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Phenotypic and Physiological Characteristics of Pistachio Bushy Top Syndrome–affected Trees

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Abstract. **Pistachio bushy top syndrome (PBTS) emerged as a new disease of pistachio in the United States in 2011. The disease, caused by two phytopathogenic *Rhodococcus* spp., affected clonally propagated 'UCB-1' pistachio rootstocks, causing a suite of symptoms including stunting, shortened internodes, swollen lateral buds, bushy growth, altered bark and root morphology, and stem galls. Symptoms developed within 1 year of planting; however, the long-term influence of PBTS on orchard productivity was unknown because conventionally trained pistachio orchards are typically first harvested after the sixth season. The goal of this project was to determine the phenotypic characteristics and productivity of PBTS-affected trees in the seventh and eighth seasons, as trees entered maturity. Symptomatic and asymptomatic trees in a PBTS-affected orchard were evaluated for bark morphology, tree size, suckering potential, yield, nut quality, photosynthetic rate, foliar chlorophyll content, foliar nutritional status, stem water potential, and bloom progression. PBTS-affected trees exhibited at least a 20% reduction in growth, more than three times more suckering, and a greater than 50% reduction in yield compared with asymptomatic trees. In addition, PBTS-affected trees exhibited greater levels of blank nuts than asymptomatic trees; however, percent edible yield was unaffected by disease status. Symptomatic trees exhibited greater variability in tree size parameters, suckering, and edible yield than asymptomatic trees. The heterogeneity of PBTS symptomatic trees undermined the value of planting clonal rootstocks, and the deleterious impact of the syndrome on both yield and nut quality demonstrates that affected trees should be rogued upon initial symptom development rather than managed to maturity.**

Pistachio (*Pistacia vera* L.) (Family: Anacardiaceae) is a dioecious, deciduous perennial tree crop native to western Asia and Asia Minor (Ferguson et al. 2008). Pistachios have a long juvenile period, reaching full bearing potential ∼10 to 12 years after planting (Ferguson et al. 2008), and exhibit a strong alternate bearing tendency (Blank 2008). All commercial orchards in the United States are grown on rootstocks selected for tolerance to disease, cold, and salinity, as well as improved yield potential (Ferguson et al. 2008). The United States has more than 527,000 acres of pistachio, and California is responsible for more than 99% of domestic production (Department of Agriculture 2024).

Pistachio bushy top syndrome (PBTS) emerged as a new problem affecting commercial pistachio orchards planted in California, Arizona, and New Mexico between 2011 and 2016 (Stamler et al. 2015a, 2015b). PBTS manifested in orchards established on clonal 'UCB-1' rootstock, inciting a suite of symptoms including shortened internodes, stunted growth, swollen lateral buds, bushy/bunchy growth, stem galls with multiple buds, and twisted roots with minimal lateral branching (Fig. 1) (Stamler et al. 2015b). Disease incidence ranged from 10% to 90% in affected orchards, and the combined symptoms of PBTS resulted in off-type trees with altered growth and development (Stamler et al. 2015b). Because of the unprecedented nature of the disease, decisions to rogue off-type trees or remove entire orchards were made without prior knowledge of the future economic productivity of PBTS-affected trees (Fig. 1C). Traditionally, pistachio orchards have their first economic harvest after the sixth year (Klonsky et al. 2008); consequently, growers were reluctant to maintain and cultivate PBTS-affected trees for years after symptom development with the uncertainty of yield and longevity of off-type trees. Conservative estimates suggest that ∼1 million trees from 14,000 ha were affected by PBTS (Stamler et al. 2015b).

This unprecedented disease was caused by two gram-positive phytopathogenic bacteria in the genus *Rhodococcus* (Stamler et al. 2015b). One bacterium, *Rhodococcus fascians* (PBTS2), is a known plant pathogen with a wide host range including herbaceous perennials (Putnam and Miller 2007) as well as some trees and shrubs (Miller and Putnam 2010; Quoirin et al. 2004). The second bacterium, a relative of *Rhodococcus corynebacterioides* (PBTS1), was first identified as a phytopathogen with its association in PBTS (Stamler et al. 2015b). The mode of pathogenicity of *R. fascians* has been studied extensively in a model isolate (strain D188) (Stes et al. 2013) that has a large conjugative linear plasmid (pFiD188) imparting virulence (Crespi et al. 1992). pFiD188 contains the *fas* virulence locus, which consists of genes involved in cytokinin production and the induction of fasciation (Crespi et al. 1994). *R. fascians* produces methylated cytokinins that are recognized by cytokinin receptors in the host plant (Radhika et al. 2015). The pathogen-derived methylated cytokinins are more stable than plant-derived cytokinins (Jameson 2019; Radhika et al. 2015) and can thus persist in the host plant, altering growth and development. As a result, the mechanism of pathogenicity of *R. fascians* has been compared with that of other phytopathogenic bacteria that induce tissue hyperplasia by production of hormones such as cytokinins and/or auxins as virulence factors (Jameson 2019; Stes et al. 2013). Polymerase chain reaction amplification of both PBTS1 and PBTS2 generated *fasD* amplicons, and the sequences were 100% homologous to those of *fasD*, a virulence gene found on the pFiD188 plasmid (Stamler et al. 2015b), suggesting similarities in the mechanisms of pathogenicity between *R. fascians* D188 and the PBTS isolates. Further analyses demonstrated that PBTS isolates contain *fasC*, *fasE*, and *fasF*—all genes located on the pFiD188 plasmid; however, similar to other isolates of

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Fig. 1. Pistachio bushy top syndrome (PBTS) is characterized by a range of symptoms on 'UCB-1' rootstock. Adjacent healthy (**A**) and PBTS symptomatic (**B**) (stunted) orchards planted concurrently and maintained with the same horticultural practices. Symptomatic orchards were removed (**C**) before maturity to mitigate potential economic loss. Symptoms included swollen nodes (**D**); cracking bark, particularly at the graft union (**E**); compact root systems with reduced branching (**F**); shortened internodes (**G**); excessive suckering (**H**); and multiple buds at nodes (**I**).

