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Is There a Risk of Replanting Pistachio in Soils From Pistachio Bushy Top Syndrome-Affected Orchards?

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With our current understanding that pistachio bushy top syndrome (PBTS) is a disease caused by a bacterial plant pathogen (*Rhodococcus* sp.), one of the main issues faced by affected growers is the question of whether replants may become infected with the pathogen. Because the epidemic of PBTS is unprecedented, it is unknown whether soilborne inoculum may infect roots and induce symptoms on replants. A series of studies have been completed, and others are underway, to address the following:

1) Identify potential inoculum sources at replant sites after removal of PBTS-affected trees?
2) Determine if the pathogen can infect roots of pistachio rootstock?
3) Assess whether the pathogen can be transmitted from infested soil to healthy replants?

Inoculum sources at replant sites:

Source-plant inoculum vs. environmental inoculum. Research efforts are underway to address potential sources of inoculum at replant sites. One major challenge is the fact that the natural distribution of *Rhodococcus* in the environment is unknown; consequently, when *Rhodococcus* is detected at a site, it is difficult to determine whether inoculum originated with the infected pistachio rootstock or whether inoculum was already present in the environment.

Root fragments from PBTS-affected plants. *Rhodococcus* sp. has been isolated from surface-sterilized roots of PBTS-symptomatic 'UCB-1' rootstocks. Consequently, root fragments remaining in the field after removal of affected trees may serve as a source of inoculum (Figure 1).

Soilborne inoculum. *Rhodococcus* sp. has been detected in soil from a PBTS-affected orchard using PCR. It is important to note that this method relies on detection of pathogen DNA in soil, and does not address whether the pathogen is viable (alive). A confounding issue is the fact that the natural distribution of the pathogen in soil is unknown; consequently, it is premature to conclude whether the pathogen DNA detected in the soil originated with PBTS-affected plants. Future survey-style studies are planned to methodically address pathogen presence/absence in orchards with and without a history of PBTS.

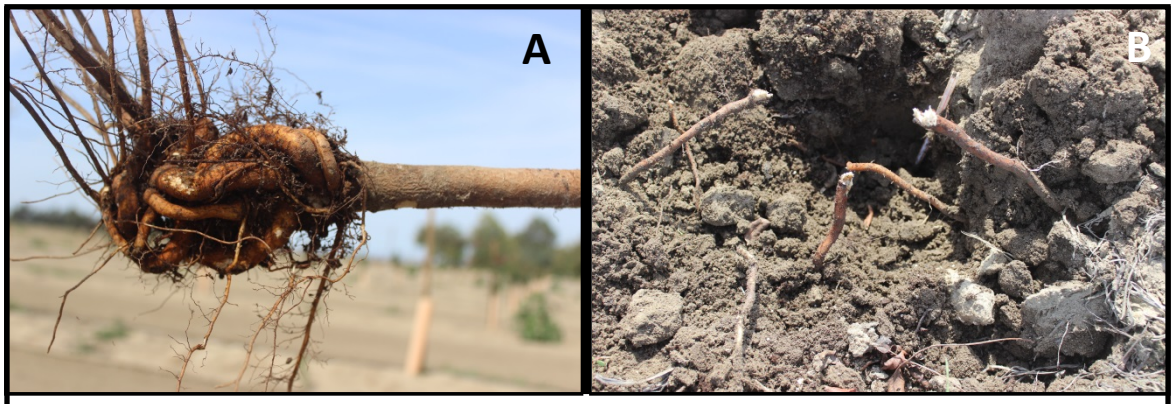

Figure 1. When plants symptomatic of PBTS (A) are removed from the field, residual root fragments remain in the soil and may serve as a source of *Rhodococcus* inoculum.

From a management perspective, this information suggests that effort be made to excavate the majority of root material when removing affected trees.

Root infectivity of *Rhodococcus* sp. on 'UCB-1' rootstock:

Because foliar inoculation was used to prove pathogenicity of *Rhodococcus* to 'UCB-1' pistachio rootstock, it is unknown whether the pathogen has the potential to infect roots. Therefore, the first step in addressing whether soilborne inoculum may affect replants is to determine susceptibility of roots to infection. As part of a replant study (see below) roots of healthy clonal 'UCB-1' plantlets were dipped into a bacterial pathogen suspension. After 6 months, *Rhodococcus* was found to have colonized and persisted on roots, and root-inoculated plants were statistically shorter and had lower shoot and root mass than that of uninfected plants (Figure 2). This finding validates the documentation of pathogenicity of *Rhodococcus* to 'UCB-1' rootstock, as described and published by the Randall laboratory, and adds root susceptibility to our understanding of the epidemiology of the pathogen on pistachio rootstock.

