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Biology and Ecology of *Rhodococcus fascians* D188, and Implications for Understanding and Managing Pistachio Bushy Top Syndrome

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With the emergence of *Rhodococcus* spp., the cause of pistachio bushy top syndrome (PBTS) in California, Arizona, and New Mexico, southern San Joaquin Valley pistachio growers welcomed a seminar by Dr. Danny Vereecke, a bacteriologist in the Department of Applied Bioscience at Ghent University in Belgium. Dr. Vereecke came to the United States as a visiting scientist in the laboratory of Dr. Jennifer Randall at New Mexico State University. Dr. Vereecke, a world expert on the ecology, pathogenicity, and molecular biology of *Rhodococcus fascians*, was hosted by her former graduate student, Dr. Isolde Francis, a new faculty member at CSU Bakersfield. The purpose of this article is to summarize the content of Dr. Vereecke's seminar, making the material available for those unable to attend and addressing the frequently-asked questions posed to farm advisors after the seminar.

Dr. Vereecke's seminar provided an overview on the interaction between *R. fascians* and model host plants, specifically *R. fascians* strain D188 (*Rf*-D188) on tobacco and Arabidopsis. *Rhodococcus fascians* is a generalist pathogen with a known host range of over 150 plant species in over 50 plant families. Prior to the association of *R. fascians* with PBTS in California, Dr. Vereecke's laboratory documented the susceptibility of *Pistacia vera* to *Rf*-D188 *in vitro* (Vereecke and Baghdadi, unpublished data). Inoculated *P. vera* developed witches' brooms or the leafy gall syndrome characteristic of diseases caused by *R. fascians* (Figure 1A). In a recent study, 3 month-old *P. vera* seedlings inoculated with isolates of *Rhodococcus* spp. from bushy top plants exhibited earlier breaking of lateral buds and greater total lateral shoot growth than uninoculated control plants (Fichtner, unpublished data) (Figure 1B). In similar studies, other woody plants, including Acacia and Poplar, were found sensitive to *Rf*-D188. This type of laboratory study is useful for determining the potential for a pathogen to affect various economically- or ecologically- important plant genera and/or species in advance of an epidemic in nature.

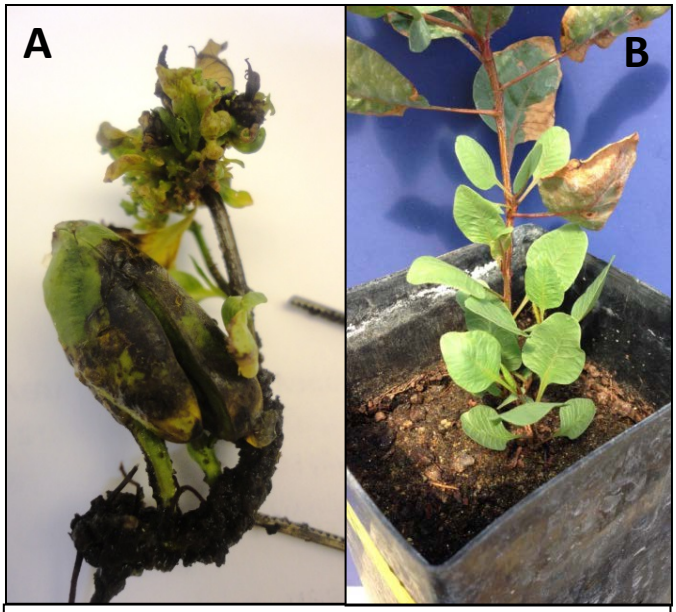

Figure 1. A) Leafy gall on *Pistacia vera* inoculated with *Rf*-D188 *in vitro* (Photo: D. Vereecke). B) Lateral bud break and shoot growth of *P. vera* seedlings inoculated with PBTS isolates of *Rhodococcus* spp. (Photo: E. Fichtner).