R. fascians (Nikolaeva et al. 2009), these *fas* genes may only be maintained in a subpopulation of cells of each PBTS isolate (Vereecke et al. 2020). Introduction of PBTS isolates to plant material has been shown to enrich the population of cells containing *fas* genes (Vereecke et al. 2020). Recent studies verified the pathogenicity of PBTS1 and PBTS2 on 'UCB-1' rootstock and compared the pathogenicity of these isolates to that of D188 on tobacco (Vereecke et al. 2020). Symptoms resulting from infection with PBTS isolates were more variable than those resulting from D188 infection, a phenomenon that may be related to variable representation of *fas* genes within the inoculum population (Vereecke et al. 2020).

Since the emergence of PBTS, additional outbreaks of phytopathogenic *Rhodococcus* spp. associated with plant disease have been reported. Like PBTS, *Rhodococcus* spp. have recently been associated with disease on other plants propagated in tissue culture, including Japanese spindle, a woody perennial (Dhaouadi et al. 2020), and *Irsine herbstii*, a herbaceous perennial (Dhaouadi et al. 2019). In addition, epiphytic populations of phytopathogenic isolates of *Rhodococcus* spp. have been found on asymptomatic almond rootstocks and pistachio trees in commercial nurseries (Dhaouadi et al. 2021), demonstrating that the presence and threat of these pathogens to orchard systems is not limited to the PBTS pathosystem. More recently, *R. fascians* has also been associated with fasciated lilies in South Korea, resulting in the destruction of 1.3 million imported bulbs (Lim et al. 2021).

Clonal 'UCB-1' rootstocks were planted to avoid the heightened variability associated with seedling 'UCB-1' rootstocks (Jacygrad et al. 2020). 'UCB-1' is an interspecies cross of *Pistacia atlantica* and *Pistacia integerrima* that was originally developed for tolerance to *Verticillium*, salinity, and frost (Ferguson et al. 2008). The 'UCB-1' seedling population, originally selected for its resistance to Verticillium wilt (Morgan et al. 1992), is well known for its heterogeneity, resulting in orchards with variable tree size. Approximately 10% to 15% of seedling 'UCB-1' rootstocks are rogued within the first year in the nursery based on poor growth and visual observation (Palmer et al. 2023). Up to 30% of 'UCB-1' seedlings are of low vigor, but these low-vigor phenotypes are generally not manifest until multiple years of growth (Jacygrad et al. 2020). Because early tree growth of the 'UCB-1' seedlings is a poor predictor of later tree size, rogueing is not a satisfactory method of achieving uniform, vigorous populations of 'UCB-1' seedlings at the orchard level (Jacygrad et al. 2020). To produce a more homogeneous product, selections from 'UCB-1' seedling populations have been placed into tissue culture and micropropagated via axillary bud proliferation to generate clonal lines of commercial 'UCB-1' clonal rootstocks. One-year-old pistachio rootstocks are typically planted in the field in February or March and then budded in summer (Holtz 2008).

With the emergence of PBTS, growers were concerned that affected rootstocks could influence yield and nut quality as well as the structural integrity of trees upon the introduction of vigorous shaking associated with mechanical harvest. Historical research on pistachio rootstocks indicates that annual yield is related positively to trunk cross-sectional area (Crane and Iwakiri 1986); therefore, growers were concerned that the smaller tree size associated with PBTS could result in reduced yield. In addition, prior studies indicate that rootstock may influence the degree of shell splitting and the percentage of blank nuts at harvest (Crane and Iwakiri 1986; Tajabadipour et al. 2006). As a result, the potential for PBTS to affect nut quality and consequent nut value are also of concern to the industry. Last, similar scion and rootstock growth rates contribute to the structural integrity of graft unions and a uniform exterior tree surface, thus facilitating harvest with trunk-shaking equipment (Kallsen and Parfitt 2011). PBTS-affected trees exhibited excessive bark cracking around the bud union (Fig. 1E) (Stamler et al. 2015b). Consequently, the structural integrity of PBTS-affected trees upon introduction of mechanical harvest with trunk shakers is also of concern to both growers and custom harvesters.

The potential influence of PBTS on the physiological characteristics of the scion are also unknown. Pistachio rootstock selections have been shown to influence micro- and macronutrient content in scion foliage (Sherafati et al. 2011), as well as leaf chlorophyll and soluble sugar contents (Ghrab et al. 2016). In other orchard systems, rootstock selection has been found to influence bloom date (i.e., Young and Houser 1980). In addition, heterogeneity of tree size resulting from rootstock vigor may influence tree water stress, as measured by midday stem water potential, with smaller trees generally having greater (less negative) water potential than larger trees initially after irrigating, but often becoming more stressed by the end of the irrigation cycle, making it difficult to satisfy the individual irrigation needs of each tree along a tree row in orchards.

