

The current status of Pistachio Bushy Top Syndrome in California

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Field surveys and observations in 2015:

- PBTS orchards (2011-2012)
- Orchards planted prior to 2011
- Replant orchards and new plantings (2015)
- Sampling at Duarte nursery
- Host range of *Rhodococcus fascians*

Field surveys and observations in 2015:

- 37 pistachio orchards visited and sampled
- 521 trees tested for this study

First Report of *Rhodococcus* Isolates Causing Pistachio Bushy Top Syndrome on 'UCB-1' Rootstock in California and Arizona

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Shortened Internodes



Image credits: Dr. J. Randall

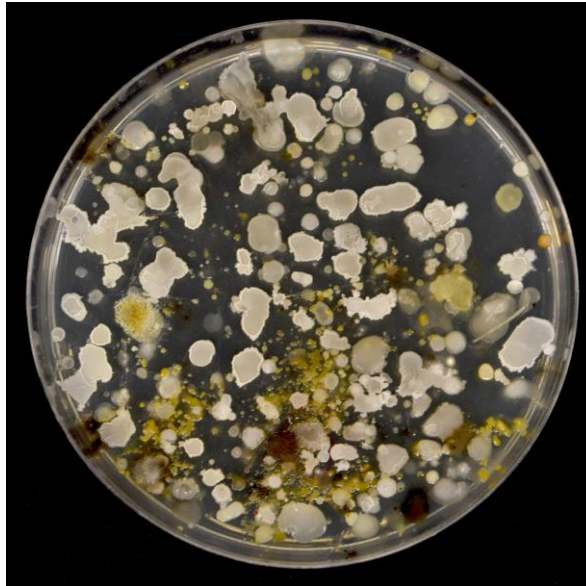
Detection of *Rhodococcus* spp.:

1:



Leaf imprint

2:



Isolation of bacterial colonies

3:



Selection of
yellow to
orange-red
colonies
(pure cultures)

4:



DNA Extraction

Based on Stamler et al. 2015

Detection of *Rhodococcus* spp.:

5:



Bacterial DNA



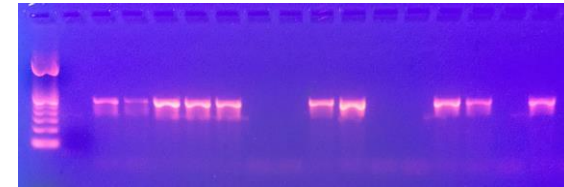
6:



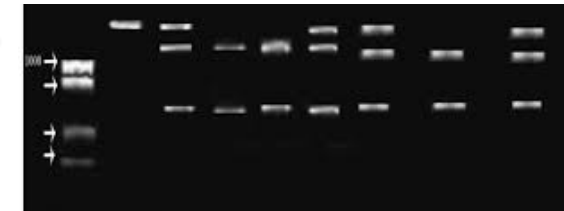
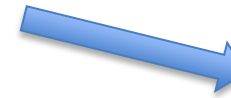
PCR



7:



vicA1497/vicA1990 specific primers
(Nikolaeva et al. 2012)



16S rRNA PCR-RFLP (Leveau's lab, UCD)

Orchard removal: 2013-2014



2011-2012 PBTS orchards :

