

Anthony Adesemoye, Akif Eskalen, Ben Faber, Gary Bender, Neil O'Connell, Craig Kallsen and Tom Shea

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."

The pathogen, disease, and symptoms

Dry root rot (DRR), a disease caused by the soil fungus *Fusarium solani*, has a long history in citrus production in California. *Fusarium solani* is a weak pathogen on citrus and can infect only when a tree is under stress. This pathogen is present in almost all citrus orchards everywhere in the world including California, which enhances its rapid exploitation where stress is present.

Possible stresses include invasion by other pathogens, such as *Phytophthora* and *Citrus tristeza virus* (CTV), wounding by gophers or other rodents, insects, or weed control operations, girdling, asphyxiation including extended saturation of the soil from over-irrigation, damage to the roots from fertilization, etc. With stress, *F. solani* begins infection by colonizing the cortical tissue of feeder roots, advances into the lower taproot and/or scaffold root, and moves up through the crown into the center of the trunk. All ages of trees are susceptible to the disease.

Symptoms may include a reddish-purple to grayish color of the roots. Infection leads to dark decay in the bark of large scaffold roots and lower crown of the trunk. However, the discoloration may sometimes be limited to a section, not the entire cross section. Trees become weak and lose ability to produce good fruit. Leaves turn yellow, then brown; dieback and wilting occur quickly, and the canopy is lost.

The progression of the disease is affected by weather conditions, and it is more rapid during hot summer. For instance, along the coast, symptoms are usually first seen after the first hot temperatures in spring following the rainy season.

Examples of symptoms found during our survey in 2010 are shown in Figure 1. Most of the previous studies conducted across California are highlighted below. The intent of looking back at previous studies is not to do a comprehensive review of them but to highlight the impacts of the disease over time in the state and connect historical reports with present findings and future potential impacts of the disease.

Fig. 1. Symptoms of dry root rot seen (A) around the crown, (B) in a cross section of a trunk, and (C) on roots.

Previous findings on dry root rot in California from the 1920s

The disease was described as "dry root rot" for the first time in California in 1920 by Barret, when it was indicated that the designation was for "convenience". The author described the symptoms and reported that different species of *Fusarium* were isolated from different cases of the disease in different sections, which indicated that "closely related, if distinguishable, types of dry root rot may be induced by several species of the genus *Fusarium*."

Subsequently, Dr. Fawcett in studying citrus gummosis reported in 1923 that the joint inoculation of *Phytophthora citrophthora* and *Fusarium* led to a wide area of dead tissues up and down the bud union. Fawcett and Klotz in 1948 acknowledged the destructive effects of *Fusarium* on citrus production.

In 1954, Klotz conducted another study and observed DRR on trees that had injuries caused by gophers, chemicals, etc., but also found DRR in places where those factors had been eliminated. The affected areas were reported to have a fish odor with leaves wilting suddenly, drying up, and dropping with extensive dieback of twigs.

Another researcher at UC Riverside, Dr. E.C Calavan, observed in 1957 that dry root rot was causing low production and the death of several trees and believed that no orchard in the state was free from DRR problems. It was shown that *Fusarium* root rot was one of the three diseases damaging most citrus trees in California, the other two being *Phytophthora* foot/root rot and gummosis caused by *P. citrophthora* and *P. nicotianae (P. parasitica)* respectively.

Calavan further observed that *Fusarium* root rot was very frequently found in wet environments and was infecting trees following *Phytophthora* infections leading to the death of two-year-old or older trees exposed to wet conditions or with mechanical injuries. It was shown that infection by *P. citrophthora* occurs in cool or temperate environments while that of *P. nicotiana* occurs in warmer environments and that it could be very severe in hot deserts, causing problems in rootstocks that were resistant to *P. citrophthora*.

The disease usually affects a few trees throughout a grove but can sometimes cause significant damage in a short time. In 1959, citrus tree collapse and deaths induced by DRR occurred to more than 1,200 young citrus trees in the Coachella Valley area. Trees were observed with internal wood rot that was mostly confined to the rootstock. It was concluded that the disease was DRR caused by *Fusarium* based on the isolation of several species of *Fusarium* as the most frequently isolated organisms from infected roots and woods, followed by confirmatory tests. It was suggested that the pathogen gained entrance into the trees either through wounds in roots or into roots that had been damaged by an application of chicken manure into the holes during planting.

Similarly, 127 out of a total 369 trees in a grove in Santa Paula died within three years (1976-1979). After conducting studies for years, Klotz reported that all common rootstocks were susceptible, including trifoliate and 'Troyer' citrange, with infection starting in the rotting fibrous feeder roots or larger roots, progressing into the main root and crown, or starting from the crown if injury occurred there. The infected wood showed dusky to black discoloration. They noted that DRR did not produce gumming that was typical of *Phytophthora* gummosis, which agreed with the 1923 observations of Dr. Fawcett.

