Soil Survival, Root Infectivity, and Management of *Rhodococcus* spp. Causing Pistachio Bushy Top Syndrome

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INTRODUCTION

Pistachio bushy top syndrome (PBTS) has emerged as a new problem affecting commercial pistachio orchards planted between 2011 and 2016 in California, Arizona, and New Mexico. The etiology of PBTS has been associated with two species of gram-positive bacteria that are closely related to *Rhodococcus fascians* (*Rf*) and *Rhodococcus corynebacterioides* (Rc). *Rf* is a known plant pathogenic bacterium that causes plant disease by producing a suite of novel cytokinins that influence plant growth and development. To our knowledge, PBTS is the first association of *Rc* with plant disease. Symptoms of PBTS have largely been observed in clonally propagated UCB-1 rootstock, and include stunting, shortened internode length, swollen nodes, gall formation, and failure to bud. Because of the unprecedented nature of PBTS, no research-based data are available to predict the long-term productivity of PBTS-affected trees in orchards. As a consequence, most growers and land managers opted to either remove entire orchards or individual trees, depending on the level of disease incidence in affected orchards. The soil survival potential of PBTS isolates of *Rhodococcus* spp. and the potential for infectivity of roots of replants in former PBTS sites are two factors that may affect the health and productivity of replants. Additionally, the risk of reuse of tree stakes from PBTS sites is of concern to growers undergoing rogueing and replant operations. Soil survival studies conducted in 2016 indicated short-term (weeks-months) survival potential of both isolates, with longevity varying based on time of year, soil moisture, and soil type. Infested soilless potting medium has also been observed to support short-term (several months) survival of the bacteria and support infectivity of roots, particularly when both isolates are present.

The observed PBTS isolate survival in soilless potting medium and the potential for roots to serve as infection courts for these phytopathogenic bacteria suggest the ease of transmission and persistence in greenhouse propagation systems. Currently, there are no products registered for the suppression of populations of plant pathogenic *Rhodococcus* spp. on the phylloplane, and the emergence of PBTS has incited commercial interest in product development for this arena.

In an effort to address land management considerations and risk analyses of growers affected by PBTS, the following objectives were addressed in 2017: i) comparative analysis of the phenotype (suckering, trunk and scaffold diameters, bark texture, and yield) of PBTS trees compared to asymptomatic trees entering maturity; ii) determination of isolate presence on tree stakes; iii) assessment of survival potential of both *Rf* and *Rc* in field soils, including the influence of depth of inoculum, time of year, soil type, and soil moisture on survival; iv) investigation of root infectivity in soilless medium; and v) influence of a foliar-applied calcium silicate and silicon dioxide (Mainstay Si, Redox Chemical, LLC) .

RESULTS

Tree phenotype data was collected from 109 trees in a seventh-leaf, PBTS-affected Golden Hills block on clonal UCB-1 rootstock in southwestern Tulare County. PBTS incidence in the block was 17.4 percent. Trees symptomatic of PBTS had significantly smaller trunk circumference (P≤0.0001), more suckers (P≤0.0001), more suckers per cm trunk circumference (P≤0.0001), and smaller total scaffold circumference (P≤0.0001). Additionally mean trunk circumference, sucker number, suckers per cm circumference, and total scaffold circumference were significantly more variable then those of asymptomatic trees (P≤0.0001). Yield data was collected at harvest from symptomatic and asymptomatic trees (N=19) chosen at random within the block. Average yield was 13.1 and 48.9 lb/tree in symptomatic and asymptomatic trees, respectively (P≤0.0001), but the variance in yield was similar between symptomatic and asymptomatic trees (P≤0.08).

Last, the rootstock-bark morphology was rated to assess the degree of “alligator skin” appearance. The “alligator skin” bark morphology was significantly correlated with reduced trunk circumference (P≤0.0001), and asymptomatic trees all exhibited the same bark morphology (no alligator skin). Symptomatic trees clumped into two different groups, indicating variability of bark morphology within the population of PBTS-symptomatic trees.

Tree stakes and plastic tree wraps on symptomatic trees in two PBTS-affected orchards (Kern County and Tulare County) were assessed for infestation with *Rhodococcus* spp. in December 2016. Actinomycetes were routinely detected on stakes (N=14) and plastic wraps (N=14); however, stakes and tree wraps only yielded two and one *Rhodococcus* colonies, respectively, indicating low levels of contamination.

Soil survival studies initiated in February 2017 indicate an interactive influence of soil series and irrigation treatment (P≤0.002) and isolate and irrigation (P≤0.01), on pathogen survival over time. In general, populations of either bacterium decreased by at least 99 percent, by April 2017, and were only detectable at trace levels by August. Soil survival studies initiated in August 2017 indicate limited survival potential of the bacteria in dry soil during summer. Neither *Rf* nor *Rc* was detectable one week after *in situ* incubation of inoculum in a commercial orchard. Similarly, in a screen-house study, neither isolate was recovered after one-week incubation in soils maintained dry. Moistening soil weekly to container capacity (mimicking irrigation events) extended survival of *Rf* to one week post-introduction, but only in the Vanguard soil series. *Rf* was not recovered beyond one week post-introduction, regardless of soil type or moisture treatment. Only trace levels of *Rc* were detected 14 weeks after introduction to the Gambogy-Biggriz soil, indicating potential for trace-level persistence.

The potential for foliar application of Mainstay Si to suppress epiphytic populations of *Rhodococcus* spp. on clonal UCB-1 pistachio rootstock, in a commercial orchard, was investigated. In a randomized complete block design, Mainstay Si- treated plants exhibited a 90 percent, 80 percent, and 87percent reduction of epiphytic *Rhodococcu*s spp. on the adaxial (P≤0.02), abaxial (P≤0.01), and total (P≤0.01) foliar populations, respectively.

CONCLUSION AND APPLICATIONS

The results demonstrate the long-term survival potential and root-infectivity of PBTS isolates of *Rhodococcus* spp. in greenhouse systems, and provide preliminary data on the use of Mainstay Si (Redox Chemical, LLC) in suppressing epiphytic pathogen populations. Soil survival studies indicate limited survival potential of the bacteria under field conditions; trace recovery of *Rhodococcus* spp. on stakes and plastic tree wraps suggest minimal risk in transmission of

disease in the reuse or recycling of these materials. Last, PBTS-symptomatic trees entering maturity exhibit more variability than healthy trees in the same block, and symptomatic trees exhibit a 73 percent reduction in yield.