Biology of *Rhodococcus fascians* strain D188

Genetic components. *Rhodococcus fascians* strain D188 contains three components bearing genetic information: a circular plasmid, a linear plasmid, and a chromosome. The circular plasmid is not involved in the bacterium's ability to cause disease on plants. Both the linear plasmid and the chromosome contain genetic information

responsible for the interaction with the plant and/or pathogenicity. The linear plasmid is the fasciation-inducing plasmid. It contains genes responsible for the pathogen's ability to enter the plant and produce the suite of cytokinins altering plant growth and development. The linear plasmid is considered essential for pathogenicity of *Rf*-D188; however, it is not yet known whether this plasmid is necessary for pathogenicity of *Rhodococcus* spp. associated with PBTS.

Epiphytic phase. The first phase in the disease cycle is the introduction of the pathogen to the plant surface followed by colonization of external plant parts. During this epiphytic phase, *Rf*-D188 produces a biofilm that protects the bacterium from abiotic stresses. Plants remain asymptomatic during the epiphytic phase, making it possible for the bacterium to evade plant health inspectors. While outside the plant, chromosomal pathogen genes confer auxin production that induces the plant to exude nutrients. The bacterium, in turn, detects the plant's response and upregulates genes (*att* operon) on the linear plasmid. The *att* gene product is secreted from the bacteria and moved systemically in the plant. The *att* gene product facilitates the pathogen's entry into the plant, thus allowing for endophytic host colonization. A wound is not needed for *Rf*-D188 to gain entry to the plant.

Endophytic phase. Populations of the bacterium residing inside the host tend to retain the linear plasmid with greater frequency than epiphytic populations. The pathogen's ability to move within the plant is unknown and may vary between hosts and pathogen isolates. Regardless of the pathogen's movement within the plant, the Att compound can move within the plant. The cytokinins cause local symptom development in the area infected with *Rf*-D188.

Cytokinin production and symptom development. Plasmid-borne genes (*fas* operon) are responsible for cytokinin production. The pathogen produces five cytokinins that alter plant growth and development. These cytokinins are similar to plant-produced cytokinins, but a subset is methylated and the plant is unable to break them down. This cytokinin production leads to activation of existing meristems and induces the formation of new meristems. *Rf*-D188 also affects the plant's vasculature and floral development. For example, infection of tobacco with *Rf*-D188 causes the vasculature to look more like that of a woody plant. In Arabidopsis, it has also been shown to affect flower development.

Case study of replant issue. In Naples, Italy, a field outbreak of *R. fascians* on tobacco caused reduced root and shoot biomass. The pathogen was transmitted by grafting naturally-infected tissue onto healthy plants. Additionally, soil at the site became infested with *R. fascians* and transmitted the pathogen to healthy tobacco in a subsequent planting (Vereecke and Zoina, unpublished data).

Comparison of *Rf*-D188 with PBTS isolates. Isolates of *Rhodococcus* associated with PBTS include isolates closely related to *Rhodococcus corynebacteriodes* and *R. fascians*. The *fas* and *att* genes have been detected in PBTS isolates, suggesting that PBTS isolates contain the linear plasmid. The genome of *Rf*-D188 has been sequenced; however, the genomes of isolates associated with PBTS are currently being investigated.

Frequently asked questions about Rhodococcus and pistachio bushy top syndrome?

1. How prevalent is R. fascians in the southwestern United States? Have other hosts been identified? The prevalence of *R. fascians* in the southwestern United States is unknown. Since the emergence of PBTS, researchers have isolated *R. fascians* from plants unassociated with the PBTS epidemic (Fichtner and Randall, unpublished data).

2. What is a plasmid?
A plasmid is a small piece of double-stranded DNA within a cell. Genes critical for pathogenicity of *Rf*-D188 are housed on the plasmid. The potential role of the plasmid in infectivity of pistachio is yet unknown.

3. How are phytopathogenic Rhodococcus species diagnosed?
Isolates of *Rhodococcus* spp. associated with PBTS are easily detected by isolation on semi-selective medium. After approximately 7-10 days, bacterial colonies are selected based on morphology and color and streaked onto

fresh medium to achieve purity, a process called sub-culturing. After colonies are purified, they are identified using multiple molecular techniques including, but not limited to sequencing the 16S region of ribosomal DNA.

4. What is an epiphyte? Can Rf cause disease as an epiphyte?
An epiphyte is an organism colonizing the outside surfaces of a plant. *Rhodococcus fascians* can cause disease as an epiphyte.

Select References

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