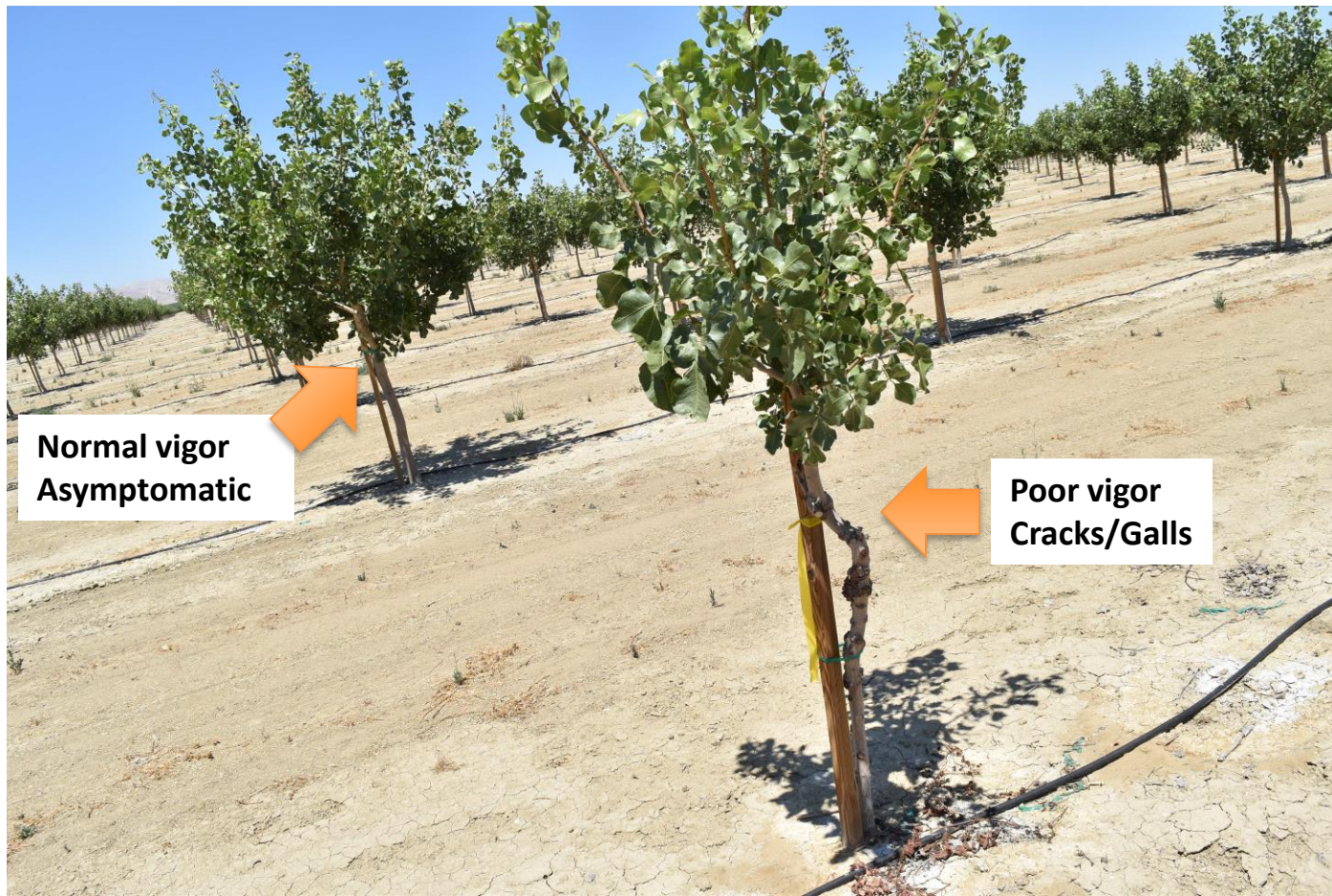
Symptomatic: cracking of the bark, galls, speckling



Asymptomatic



2011-2012 PBTS orchards:



2011-2012 PBTS orchards:

- In PBTS orchards 15 to 50 % of trees had galls/cracks below the graft union
- 100% correlation between bark cracks/galls on trunk and poor vigor



2011-2012 PBTS orchards :

0



1



2



3



4



5



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2011 PBTS orchard:



- Vigorous scion
- No cracking/galls
- Suckers with normal UCB1 phenotype

- Poor vigor scion
- Cracking/galls
- Suckers with "bushy" morphology



2011 PBTS orchard:



- Vigorous scion
- No cracking/galls
- Suckers with “bushy” morphology

- Poor vigor scion
- Cracking/galls
- Suckers with normal UCB-1 phenotype



Symptomology: Bushiness/Stunting



Symptomology: Bushiness/Stunting



2011 PBTS orchard:



2013 PBTS orchards:



2011-2013 PBTS orchards: testing for *Rhodococcus* spp.