Our study was designed to assess the long-term influence of PBTS on tree characteristics and orchard productivity as trees enter maturity, allowing future growers to make informed, research-based decisions in the advent of subsequent PBTS outbreaks. The influence of PBTS on phenotypic characteristics including trunk bark anatomy, trunk and scaffold diameter, and yield and nut quality were evaluated on trees in their seventh and eighth field seasons. Suckering potential was evaluated on trees in the seventh and ninth field seasons. In addition, the influence of PBTS on physiological characteristics, including foliar nutritional status, photosynthesis, foliar chlorophyll content, and midday stem water potential, was assessed.

Fig. 2. Rootstocks were ranked according to bark anatomy, with 0 point (**A**) designating trees with generally smooth bark and no cracking, and 1 (**B**), 2 (**C**), and 3 (**D**) points characterizing trees with increasing depth of grooves or cracking in rootstock bark.

Materials and Methods

Site description. The study was conducted in a commercial block of ‘Golden Hills’ on clonal ‘UCB-1’ rootstock in southwestern Tulare County, CA, USA. The orchard was planted in 2011 with trees from a single clonal rootstock source and was one of the first orchards in which the irregular plant morphologies associated with PBTS were observed. Incidence of PBTS in the experimental block was 17.4%. PBTS symptomatic trees were removed in 2016 and 2017 and replanted with budded trees on seedling ‘UCB-1’ rootstock; however, a portion of the block (∼2 ha) was reserved for research purposes. All trees were managed similarly regardless of PBTS status.

Tree measurements. In Mar 2017, 109 trees (90 asymptomatic and 19 symptomatic) were flagged for repeated data collection over time. In Apr 2017 and Apr 2018, trunk diameter was measured on each flagged tree just above the graft union, and the caliper diameter of each scaffold was measured. The total scaffold diameter per tree was determined by summation of all scaffold diameters on each tree. Rootstock bark texture was assessed on each tree using a rating scale of 0 to 3 points, with trees ranked 0 point having relatively smooth bark and those ranked 3 points having a cracked topography (Fig. 2). In May 2017, the number of rootstock suckers was counted on each tree. Rootstock suckers were excised routinely during the 2018 season; consequently, sucker data were not captured in 2018, but were again gathered in May 2019. Suckering frequency data were calculated both as the number of suckers per tree and the number of suckers per unit tree circumference to make comparisons across trees of different sizes.

Yield measurement. A subsample of trees was selected at random for harvest (n = 19 symptomatic and n = 19 asymptomatic). Yield data were collected on these same 38 trees over two successive years. Because of concern regarding the structural integrity of the graft union of PBTS trees, all trees were hand-harvested. Trees were harvested on 1 Sep 2017 and 4 Sep 2018 using mallets and poles to dislodge the nuts and capture the crop on tarps. Large debris (leaves and twigs) were removed from the crop, and the total mass of nuts from each tree was determined in the field. From each tree, a 9-kg subsample was collected for commercial grading. For trees yielding less than 9 kg, the sample consisted of the entire yield from the tree. Subsamples were brought to a commercial processor for grading, and the percent edible yield and percentage of blanks were determined.

Bloom progression assessment. In 2018, the progression of bloom was documented by tagging branches on four symptomatic and four asymptomatic female trees. Eight to 11 shoots were tagged on each tree, and buds

