

Pistachio Bushy Top Syndrome: The Association Between *Rhodococcus sp.* and Clonal UCB-1 Rootstock in Arizona and California

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Clonally propagated 'UCB-1' (*Pistacia atlantica* x *P. integerrima*) rootstock is an interspecific hybrid that is widely used by the pistachio industry. In the last three years, a large number of clonally-propagated 'UCB-1' pistachio rootstocks newly planted in California and Arizona orchards exhibited symptoms that include 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. The percentage of abnormal rootstocks within affected orchards varied from 8% to 63% 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.

Field samples along with ungrafted 'UCB-1' trees obtained from three different commercial sources were evaluated for pathogens. Asymptomatic and PBTS symptomatic 'UCB-1' trees from fields and nurseries were screened using optical and electron microscopy, PCR amplification, DNA sequencing analysis, and selective culturing techniques. PCR amplification using P1/P7 primers targeted to phytoplasmas [4] generated non-target amplicons from symptomatic field and nursery samples that were 99% similar to *Rhodococcus sp.* 16S rDNA (genbank ID KJ677036). Samples were subsequently screened using LAMP and PCR primers previously developed for *R. fascians* virulence genes [3]. PCR and sequence analysis using chromosomal virulence locus *vicA* [1] resulted in amplicons of the expected size that were 99% similar to *R. fascians* (genbank ID KJ677035). Transmission electron microscopy of PBTS symptomatic trees revealed signs of cell wall lysis and other histological abnormalities. Unidentified microbes were visualized both intercellularly and intracellularly. Live/dead staining for bacteria on leaf surface tissue analyzed via laser scanning confocal microscopy revealed a potential colonization of the leaf surface by clusters of living microbes. SYTO-9 (485/498) and propidium iodide (488/617) were used and 5 μ m images stacks were collected. Fresh leaf prints generated bacterial cultures on selective mD2 media from nursery and symptomatic field samples. These cultures resulted in the growth of yellow-orange, gram-positive colonies that were *vicA* PCR positive and pleomorphic in nature.

R. fascians previously known as *Corynebacterium fascians* is a known plant pathogen with a broad host range. Symptoms from this bacterium are often confused with those caused by phytoplasmas, viruses, *Agrobacterium tumefaciens*, and latent hormone effects in nursery settings [2]. *R. fascians* can be present on plants as an asymptomatic epiphyte or may gain entry into the plant where it modulates phytohormone activity that results in stunted growth, shortened internodes, bushy or bunchy top appearance, leaf galls, and modified root development [5]. While the genetic organization of *R. fascians* genotypes remains unclear, the presence of plasmid borne virulence factors may or may not be essential for phytopathogenicity. It is not known whether the *Rhodococcus sp.* isolated from 'UCB-1' rootstock is responsible for any/all of the observed symptoms or if it is a part of a larger disease complex.

References:

- [1] E. Nikolaeva, et al. *Plant Health Progress*. (2012) 10.1094/PHP-2012-0227-02-RS
- [2] M.L. Putnam, et al. *Plant Disease*. 91 (2007) 9:1064-1076
- [3] M. Serdani, et al. *Plant Disease*. (2013) 97:517-529
- [4] C.D. Smart, et al. *Applied and Environmental Microbiology*. (1996) 2988-2993
- [5] E. Stes, et al. *Annu. Rev. Phytopathol.* (2011) 49:69-86
- [6] Acknowledgements: Mid Valley Ranch, North Bowie Farming, AZ. New Mexico State University Agricultural Experiment Station.

