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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.

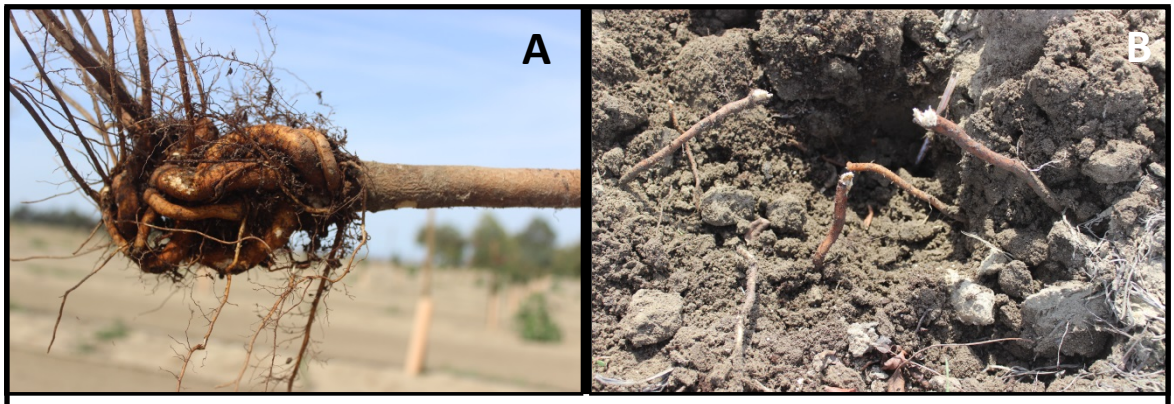

Figure 1. When plants symptomatic of PBTS (A) are removed from the field, residual root fragments remain in the soil and may serve as a source of *Rhodococcus* inoculum.

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.

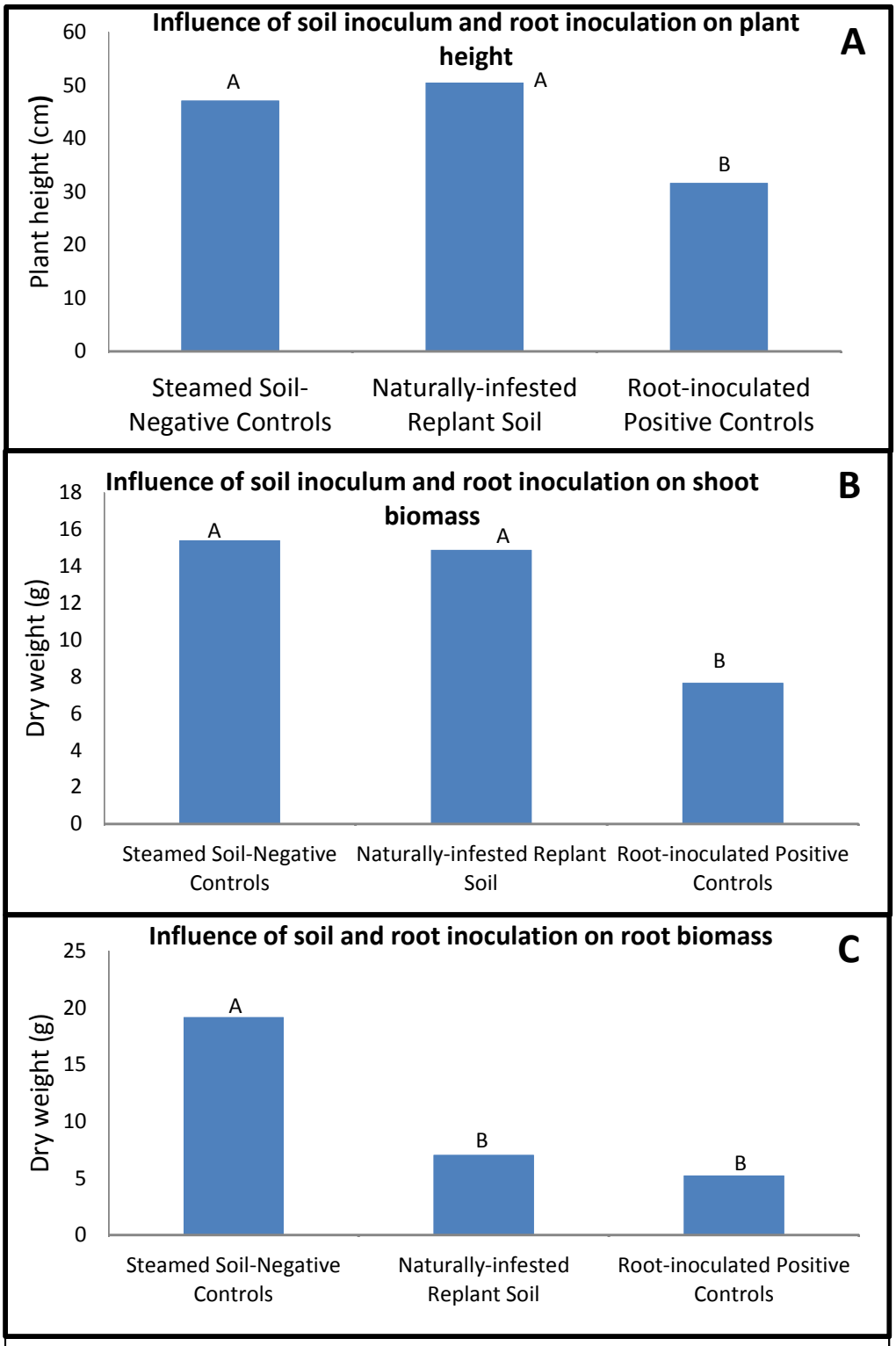

Figure 2. UCB-1 clonal rootstocks were incubated in naturally-infested replant soil collected from a PBTS site in Tulare County. Plant height (A), shoot biomass (B) and root biomass (C) were assessed were compared to plants grown in steamed/disinfested replant soil and root-inoculated plants.

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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