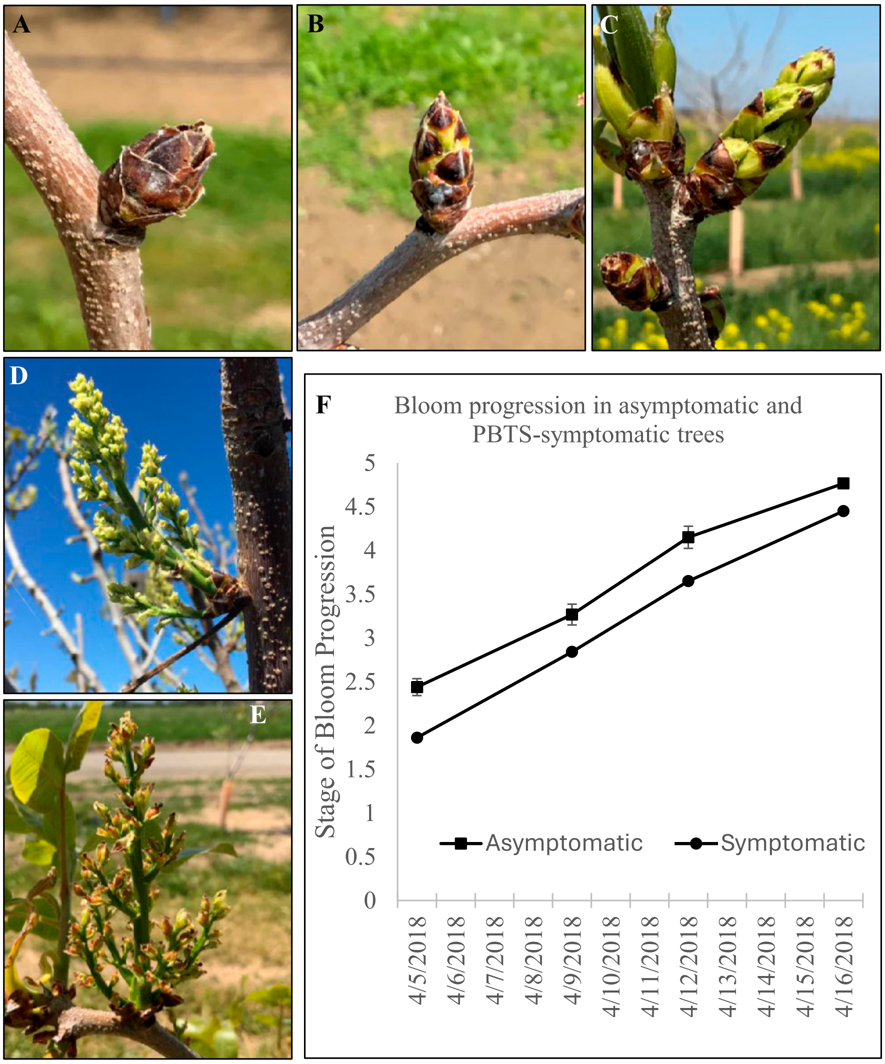

Fig. 3. Stages of bud development: first stage, total brown (**A**); second stage, beginning green (**B**); third stage, extended green (**C**); fourth stage, opening (**D**); and fifth stage, nut set (**E**). Bloom progression in asymptomatic and PBTS symptomatic trees (**F**).

(50 buds per tree) on tagged branches were observed for 11 d. The bloom progression was documented using a numerical scale of stages 1 to 5 (Fig. 3), and each bud was monitored and rated on the following days: 5, 9, 12, and 16 Apr. The numerical five stages of bloom correspond to the following phenological stages of bloom: stage 1, total brown; stage 2, beginning green; stage 3, extended green; stage 4, opening; and stage 5, fruit setting.

Table 1. Trunk and scaffold characterization[i] of symptomatic and asymptomatic trees in a pistachio bushy top syndrome–affected orchard in Tulare County, CA, USA, in the seventh and eighth field seasons.

	Trunk diam		Sum scaffold diam	
Season, year	Mean (cm)	Variance (s^2)	Mean (cm)	Variance (s^2)
Seventh season, 2017				
Asymptomatic[ii]	10.7	0.5	22.2	9.2
Symptomatic	8.6	1.9	13.7	14.2
P value[iii]	≤0.0001	≤0.0001	≤0.0001	≤0.0001
Eighth season, 2018				
Asymptomatic[ii]	11.8	0.61	25.3	54.8
Symptomatic	8.7	1.5	15.7	16.4
P value[iii]	≤0.0001	≤0.0001	≤0.0001	≤0.0001

[i] In Apr 2017 and 2018, the trunk caliper diameter above the graft union was measured as well as the caliper diameter of each scaffold. The sum of the scaffold diameters was calculated to serve as an estimate of canopy size.

[ii] Asymptomatic trees, n = 90; symptomatic trees, n = 19.

[iii] Mean trunk diameters and sum of scaffold diameters were compared with a Welch two-sample *t* test, and sum variances of each variable were compared using a one-sided F-test.

Physiological characteristics. Ten symptomatic and 10 asymptomatic trees were selected randomly for the measurement of photosynthesis rate, foliar chlorophyll content, midday stem water potential, and foliar nutrient analysis. Net photosynthetic assimilation rate and midday stem water potential were measured on 9 Sep 2019 using the LI-6400XT Portable Photosynthesis System (LI-COR, Lincoln, NE, USA), with a photosynthetically active radiation of 1400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a block temperature of 23 °C, a reference CO_2 level of 400 ppm, and ambient humidity. Fully expanded terminal leaflets (three replicate leaflets per tree) on nonbearing branches in maximum sun exposure positions were selected for photosynthesis measurements. For the midday stem water potential measurement, a single leaf in an interior, shaded location on the northeast side of the tree was chosen on each data tree. Foliar chlorophyll content was measured on fully expanded terminal leaflets in full sun exposure positions on 9 Sep 2019 (three leaflets per tree) and again on 28 Oct 2019 (4 leaflets per tree) using a chlorophyll concentration meter (Apogee Instruments, Logan, UT, USA).

Foliar nutritional analysis. In Jul 2019, leaves were collected from individual symptomatic (n = 19) and asymptomatic (n = 19) trees for foliar analysis of percent nitrogen, phosphorous, potassium, calcium, magnesium, chloride, and sodium, and parts per million of zinc, manganese, iron, copper, and boron. Twenty-five leaves were collected from the midcanopy of each of four quadrants of the canopy, for a total of 100 leaves collected per tree. Samples were sent to a commercial analytical laboratory for analysis.

Statistical analysis. All the statistical analyses were conducted in RStudio (ver. 2.14.0; R Foundation for Statistical Computing, Vienna, Austria). Statistical analyses for trunk diameter, sum of scaffold diameters, sucker frequency, suckers per unit circumference, yield, foliar nutrition, photosynthesis, chlorophyll content, water potential, percent edible yield and percentage of blanks were performed using a one-sided F-test for evaluating true difference in variance between asymptomatic and symptomatic trees, and using a Welch two-sample *t* test for investigating true difference in means. For the bloom progression, the average stage of bloom per shoot and tree was determined using a linear mixed-effects model, with time as a repeated measure.

Results

A total of 109 trees within the research block were categorized for the degree of bark cracking. The frequency of trees with a bark categorization of 0 to 3 within the plot was as follows: 0 point (n = 90), 1 point (n = 5), 2 points (n = 11), and 3 points (n = 3). Trees given the categorical ranking of 0 point for bark morphology were categorized as asymptomatic; asymptomatic trees represented 82.6% of the plot. Symptomatic trees (ranked 1–3 points in bark morphology) represented 17.4% of the plot. Because of the unequal distribution

Table 2. Suckering potential of symptomatic and asymptomatic trees in a pistachio bushy top syndrome–affected orchard in Tulare County, CA, USA, in the seventh and ninth field seasons.