From a management perspective, root susceptibility to Rhodococcus suggests the potential for soilborne inoculum to infect replants via the root system.

Potential for replant soils to transmit *Rhodococcus* to healthy replants:

Based on observational evidence, it is possible for healthy replants to become infected with *Rhodococcus* when planted in PBTS-affected orchards (see article in this newsletter edition). A controlled study has been conducted to verify the risk of soilborne inoculum to replants.

In November 2014, within 2 weeks of removal of PBTS-symptomatic trees from a Tulare Co. orchard, soil samples were collected from the holes from which plants were removed. Soil samples were collected from 20 holes and mixed to evenly distribute any residual inoculum. To establish a 'negative' control of uninfested soil, a portion of soil was steamed for 1 hour on two consecutive days. To establish a 'positive' control, roots of 'UCB-1' clonal plantlets were dipped in a bacterial suspension of *Rhodococcus* sp. The experiment contained three treatments with 'UCB-1' clonal rootstocks planted in either steamed/uninfested soil, naturally-infested replant soil, or root-inoculated prior to planting in replant soil.

After 6 months, plants grown in naturally-infested soil, and root-inoculated plants, exhibited root colonization by *Rhodococcus* sp. The pathogen was not recovered from the roots of plants grown in steamed replant soil.

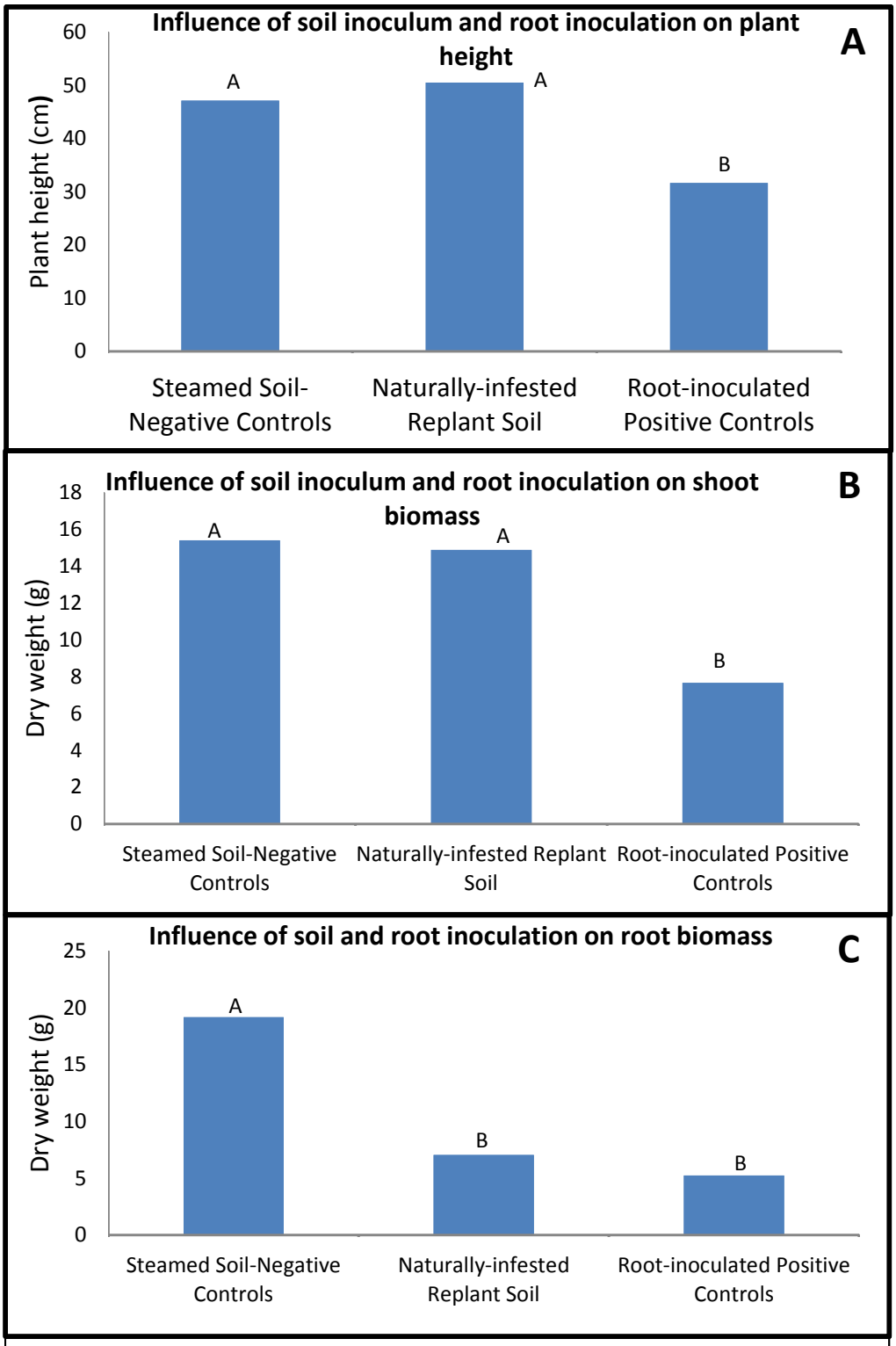

Figure 2. UCB-1 clonal rootstocks were incubated in naturally-infested replant soil collected from a PBTS site in Tulare County. Plant height (A), shoot biomass (B) and root biomass (C) were assessed were compared to plants grown in steamed/disinfested replant soil and root-inoculated plants.

Plants grown in naturally-infested replant soil exhibited statistically similar height (Figure 2A) and shoot mass (Figure 2B) to those grown in uninfested soil. Root mass, however, was significantly lower in plants grown in naturally-infested soil as compared to the those grown in uninfested soil (Figure 2C; Figure 3A and B). Root-inoculated positive control plants were significantly shorter (Figure 2A), and exhibited lower shoot (Figure 2B) and root mass (Figure 2C) than plants grown in uninfested soil. Root mass was statistically similar between root-inoculated plants and plants grown in naturally-infested soil (Figure 2C).

Figure 3. 'UCB-1' clonal plants grown in uninfested replant soil (A) exhibited significantly more root mass than those grown in naturally-infested (B) replant soil. *Rhodococcus* was reisolated from the roots of replants grown in naturally-infested soil, but not from those grown in uninfested soil.

From a management perspective, the results of the replant study, combined with observational evidence, suggest a risk of soilborne inoculum to replants in PBTS-affected orchards.

