

Pistachio Bushy Top Syndrome
Randall Laboratory, New Mexico State University
By Dr. Jennifer Randall 06/30/14

Good morning from the Land of Enchantment where the high temperature today is a balmy 109°F, but thankfully it is a ‘dry’ heat.

A summary of the results from my laboratory along with my colleagues on the progress made in regards to the association of *Rhodococcus* sp. with Pistachio Bushy Top Syndrome may be useful as I know many people are confused by some of the reports.

First, what is Pistachio Bushy Top Syndrome?

In the last three years, a large number of clonally-propagated ‘UCB-1’ pistachio rootstocks planted in California and Arizona orchards exhibited symptoms that included shortened internodes, stunted growth, swollen lateral buds, bushy/bunchy growth pattern, and twisted roots with virtually no lateral branching. In California, many of these trees also had stem galls. These stem galls are characterized by having multiple buds arising from the gall. The percentage of abnormal rootstocks within affected orchards varied from 8% to 80% with approximately 30% budding success in the field. Within one to two years of t-budding, many trees exhibited unusual bark cracking around the bud-union. We have termed ‘Pistachio Bushy Top Syndrome’ (PBTS) for describing the suite of symptoms exhibited by these trees.

What is the cause of Pistachio Bushy Top Syndrome (PBTS)?

The cause of Pistachio Bushy Top Syndrome is at the moment unknown.

Research efforts by our laboratory at New Mexico State University have found an association of *Rhodococcus* spp. with symptomatic Pistachio Bushy Top trees. However, an association does not mean *Rhodococcus* sp. is the cause of the symptoms. We are currently conducting tests to determine if the *Rhodococcus* sp. is responsible for these symptoms.

What testing has been accomplished in New Mexico?

Our laboratory in New Mexico has tested around 300 trees that include field trees from affected and non-affected orchards in California, Arizona, and four independent nurseries. Our testing included DNA analyses from pistachio trees, bacteria isolation and DNA analyses of these bacteria, microscopy analysis including light microscopy, confocal microscopy, and transmission electron microscopy. We found an association of *Rhodococcus* sp. with trees exhibiting PBTS in affected orchards. We are currently testing to determine if these bacterial isolates can cause PBTS symptoms on pistachio trees and other woody tree species.

What is *Rhodococcus* sp.?

Rhodococcus is a genus of bacteria that contains many related but distinct species. These species are gram positive and their genome has a high GC content. For us scientists, DNA with high GC content means that it is a little more ‘tricky’ to work with when performing some of our DNA tests and sequencing. Several of these *Rhodococcus* species are environmentally advantageous as they have been utilized for biodegradation of harmful products. However, there are also known pathogens from this genus. These include *Rhodococcus equi*, which affects horses and humans and *Rhodococcus fascians* a known plant pathogen with a broad host range. *R. fascians* was previously known as *Corynebacterium fascians*. Symptoms from this bacterium are often confused with those caused by phytoplasmas, viruses, *Agrobacterium tumefaciens*, and latent hormone effects in nursery settings (Putnam and Miller, 2007). *R. fascians* can be present on plants as an asymptomatic epiphyte (a bacteria living on the outside of the plant that does not cause symptoms) or may gain entry into the plant where it modulates hormone activity, resulting in stunted growth, shortened internodes, bushy or bunched top appearance, leaf galls, and modified root development. Genetic analyses from our laboratory indicate that we have identified a species of *Rhodococcus* that is 99% similar to *R. fascians* and another species that is also similar to *R. fascians*. We are performing in-depth genetic analyses of both of these *Rhodococcus* spp.

What have other laboratories found in regards to this association?

Dr. Maher Al Rwahnih from Foundation Plant Sciences at UC-Davis reported on June 9th that their laboratory had also found *Rhodococcus* sp. on samples from the nursery and from field samples (12 out of 31 trees from the field). His results indicate that the chromosomal virulence gene *vicA* that he amplified and sequenced was 98-99% similar to *R. fascians*. This matched the results from NMSU.

Melodie Putnam, Oregon State University, reported on June 27th that her laboratory also found the presence of *Rhodococcus fascians*, and *Rhodococcus* sp. from nursery plants. She was unable to isolate *Rhodococcus* from the field samples that were sent to her.

They also reported that their results were **negative** when testing for the **plasmid** associated genes that carry the ability to cause disease on herbaceous plants in nursery settings.

What is a plasmid?

A plasmid is a piece of DNA that is not part of the bacterial chromosome. Plasmids can replicate on their own, independent of the bacterial chromosome and can be transferred between bacteria. Plasmids can carry important information for the bacteria. For example, the pathogen that causes crown gall, *Agrobacterium tumefaciens*, has a plasmid that is required for pathogenicity. The most commonly studied strain of *R. fascians* (D188) has a plasmid that is recognized to be responsible for pathogenicity in herbaceous plant species. This plasmid has been the focus of study by many groups since the 1970’s. The plasmid contains genes that produce cytokinins, plant hormones that are responsible for shoot proliferation. The presence of the plasmid in *R. fascians* indicates the bacteria may be pathogenic (meaning that it can cause disease and disease symptoms) when it is associated with herbaceous plant species. Plasmids can be unstable and due to different conditions during growth may not be transferred to the next generation of bacteria. Also, in regards to *R. fascians*,

studies have shown that leaves can have different populations of *R. fascians* with and without the plasmid. Does the absence of the plasmid mean that it is not pathogenic or virulent on pistachio? Not necessarily. Data shared by private communication with Melodie Putnam indicates that there are *Rhodococcus fascians* isolates that either lack the plasmid or have major re-arrangements of the plasmid and are still virulent on herbaceous plant species. In addition, the presence of a plasmid is no indication that the bacterium is pathogenic, since non-pathogenic strains may carry a plasmid, and yet lack the genes necessary to cause disease. The *Rhodococcus sp.* we have isolated may be genetically different from the *R. fascians* that infects herbaceous plants. We do not yet have enough information to know whether or not these *Rhodococcus sp.* isolated from these PBTS trees are pathogenic or virulent. We are currently performing in-depth genetic analyses on these *Rhodococcus spp.* The bacterial isolates are currently being tested both at New Mexico State University and Oregon State University to determine if they can cause symptoms on healthy pistachio trees.

We fully understand that many groups and individuals associated with the issue of PBTS are eager for results from the scientific community. We are too! However, it would be both irresponsible and unprofessional to draw conclusions (either positive or negative) on the research prior to completing the experiments, which is why simple precautionary measures such as disinfecting pruning and propagating tools should be suggested to growers during the investigative process until a pathogen can be confirmed or absolutely refuted. Because we are investigating something new, no one knows exactly how long is required or what the results will show. The experiments in work may take weeks or months to complete and must be performed rigorously and accurately.

Craig Kallsen, Citrus, Pistachios/Subtropical Horticulture Advisor
cekallsen@ucdavis.edu or 661-868-6221

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