	No. of rootstock suckers per tree[i]		No. of rootstock suckers per unit trunk circumference[ii]	
Season, year	Mean (no.)	Variance (s^2)	Mean (no./cm)	Variance (s^2)
Seventh season, 2017				
Asymptomatic	6.8	26.2	0.19	0.02
Symptomatic	25.1	121.7	0.88	0.20
P value[iii]	≤0.0001	≤0.0001	≤0.0001	≤0.0001
Ninth season, 2019				
Asymptomatic	1.7	3.7	0.04	0.002
Symptomatic	15.6	77.8	0.51	0.106
P value[iii]	≤0.0001	≤0.0001	≤0.0001	≤0.0001

[i] The suckering potential of symptomatic (n = 19) and asymptomatic (n = 90) trees was assessed in May 2017 and May 2019. The number of suckers on each rootstock was counted.

[ii] The number of suckers per centimeter trunk circumference was calculated to account for variability resulting from tree size.

[iii] Means were compared with a Welch two-sample *t* test, and sum variances of each variable were compared using a one-sided F-test.

of the number of trees in each bark morphology category, the bark morphology rankings were not used as independent variables to assess for a putative relationship of bark morphology to dependent variables.

Trees symptomatic of PBTS had significantly smaller mean trunk diameters and mean total scaffold diameter than asymptomatic trees during both years ($P \leq 0.0001$) (Table 1). In 2017 and 2018, symptomatic trees exhibited 20% and 26% smaller trunk diameters than asymptomatic trees, respectively. Total scaffold diameter was 38% less than that of asymptomatic trees in both years (Table 1). The sum variance (s^2) of both trunk circumference and total scaffold diameter was greater for symptomatic trees than asymptomatic trees ($P \leq 0.0001$) in both years (Table 1).

Symptomatic trees had statistically more suckers ($P \leq 0.0001$) per tree and more suckers per unit tree circumference ($P \leq 0.0001$) than asymptomatic trees in 2017 and 2019 (Table 2). Symptomatic trees had 3.7 and 9.2 times the number of suckers of asymptomatic trees in 2017 and 2019, respectively (Table 2). In addition, symptomatic trees had more variability in sucker frequency and the number of suckers per unit of trunk circumference than asymptomatic trees ($P \leq 0.0001$) in both years (Table 2).

Progression of bloom was significantly influenced by PBTS over time. Symptomatic trees were consistently behind asymptomatic trees in bloom progression (Fig. 3). Bloom progress of symptomatic trees was generally 2 to 3 d behind that of asymptomatic trees throughout bloom.

PBTS had a significant effect on both yield and nut quality. PBTS symptomatic trees exhibited 73% and 55% lower yields than asymptomatic trees in 2017 and 2018, respectively (Table 3). The percentage of blanks was significantly greater in samples from symptomatic trees than asymptomatic trees in both years ($P \leq 0.05$) (Table 4). Percent edible yield was statistically less in symptomatic trees than asymptomatic trees in 2017, but not in 2018 (Table 4). The variability in yield per tree was similar between symptomatic and asymptomatic trees in both years (Table 3). The percentage of blanks was more variable in samples from symptomatic trees than asymptomatic trees in 2017, and percent edible yield was more variable in samples from symptomatic trees in both years (Table 4).

Midday stem water potential and net photosynthetic assimilation rate did not differ between symptomatic and asymptomatic trees (Table 5) in Sep 2019. Foliar chlorophyll was different between asymptomatic and symptomatic trees at the 28 Oct 2019 sample time, but not at the 9 Sep 2019 sample time (Table 5). In late October, symptomatic trees had 11% less foliar chlorophyll content than asymptomatic leaves (Table 5). Only two nutrients, Ca and Mg, varied significantly in foliar samples collected from asymptomatic and symptomatic trees ($P \leq 0.001$). Mean foliar Ca levels were 2.21% and 1.99% in asymptomatic and symptomatic trees, respectively, and mean foliar Mg levels were 0.54% and 0.49% in asymptomatic and symptomatic trees, respectively. Consequently, symptomatic trees had 10% and 9% lower levels of foliar Ca and Mg, respectively.

Table 3. Yield of symptomatic and asymptomatic trees in a pistachio bushy top syndrome–affected orchard in Tulare County, CA, USA in the seventh and eighth field seasons.

	Yield[i]	
Season, year	Mean (kg/tree)	Variance (s^2)
Seventh season, 2017		
Asymptomatic	22.2	9.8
Symptomatic	5.9	23.5
P value[ii]	≤0.0001	≤0.08
Eighth season, 2018		
Asymptomatic	31.9	40.8
Symptomatic	14.2	57.5
P value[ii]	≤0.0001	≤0.23

[i] Yields of individual asymptomatic (n = 19) and symptomatic (n = 19) trees were assessed by hand harvest 1 Sep 2017 and 4 Sep 2018.

[ii] Means were compared with a Welch two-sample *t* test, and variances of each variable were compared using a one-sided F-test.

Discussion

The results of our study indicate an adverse and progressive impact of PBTS on tree size, with symptomatic trees growing at a slower rate than asymptomatic trees. The influence of PBTS on tree size may be the primary factor responsible for the reduction in yield associated with the syndrome. This is supported by the similar photosynthetic rates between symptomatic and asymptomatic trees. In the crown gall pathosystem in walnut, *Agrobacterium tumefaciens* has been found to reduce tree size and consequent yield (Epstein et al. 2008). Walnut yield is reduced in direct relation to the percentage of the rootstock circumference colonized by gall tissue (Epstein et al. 2008). PBTS caused a 73% and 55% decrease in yield in 2017 and 2018, respectively, which translates to an annual loss of revenue of more than $76 per infected tree, if nuts are valued at $2.12/lb. (Baldwin et al. 2020).