Over time, researchers isolated many organisms from infected plants; however, inoculation experiments with *Fusarium* often failed to produce symptoms of DRR; thus, in 1980 Menge believed that the disease was a complex.

Two years later, Bender and others suggested that plugging of xylem elements in the root and trunk was responsible for the rapid wilting and death of infected trees. Symptoms they observed included decay with dark color, drying, and cracking of trees, purple staining of the cambium, leaf yellowing, wilting, and dying.

Bender conducted some experiments, and their results corroborated prior studies that *F. solani* was the cause of DRR, although the pathogen alone did not infect citrus trees except after pre-disposition through a combination of stresses. The stresses included heat, infections by *Phytophthora*, poor aeration, overwatering, excessive fertilizer and/or manure, careless use of chemicals, and mechanical injury. In one study reported in Bender's Ph.D. dissertation, when a tree was cut and inoculated, trees appeared to heal quickly and prevented invasion from the pathogen. However, invasion was recorded when taproots just above the inoculation point were girdled completely with a 2 mm-wide cut. Bender suggested that internal girdling at the graft union caused by Tristeza virus, and perhaps graft union incompatibilities found with macrophylla and trifoliate rootstocks, might be predisposing trees to infection by *Fusarium*.

In 2003, Sakovich traced the history of DRR in Ventura County to the 1950s. He noted that many trees collapsed due to DRR without any apparent predisposing factor. He therefore reasoned that there might be other factors which are yet to be identified since the disease could only occur when there were pre-disposing factor(s). In the studies conducted in 20 orchards, no soil nutrient element correlated as a predisposing factor for the disease.

After a study conducted on citrus quick decline, Eskalen and others suggested in 2010 that an alliance between *Citrus tristeza virus* (CTV) and *F. solani* played a role in quick decline. They recovered *F. solani* only from trees which had been stressed by CTV, which had facilitated rapid infection by the DRR pathogen.

Our current efforts

The studies highlighted above demonstrate that consistent attention has been given to DRR but much remains unknown about the disease, and progress to develop methods for its management has been slow.

The interactions of *F. solani* with *Phytophthora* spp. and other biotic and abiotic factors remains an unclear dynamic in the DRR complex. The seasonality in the activity of the two *Phytophthora* species (*P. nicotianae* and *P. citrophthora*) involved in DRR infestation, according to many studies, makes the situation even more complex. Our objective was to study the seasonal occurrence and survival of *F. solani*, *P. nicotianae* and *P. citrophthora* in irrigated citrus in California. The study includes understanding the distribution of *F. solani*, *Phytophthora* spp. and other factors associated with DRR throughout the year.

Sample collection and analyses

Infected trees were rated for disease severity using a rating scale of 1 to 5 to describe the level of the disease in each orchard. On our scale, 1 refers to "healthy" trees (without any visible symptoms); 2 are trees with golden color of leaves; 3 are trees with obvious canopy reduction; 4 is the wilting stage; and 5 is the advanced stage of drying leaves and dying trees.

Shoot, root, and soil samples were collected from orchards in eight citrus growing counties in California, namely Fresno, Kern, Riverside, San Diego, San Luis Obispo (SLO), Santa Barbara, Tulare, and Ventura. 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 if a correlation among soil characteristics and the disease existed.

Small pieces of tissue from symptomatic root and branch samples were plated onto potato dextrose agar (PDA) amended with 0.01% tetracycline (PDA-tet) to isolate fungi. Root samples were also plated onto a medium named PARPH to isolate *Phytophthora* species. Pure cultures of fungal and *Phytophthora* isolates were obtained. More than 250 isolates were collected in the first sampling conducted in the spring/early summer of 2010.

Morphology was used to screen out fungal saprophytes and oomycetes other than *Phytophthora* species. Isolates were examined under the microscope, and pictures of mycelia and spores, including macro and microconidia of *Fusarium*, were taken. Isolates were screened to a final number of 82 (60 *Fusarium*, 18 *P. citrophthora*, and 4 *P. nicotianae*). Some pictures of the morphology and spores are displayed in Figure 2.

Selected isolates were further analyzed using molecular methods. Genomic DNA was extracted and used in a polymerase chain reaction (PCR) test. Three different molecular markers (ITS4/5, Bt2a/2b, and EF1-728F/EF1-986R) were used for correct identification of *Fusarium* isolates. Two of these primer pairs were enough to fully resolve the identity of *Phytophthora* isolates. The PCR products obtained were sequenced at the UCR Genomics Core Sequence Facility, and the sequences were further analyzed. The next set of about 180 isolates collected during winter 2010-2011 is being screened currently.