The duration of *Rhodococcus* persistence in soil is currently unknown. Research efforts are currently underway to improve techniques for pathogen detection, which will, in turn, allow for the design and implementation of studies addressing soil survival and disinfestation options.

Select Reference

Stamler, R. A., Kilcrease, J., Fichtner, E., Kallsen, C., Cooke, P., Heerema, R. J., & Randall, J. First Report of Rhodococcus isolates causing Pistachio Bushy Top Syndrome on 'UCB-1'rootstock in California and Arizona. *Plant Disease*. http://dx.doi.org/10.1094/PDIS-12-14-1340-RE

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Initial Observations From Orchards Affected With Pistachio Bushy Top Syndrome: Implications for Management

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Recovery of Rhodococcus sp. from roots of replants

In July 2014, 'UCB-1' clonal trees symptomatic of pistachio bushy top syndrome (PBTS) were removed from a western Fresno County orchard and replants were immediately introduced to the holes of removed trees. The replants were also 'UCB-1' clonal rootstocks; however, they were purchased from a nursery with no known history of infestation with *Rhodococcus*. After 6 months duration in the former bushy-top holes, 6 asymptomatic replants were destructively sampled to determine presence of *Rhodococcus*. Roots were surface sterilized in diluted bleach solution prior to maceration of root tissue for isolations. *Rhodococcus* was recovered from one out of 6 samples, thus demonstrating that the pathogen may infect root tissues. The source of primary inoculum for this root isolate is unknown. The prevalence of *Rhodococcus sp*. in California agro-ecosystems is yet unknown; however, the pathogen has been isolated from commercial walnut orchards in the Central Valley. Consequently, at this time it is unknown whether the primary inoculum inciting this root infection was from the removed PBTS-affected plants or from environmental inoculum.

Recovery of Rhodococcus fascians from rootstock suckers of trees replanted into locations previously occupied by trees with pistachio bushy-top syndrome.

As part of the objectives of a grant provided by California growers through the California Pistachio Research Board, we were able to make an initial investigation on the fate of new rootstocks or budded tree planted into holes previously occupied by trees showing advanced PBTS. We want to make it clear that this was not a scientifically designed study. Our investigation began many months after the growers in these various orchards replaced PBTS trees. The objective of this activity was to obtain some initial information associated with the risk of replanting into holes previously occupied by PBST trees. Diagnosis of *Rhodococcus fascians* was based on culturing and PCR techniques. This investigation involved three orchards. The original trees, which showed severe PBTS symptoms, all came from one nursery, the replants from other nurseries.