The size difference between symptomatic and asymptomatic trees may be explained in part by the relative irrigation levels observed by trees along a row. The irrigation was managed to meet the evapotranspiration demands of the large (asymptomatic) trees; therefore, the smaller (symptomatic) trees were overwatered and will continue to be increasingly overwatered over successive seasons.

Nut quality was also adversely affected by PBTS. One measure of nut quality for pistachio is the percentage of blank nuts in a sample, meaning nuts that consist of a shell with no edible nut inside. Samples of nuts from PBTS symptomatic trees had 97% and 27% more blank nuts than asymptomatic trees in 2017 and 2018, respectively. Reduced pollination success is the most likely cause of the heightened frequency of blank nuts produced in symptomatic trees. The 2- to 3-d delay in female bloom progression in symptomatic trees may influence the duration of overlap of male and female bloom. This time delay would also result in different environmental conditions present during peak flower receptivity. In our study, bloom progression was only assessed for female trees, thus the influence of PBTS on catkin maturation is unknown. In 2017, the edible yield was also 7% less in samples from symptomatic trees than asymptomatic trees. Growers only receive payment for filled nuts, with nuts containing split shells receiving the highest price from processors (Baldwin et al. 2020). Blank nuts have zero financial value; therefore,

Table 4. Percent edible yield and percent blanks were compared on symptomatic and asymptomatic trees in a pistachio bushy top syndrome–affected orchard in Tulare County, CA, USA, in the seventh and eighth field seasons.

Season, year	Blank nuts[i] Mean (%)[ii]	Variance (s^2)[iii]	Edible yield[i] Mean (%)	Variance (s^2)
Seventh season, 2017				
Asymptomatic[iii]	1.8	0.52	34.5	7.0
Symptomatic	3.5	5.61	32.2	28.1
P value[iii]	≤0.004	≤0.00001	≤0.05	≤0.01
Eighth season, 2018				
Asymptomatic	1.1	0.15	39.8	0.83
Symptomatic	1.4	0.31	40.0	6.9
P value[iii]	≤0.04	≤0.07	≤0.58	≤0.0001

[i] The percentage of blanks and edible yield is a measure of nut quality. Both parameters were determined by grading at a commercial processor.

[ii] Means were compared with a Welch two-sample *t* test, and variances of each variable were compared using a one-sided F-test.

[iii] Samples were collected from each individual asymptomatic (n = 19) and symptomatic (n = 19) tree at harvest.

factors promoting increased levels of blank nuts at harvest affect the economic returns to growers and reduce the profitability of pistachio farming operations. The combined influence of PBTS on both yield and nut quality indicate a significant impact of the syndrome on the economic sustainability of affected orchards.

Our results also validate grower accounts of increased orchard management costs associated with PBTS symptomatic trees. PBTS symptomatic trees produced up to nine times more suckers than asymptomatic trees, a growth characteristic that requires extensive hand labor for removal of excess growth. Another management cost associated with PBTS included a greater rate of rebudding of trees resulting from failure of the graft union (Stamler et al. 2015b).

Several of the measured physiological parameters differed significantly between symptomatic and asymptomatic trees. For example, chlorophyll levels in late October were significantly higher in asymptomatic trees than symptomatic trees. Symptomatic trees appeared to enter senescence earlier than asymptomatic trees, a factor that may explain the difference in foliar chlorophyll level at that time of year. In addition, foliar nutritional status of Mg and Ca was greater in asymptomatic trees than symptomatic trees. In the test orchard, the fertility program was designed to provide both nutrients at sufficient levels to meet tree needs, and both nutrients are available for root uptake via mass flow. Consequently, the lower levels of foliar Ca and Mg in symptomatic trees is likely related to the off-type architecture of the root system of PBTS symptomatic trees—namely, the reduced lateral root branching on affected trees (Stamler et al. 2015b), which may limit the area available for uptake of nutrients via mass flow.

Asymptomatic trees were less variable than PBTS-affected trees in tree size, sucker-ing potential, and degree of bark cracking. All asymptomatic trees were ranked as 0 point for bark morphology, whereas symptomatic trees were variable in the degree of bark cracking. The low variability in phenotypic characteristics of asymptomatic trees is consistent with the expected performance of clonal rootstocks. The variability of PBTS-affected trees is consistent with the expected manifestation of disease symptoms that may vary based on parameters affecting the disease progress curve, including the frequency of virulence gene representation, such as *fas*, in the primary inoculum. Upon the emergence of PBTS, and before the detection and proof of pathogenicity of PBTS isolates of *Rhodococcus* spp., a genetic mutation in the clonal line was hypothesized as a potential cause of PBTS. The heightened variability of PBTS symptomatic trees in comparison with asymptomatic trees supports the evidence of a disease-based causality to PBTS over the proposed mutation-based causality, because clonal populations of a mutated clonal line should still be homogeneous, albeit off-type. In addition, a second outbreak of PBTS was associated with a different source of clonal 'UCB-1' rootstock several years after the first outbreak. The symptoms of PBTS were identical in both outbreaks, yet the clonal lines were not shared between sources, thus supporting the evidence of disease-based causality to the syndrome. Since the outbreak of PBTS, researchers have quantified the variability of the seedling 'UCB-1' population (Jacygrad et al. 2020), underscoring the value of true-to-type clonal lines of this rootstock. Genetic markers associated with tree vigor in 'UCB-1' seedling populations were recently identified, providing new opportunities for marker-assisted selection of superior 'UCB-1' seedling rootstocks (Palmer et al. 2023). The combination of advances in marker-assisted breeding and enhanced phytosanitary practices to prevent future disease outbreaks in micropropagated systems would help facilitate the development and rapid propagation of superior, pathogen-free rootstock selections for growers.