Results and new activities

Preliminary results indicate that in addition to *F. solani* and two species of *Phytophthora* (*P. citrophthora* and *P. nicotiana*), *F. oxysporum*, and *F. proliferatum* are major organisms associated with DRR. Results also indicate correlation between season and isolation of *Phytophthora* spp. For example, only two isolates have been isolated so far from the winter samples compared to 22 from the spring/early summer samples. Previous reports indicate that *P. nicotianae* and *P. citrophthora* are affected by sugar-starch availability dynamics in summer, winter, and spring months. More information about the seasonal behavior of the two *Phytophthora* species will contribute to better understanding of the mechanism of DRR.

Another finding is that there is currently no correlation between nutrient content and the disease. Pathogenicity tests are currently being conducted on

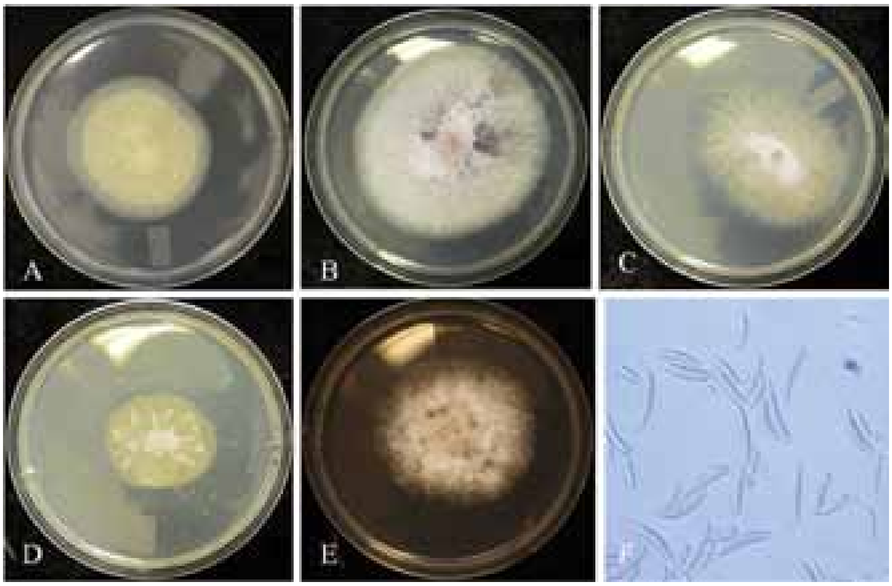

Fig. 2. Pictures of plates: (A) *Fusarium solani*, (B) *F. oxysporum*, (C) *F. proliferatum*, (D) *Phytophthora citrophthora*, (E) *P. nicotiana*, (F) Macro and microconidia of *F. solani*.

representatives of the identified isolates in a lath house at UC Riverside using healthy trees of 'Early Cutter' Valencia and 'Allen Eureka' lemon. If DRR symptoms develop on the trees, it will confirm the roles of each isolate in DRR.

Disease management and looking into the future

In 2010, many trees infected with DRR were dying throughout citrus growing areas, as can be seen in those shown in Figures 1 and 3. Thus, DRR remains a concern, and a well- designed management plan is needed.

Irrigation should be done with care in which application matches tree water requirements, and the trunk is kept dry. Attention should be paid to drainage because water should not be allowed to stand in contact with the tree crown for an extended period of time. Movement of equipment facilitates spread of the pathogen, so equipment should be well cleaned before moving it between orchards. Proper fertilization will minimize stress and opportunity for the disease; insufficient or excessive nutrient applications should be avoided. Rodent control and the careful use of herbicides and other chemicals will also be helpful. Sanitation is important; keeping the immediate environment of wounded and/or infected trees clean and dry will help to reduce or prevent transmission of pathogen. Total removal of infected trees is not recommended at early stages but should be done in advanced stages. (Some trees may recover from the early stages of the infection.) Management of *Phytophthora* should be part of the focus in DRR management.

There is a need for integrated pest (disease) management (IPM) and control methods based directly upon knowledge of the pathogen and the development cycle of DRR. The use of IPM to control pathogens is a more environmentally friendly alternative than fungicides. The control strategy for dry root rot that integrates both cultural and biological methods should be studied. For instance, calcium is an important nutrient that has been utilized in the control of *Phytophthora* through manipulation of zoospore production. Lessons from those studies should be applied to citrus. Better management

Fig. 3. Infected trees - (A) Lemon in Ventura County and (B) Navel orange in Tulare County.

of DRR through IPM should be implemented through appropriate execution of cultural practices, nutrient management, biological control, and fungicide application.

Project Leader Dr. Akif Eskalen is a UC Cooperative Extension Specialist and Plant Pathologist based at UC Riverside, Dr. Anthony Adesemoye is a Postdoctoral Scholar, Department of Plant Pathology and Microbiology, UC Riverside; Dr. Ben Faber, Dr. Gary Bender, Neil O'Connell and Craig Kallsen are Farm Advisors, UC Cooperative Extension in Ventura, San Diego, Tulare and Kern counties, respectively. Tom Shea is a Staff Research Associate, UC Cooperative Extension, Riverside.

CRB research project reference number 5300-140 (5400-140 in FY2011-2012).

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