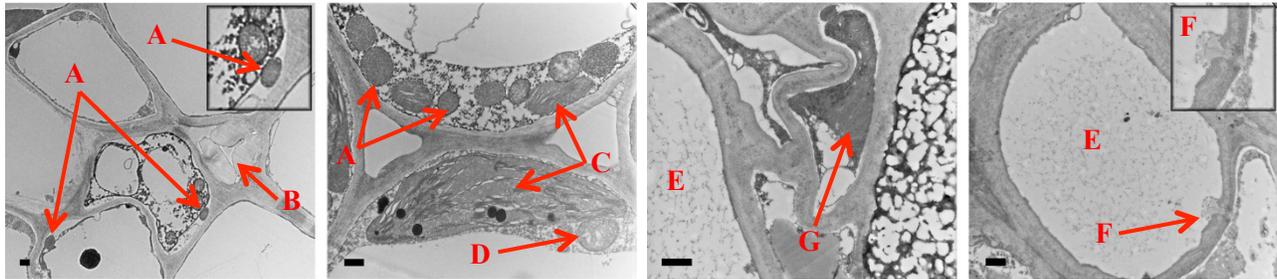


Fig. 1. (Above) PBTS symptomatic *Pistacia atlantica x integerrima* (UCB-1) leaf petiole vascular tissue transmission electron micrographs. A; Microbial bodies. B; Collapsed cells. C; Chloroplasts. D; Mitochondria. E; P-proteins. F; Localized cell wall lysis. G; Condensed cytoplasm. Scale bars, 500 nm.

Fig. 2. (Right) *Pistacia atlantica x integerrima* (UCB-1) leaf petiole vascular tissue transmission electron micrographs. Left panels indicate healthy tissue non-symptomatic of PBTS. Right panels indicate symptomatic tissue showing signs of pathogenic infection. A; collapsed phloem cells. B; Condensed cytoplasm. Scale bar, 2 μ m.

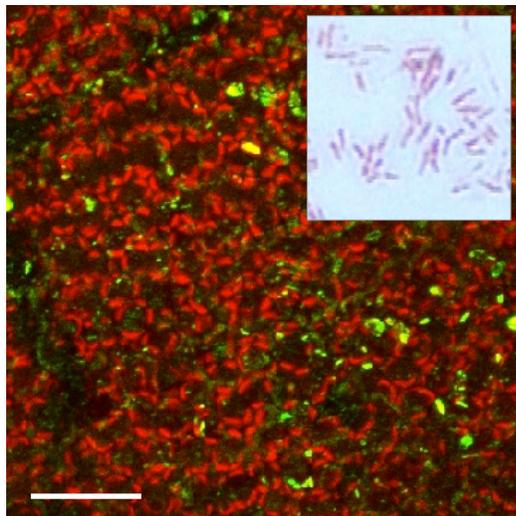
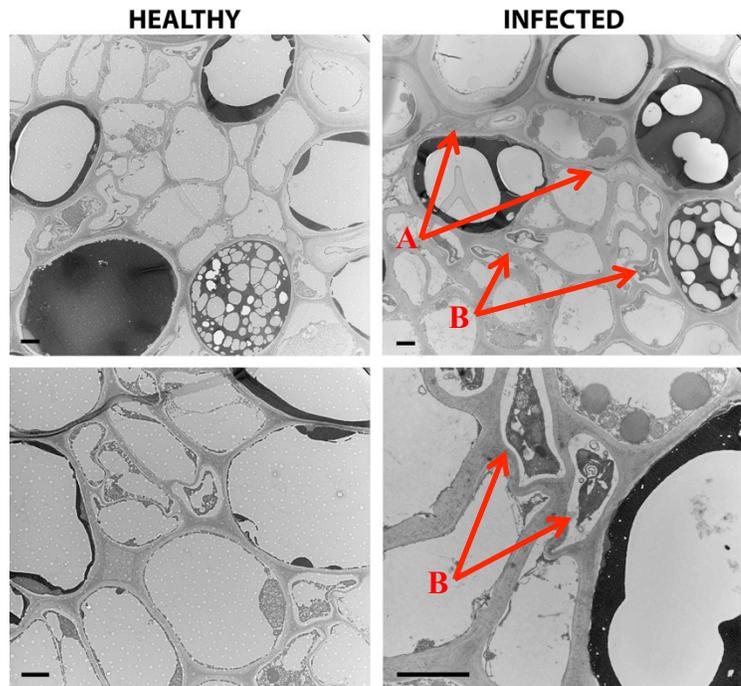


Fig. 3 (Left) PBTS UCB-1 leaf surface tissue imaged using a live/dead bacterial stain containing propidium iodide and SYTO-9. Green color indicates live microbes, red color indicates dead microbes and autofluorescence. Scale bar, 30 μ m. Upper right corner panel demonstrates gram stain of yellow-orange bacterial colony. Pleomorphic, gram-positive rods and clubs are shown (NTS).