Table 5. Midday stem water potential, net photosynthetic assimilation rate, and foliar chlorophyll content were assessed on symptomatic and asymptomatic trees in a pistachio bushy top syndrome–affected orchard in Tulare County, CA, USA, in the ninth field season.

Variable	9 Sep 2019 Midday stem water potential (bars)	Net photosynthetic assimilation rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)[i]	Foliar chlorophyll ($\mu mol \cdot m^{-2}$)[ii]	28 Oct 2019 Foliar chlorophyll ($\mu mol \cdot m^{-2}$)
Asymptomatic[iii]	7.2	18.2	602.7	485.4
Symptomatic	7.1	16.7	599.5	430.8
P value[iv]	0.29	0.11	0.42	0.02

[i] Net photosynthetic assimilation rate was measured on three replicate, fully expanded terminal leaflets on nonbearing branches in full sun–exposed positions on each tree.

[ii] Foliar chlorophyll content was measured on fully expanded terminal leaflets in full sun exposure positions on three and four leaflets per tree in September and October, respectively.

[iii] Physiological measurements were taken on pistachio bushy top syndrome symptomatic (n = 10) and asymptomatic (n = 10) trees.

[iv] Means were compared with a Welch two-sample *t* test, and variances of each variable were compared using a one-sided F-test.

References Cited

Baldwin R, Battany M, Beede R, Brar GS, Culumber M, Ferguson L, Fichtner E, Gordon P, Hanson B, Haviland D, Hembree K, Kallsen CE, Marino G, Sanden B, Michailides TJ, Trouillas F, Zazzaria D, Harris L, Stewart D, Sumner DA. 2020. University of California Agriculture and Natural Resources Cooperative Extension Agricultural Issues Center UC Davis Department of Agricultural and Resource Economics sample costs to establish and produce pistachios. University of California Press, Davis, CA, USA.

Blank S. 2008. Economic consideration in pistachio production, p 1–10. In: Ferguson L (ed). Pistachio production manual (5th ed). Omnipress, Madison, WI, USA.

Crane JC, Iwakiri BT. 1986. Pistachio yield and quality as affected by rootstock. HortScience. 21(5):1139–1140. https://doi.org/10.21273/HORTSCI.21.5.1139.

Crespi M, Messens E, Caplan AB, van Montagu M, Desomer J. 1992. Fasciation induction by the phytopathogen *Rhodococcus fascians* depends upon a linear plasmid encoding a cytokinin synthase gene. EMBO J. 11(3):795–804. https://doi.org/10.1002/j.1460-2075.1992.tb05116.x.

Crespi M, Vereecke D, Temmerman W, Van Montagu M, Desomer J. 1994. The *fas* operon of *Rhodococcus fascians* encodes new genes required for efficient fasciation of hast plants. J Bacteriol. 176(9):2492–2501. https://doi.org/10.1128/jb.176.9.2492-2501.1994.

Department of Agriculture. 2024. 2022 Census of agriculture. United States summary and state data, vol. 1. Geographic area series, part 51. AC-22-A-51. United States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS), Washington, DC.

Dhaouadi S, Hamdane AM, Bahri BA, Rhouma A, Fichtner EJ. 2019. First report of *Rhodococcus* spp. isolates causing stunting and lateral stem proliferation of *Iresine herbstii* 'Aureo-Reticulata' in Tunisia. Phytopathol Mediterranea. 58:391–394. https://doi.org/10.14601/Phytopathol_Mediter-10626.

Dhaouadi S, Hamdane AM, Rhouma A. 2021. Isolation and characterization of *Rhodococcus* spp. from pistachio and almond rootstocks and trees in Tunisia. Agronomy. 11(2):355. https://doi.org/10.3390/agronomy11020355.

Dhaouadi S, Mougou AH, Gleason ML, Rhouma A, Fichtner EJ. 2020. First report of bushy stunt of Japanese spindle caused by *Rhodococcus* spp. in Tunisia. Plant Dis. 104:1250. https://doi.org/10.1094/PDIS-10-19-2066-PDN.

Epstein L, Kaur S, McKenna JR, Grant JA, Olson WH, Reil WO. 2008. Crown gall can spread between walnut trees in nurseries and reduce future yields. Hilgardia. 62(3):111–115. https://doi.org/10.3733/ca.v062n03p111.

Ferguson L, Polito V, Kallsen C. 2008. The pistachio tree: Botany and physiology, p 40–42. In: Ferguson L (ed). Pistachio production manual (5th ed). Omnipress, Madison, WI, USA.

Ghrab M, Chelli-Chaabouni A, Mimoun MB. 2016. Behaviour of scion–rootstock combinations of pistachio under Mediterranean climate. Acta Hortic. 1109:155–160. https://doi.org/10.17660/ActaHortic.2016.1109.25.

Holtz B. 2008. Rootstock production and budding, p 78–83. In: Ferguson L (ed). Pistachio production manual (5th ed). Omnipress, Madison, WI, USA.