In Orchard 1, trees were originally planted in 2013 and the entire orchard removed approximately 8 months later based on PBTS symptoms. The holes remained vacant for approximately 4 to 5 months and then replanted with new rootstocks in 2014 and fall grafted. Three pooled or bulked samples of rootstock suckers from 10 trees each were tested for Rhodococcus in April 2015. Two of these samples were found to be positive for *Rhodococcus*.

In Orchard 2, the original trees were planted in 2013 and the entire orchard removed based on the prevalence of PBTS trees in the orchard in April 2014. Immediately after tree removal, rootstocks, obtained from two different nurseries, were planted into these holes. In April of 2015, four pooled samples from 20 trees each were analyzed. One of these four samples was positive for *Rhodococcus*.

In Orchard 3, the original trees were planted in 2011. Many original trees remain in the orchard. Trees with obvious PBTS were removed in 2014 and immediately replanted with budded trees from a different nursery. Four samples of rootstock suckers from 5 to 15 trees each were analyzed and none found positive for *Rhodococcus*.

At this time, we do not know the eventual fate of large, healthy replant trees that test positive for *Rhodococcus* as a result of being planted in locations previously occupied by a PBTS trees. None of these trees tested in the three orchards had visually-obvious PBTS symptoms. A continuing concern, and previously described in the February 2015 edition of the UCCE Kern Co. newsletter (http://cekern.ucanr.edu/news_80/Pistachio_Notes_Newsletter/?newsitem=54750), are mature trees that abort most of their crop, by mid-June. To date, the only obvious difference between trees in these orchards is the presence of *Rhodococcus* on the bark, leaves and flowers of those that abort the crop. A fourth orchard with this mature tree nutlet drop was visited earlier this week.

Initial suggestions for growers with Rhodococcus-affected orchards:
Based on observational evidence gathered from farm calls and laboratory isolation of the pathogen, it is advisable for growers to mitigate the risk of potential infection of replants from residual inoculum in affected orchards. Therefore, observational evidence suggests that replants should not be placed in the same holes that were formerly occupied by symptomatic plants.

Secondly, it may be appropriate in orchards where significant tree removal occurred as a result of PBTS and in which original trees were retained and look symptomless, to closely observe these trees for early nutlet abortion as they come into bearing.

Is There a Risk of Transmitting *Rhodococcus* Between Plants or Orchards on Infested Pruning Equipment?

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With the recent elucidation that plant pathogenic bacteria in the genus *Rhodococcus* are responsible for the new disease of 'UCB-1' pistachio rootstock called pistachio bushy top syndrome (PBTS), researchers now seek to understand the epidemiology of the disease for prevention of transmission to unaffected plants and orchards. In direct response to growers' concern over the potential for pathogen transmission on pruning equipment, a greenhouse experiment was designed and implemented in November 2014.

To address the pathogen-transmission potential on infested tools, healthy 'UCB-1' clonal rootstocks were obtained from a commercial nursery. Plants were tested for presence of *Rhodococcus* bacteria by pressing leaves on selective medium prior to initiating the study; *Rhodococcus* was not recovered from any of the plants. Plants were cut with pruners that were prepared with each of three treatments: 1) uninfested/negative control, 2) naturally-infested pruners, and 3) artificially-infested pruners/positive control. Uninfested pruners were sprayed with 95% ethanol and flamed prior to making cuts on test plants. A positive control treatment was established by dipping pruners in a bacterial suspension of two isolates of *Rhodococcus* that had been recovered from pistachio bushy top plants. The naturally-infested pruners were run through naturally-infected plant material collected from a commercial pistachio orchard in Tulare County, CA. The naturally-infected material was collected the same day as treatments were implemented on plants. Twenty replicate plants were utilized for each treatment and treatments were geographically separated within the greenhouse to prevent transmission from infested plants to negative controls.

After approximately 5 months, each plant was sampled for epiphytic populations of *Rhodococcus*. Putative *Rhodococcus* isolates were subcultured and sent to the Randall laboratory at New Mexico State University for confirmation of identity. Based on visual observation, there are no differences in aboveground growth and development between treatments; however, epiphytic populations of

Rhodococcus have been detected on 20% (5/20) and 15% (3/20) of plants cut with naturally-infested and artificially-infested pruners, respectively. The pathogen has not been recovered from the negative control plants. Further work with be conducted to determine whether treatments affected plant height, shoot, and root biomass; the results will be presented in a future newsletter edition.

The results of this study indicate that *Rhodococcus* is transmissible on pruning tools. Consequently, to mitigate spread of the pathogen from infected to uninfected plants, both within and between orchards, growers should instruct pruning crews to disinfest tools between trees. Additionally, growers unaffected by pistachio bushy top syndrome are advised to request pruners disinfest all tools prior to entering unaffected blocks.

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