

## Special *Rhodococcus* Edition

### ***Rhodococcus* in the news!**

*Rhodococcus fascians* appears to be well-on its way toward getting the recognition it deserves. See CDFA's effort to get input on making *Rhodococcus fascians* a permanent class C pest of pistachio: <http://blogs.cdfa.ca.gov/Section3162/?tag=rhodococcus-fascians>

### **Private laboratories now taking plant samples for *Rhodococcus* sampling**

I am currently aware of two private laboratories taking samples for diagnosing *Rhodococcus* in pistachio. They are CSPLabs and AL&L Crop Solutions. These labs sent me the following contact and sampling information. *\* Discussion of research findings necessitates using company names. This does not constitute company endorsement, nor does it suggest companies not listed would not be suitable for use.*

#### **\*CSPLABS**

Sukhi Pannu, of CSPLabs tells me that all the information necessary for submitting a sample to his laboratory is on his blog <http://www.csplabs.com/blog/> . If you are not an active blogger CSPLabs office phone is 916-655-1581. The cost involves a \$60 setup fee and then \$30 dollars for each subsequent sample.

#### **\*AL&L Crop Solutions (Vacaville, CA)**

Contact: Anna-Liisa Fabritius, Plant Pathologist  
Tel: (530) 387-3270 or (707) 693-3050  
Email: [info@allcropsolutions.com](mailto:info@allcropsolutions.com)  
Website: [www.allcropsolutions.com](http://www.allcropsolutions.com)

Sampling for *Rhodococcus* assay in pistachios:

From symptomatic trees: select symptomatic plant tissues: branches with shortened internodes, leaves, enlarged buds, galls.

From asymptomatic (non-symptomatic) trees: collect branches and leaves from several different locations (minimum of three (3) per tree).

To avoid cross-contamination between samples, wear single-use plastic gloves and change them after each sample. Sterilize shears between different samples by dipping them into 70% ethanol, 10% bleach or Lysol-solution.

Place each sample into a gallon-size zip-lock bag. Label bag accordingly. Keep samples cool: Place blue ice pack in a cooler together with the samples. Wrap it in newspaper so that it won't touch the samples.

Fill in a sample submission form and send it with your sample. Ship your samples over night to the laboratory:

*AL&L Crop Solutions*

*7769 N. Meridian Rd.*

*Vacaville, CA 95688*

## **UPDATES ON SOME RECENT INITIAL INVESTIGATIONS INTO THE TRANSMISSION OF THE DISEASE-CAUSING ORGANISM OF PISTACHIO BUSHY-TOP SYNDROME**

As part of the objectives of a grant provided by California growers through the California Pistachio Research Board, some initial investigations were conducted into possible means of transmission of *Rhodococcus fascians* in pistachio orchards. Results of these studies follow in two separate articles.

### **ARTICLE 1: Initial observations from orchards affected with pistachio bushy top syndrome: implications for replanting**

#### **AUTHORS**

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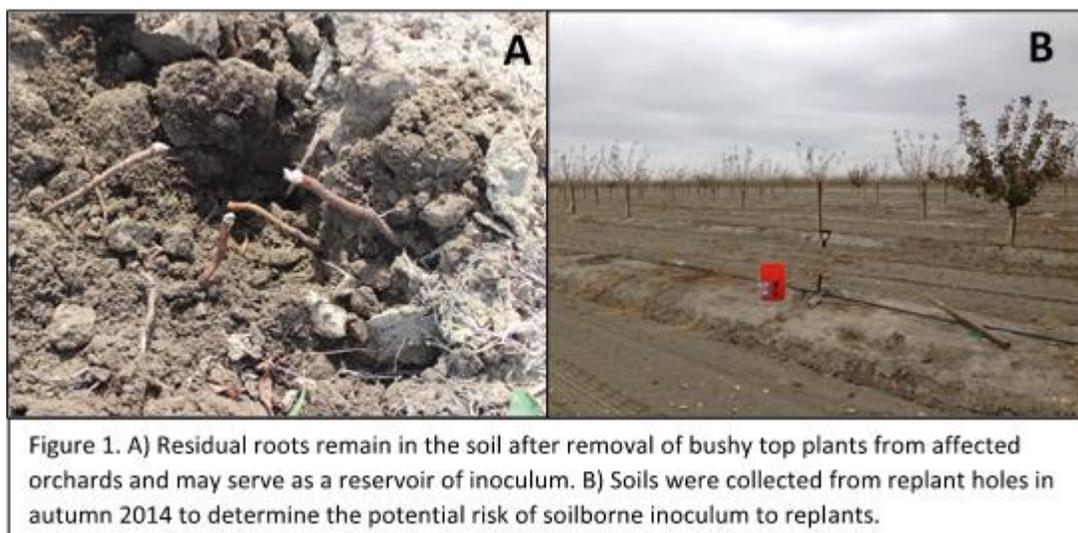
Dr. Jennifer Randall, Research Associate Professor, New Mexico State University

#### **Recovery of *Rhodococcus sp.* from roots of replants**

In July 2014, UCB-1 clonal trees symptomatic of pistachio bushy top syndrome 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 dilute 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 bushy top plants or from environmental inoculum.

*Rhodococcus* has been isolated from the roots of trees symptomatic of pistachio bushy top syndrome. When infected trees are removed from orchards, residual roots remain in the ground as a potential source of inoculum (Figure 1A). Consequently, a main goal of our research program is to address the risk of soil borne inoculum to the health and productivity of replants. Currently, two studies are underway to address whether replants may be colonized by soil borne inoculum from naturally-infested soil (Figure 1B) and whether resulting colonization may impact plant growth and development; the results will be available in summer 2015.



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

We did some initial testing for the presence of *Rhodococcus fascians* on new rootstocks or budded trees replanted into holes previously occupied by trees showing advanced pistachio bushy top syndrome (abbreviated as 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 by the Randall laboratory at New Mexico State University. 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-sucker leaves (from 10 replanted trees in each sample) were tested for *Rhodococcus* in April 2015. Two of these pooled 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 of rootstock-sucker leaves (from 20 trees in each sample) 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 pooled rootstock-sucker leaves from 5 to 15 replanted trees each were analyzed and none found positive for *Rhodococcus*.

As mentioned above, at this time it is unknown whether the primary inoculum inciting infection was from the removed bushy top plants or from environmental inoculum. As well, we do not know the eventual fate of large, healthy replant trees that test positive for *Rhodococcus*. 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 this newsletter : [http://cekern.ucanr.edu/news\\_80/Pistachio\\_Notes\\_Newsletter/?newsitem=54750](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 and samples were taken (but analysis is not yet complete).

#### 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.

### **ARTICLE 2: Is there a risk of transmitting *Rhodococcus* between plants or orchards on infested pruning equipment?**

#### Authors

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Jennifer J. Randall, PhD., Research Associate Professor, New Mexico State University

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 will 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 disinfect tools between trees. Additionally, growers unaffected by pistachio bushy top syndrome are advised to request pruners disinfect all tools prior to entering unaffected blocks.

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