Jacygrad E, Preece JE, Palmer WJ, Michelmore R, Golino D. 2020. Phenotypic segregation of seedling 'UCB-1' pistachio rootstock. Trees. 34(2):531–541. https://doi.org/10.1007/s00468-019-01934-2.

Jameson PE. 2019. Virulent *Rhodococcus fascians* produce unique methylated cytokinins. Plants. 8(12):582. https://doi.org/10.3390/plants8120582.

Kallsen CE, Parfitt DE. 2011. Comparisons of scion/rootstock growth rates among U.S. pistachio cultivars. HortScience. 46(2):197–200. https://doi.org/10.21273/HORTSCI.46.2.197.

Klonsky KM, Beede RH, Kallsen CE, Holtz BA, Ferguson L, DeMoura RL. 2008. Sample costs to establish and produce pistachios (low-volume irrigation), p 11–39. In: Ferguson L (ed). Pistachio production manual (5th ed). Omnipress, Madison, WI, USA.

Lim YJ, Kong HG, Lee H, Kim HR, Park DH. 2021. First report of *Rhodococcus fascians* causing fasciation of lilies (*Lillium longiflorum*) in South Korea. Plant Dis. 105(4):1190. https://doi.org/10.1094/PDIS-10-20-2288-PDN.

Miller ML, Putnam ML. 2010. *Sorbaria sorbifolia* is a new host for *Rhodococcus fascians*. Plant Health Progress. 11(1). https://doi.org/10.1094/PHP-2010-0408-01-BR.

Morgan D, Epstein PL, Ferguson L. 1992. Verticillium wilt resistance in pistachio rootstock cultivars: Assays and an assessment of two interspecific hybrids. Plant Dis. 76(3):310–313. https://doi.org/10.1094/PD-76-0310.

Nikolaeva EV, Park S-Y, Kang S, Olson TN, Kim SH. 2009. Ratios of cells with and without virulence genes in *Rhodococcus fascians* populations correlate with degrees of symptom development. Plant Dis. 93(5):499–506. https://doi.org/10.1094/PDIS-93-5-0499.

Palmer W, Jacygrad W, Sagayaradj S, Cavanaugh K, Han R, Bertier L, Beede B, Kafkas S, Golino D, Preece J, Michelmore R. 2023. Genome assembly and association tests identify interacting loci associated with vigor, precocity, and sex in interspecific pistachio rootstocks. G3. 13(2):jkac317. https://doi.org/10.1093/g3journal/jkac317

Putnam ML, Miller ML. 2007. *Rhodococcus fascians* in herbaceous perennials. Plant Dis. 91(9):1064–1076. https://doi.org/10.1094/PDIS-91-9-1064.

Quoirin M, Bona C, de Souza EF, Schwartsburd PB. 2004. Induction of leafy galls in *Acacia mearnsii* De Wild seedlings infected by *Rhodococcus fascians*. Braz Arch Biol Technol. 47(3):339–346. https://doi.org/10.1590/S1516-89132004000300002.

Radhika V, Ueda N, Tsuboi Y, Kojima M, Kikuchi J, Takuji K, Sakakibara H. 2015. Methylated cytokinins from phytopathogen *Rhodococcus fascians* mimic plant hormone activity. Plant Physiol. 169(2):1118–1126. https://doi.org/10.1104/pp.15.00787.

Sherafati A, Hokmabadi H, Taheri M. 2011. Effects of some rootstocks on mineral nutrient uptake in two pistachio cultivars (*Pistacia vera* L.). Acta Hortic. 912:197–201. https://doi.org/10.17660/ActaHortic.2011.912.28.

Stamler RA, Heerema RJ, Randall JJ. 2015a. First report of phytopathogenic *Rhodococcus* isolates on pistachio bushy top syndrome 'UCB-1' rootstock in New Mexico. Plant Dis. 99(12):1854–1855. https://doi.org/10.1094/PDIS-04-15-0471-PDN.

Stamler RA, Kilcrease J, Kallsen C, Fichtner EJ, Cooke P, Heerema RJ, Randall JJ. 2015b. First report of *Rhodococcus* isolates causing pistachio bushy top syndrome on 'UCB-1' rootstock in California and Arizona. Plant Dis. 99(11):1468–1476. https://doi.org/10.1094/PDIS-12-14-1340-RE.

Stes E, Francis I, Pertry I, Dolzblasz A, Depuydt S, Vereecke D. 2013. The leafy gall syndrome induced by *Rhodococcus fascians*. FEMS Microbiol Lett. 342(2):187–194. https://doi.org/10.1111/1574-6968.12119.

Tajabadipour A, Panahi B, Zadehparizi R. 2006. The effects of rootstock and scion on early splitting and cracked hull of pistachio. Acta Hortic. 726:193–198. https://doi.org/10.17660/ActaHortic.2006.726.29.

Vereecke D, Zhang Y, Francis IM, Lambert PQ, Venneman J, Stamler RA, Kilcrease J, Randall JJ. 2020. Functional genomics insights into the pathogenicity, habitat fitness, and mechanisms modifying plant development of *Rhodococcus* sp. PBTS1 and PBTS2. Front Microbiol. 11:14–23. https://doi.org/10.3389/fmicb.2020.00014.

Young E, Houser J. 1980. Influence of Siberian C rootstock on peach bloom delay, water potential, and pollen meiosis. J Amer Soc Hort Sci. 105(2):242–245. https://doi.org/10.21273/JASHS.105.2.242.