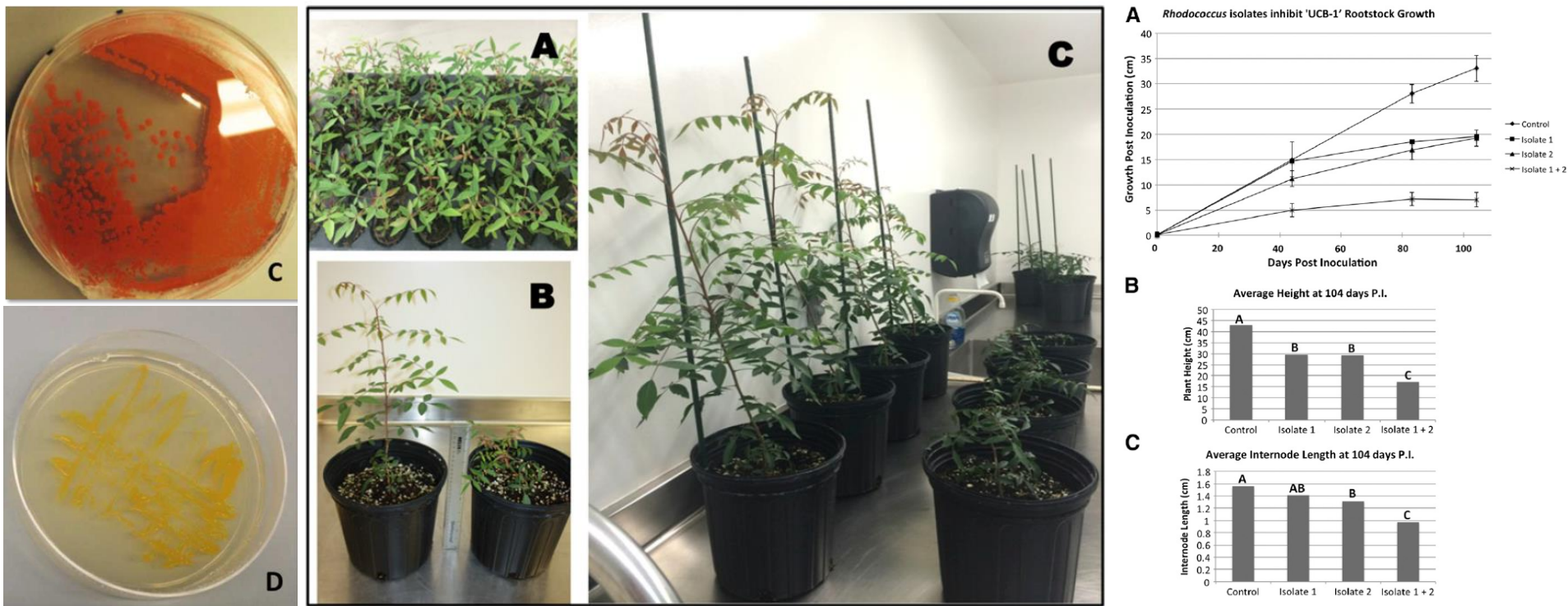
➤ Sampling results

| Number of trees sampled per symptom category in PBTS orchards | Number of trees positive for Rf |
|---|---------------------------------|
| Cracking Bark, poor vigor trees = 102 | 23 (22.5%) |
| Bushy Trees = 11 | 2 (18.2%) |
| Asymptomatic Trees = 50 | 3 (6%) |
| Mostly poor vigor trees, no cracking = 30 | 6 (20%) |
| Total: 193 | 34 (17.6%) |

Very low amount of the bacterium on plants...

Sampling results: testing for *Rhodococcus* spp.

- We never isolated *Rhodococcus* isolate 1 (red) (*R. corynebacterioides*)



Stamler et al. 2015

Orchards prior to 2011: testing for *Rhodococcus* spp.

➤ Test results

All trees were asymptomatic...

| Orchard # | County | Planting year | Number of tree sampled | Number of trees positive for Rf |
|-----------|--------|---------------|------------------------|---------------------------------|
| 15-87 | Fresno | 1995 | 10 | 4 (40%) |
| 15-51 | Kern | 2003 | 10 | 0 |
| 15-52 | Kern | 1992 | 10 | 0 |
| 15-53 | Kern | 2005 | 10 | 0 |
| 15-55 | Kern | 1995 | 10 | 0 |
| 15-56 | Kern | 2002 | 10 | 0 |
| 15-58 | Kern | 1997 | 5 | 0 |
| 15-75 | Kern | 2007 | 10 | 0 |
| 15-93 | Merced | 2010 | 1 | 0 |
| | | | Total Trees: 76 | 4 (5.25%) |



Callus on shaker injury: testing for *Rhodococcus* spp.



- 2009 orchard
- 25 symptomatic trees sampled (Bark, Suckers, Scion)
- 8 positives (*vicA*) (5 bark + 3 suckers)

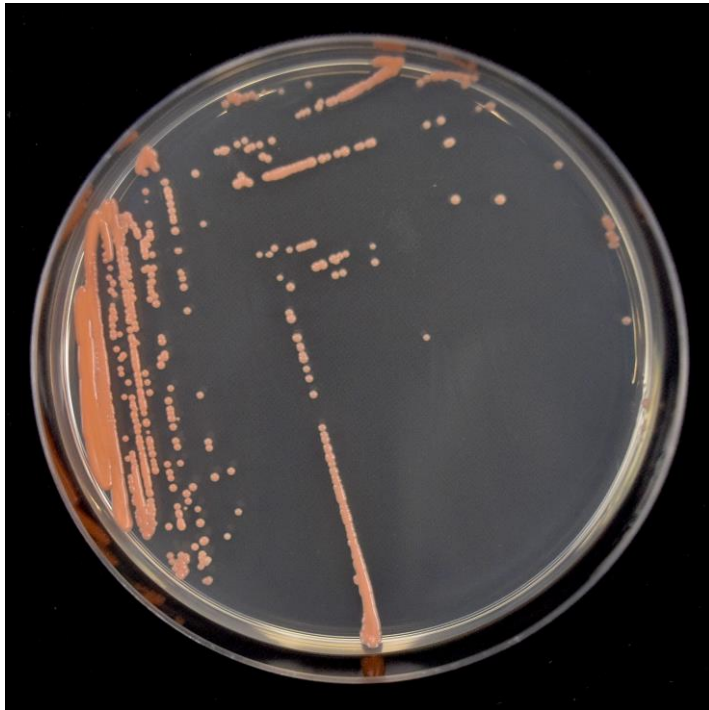
Nut drop orchard: testing for *Rhodococcus* spp.



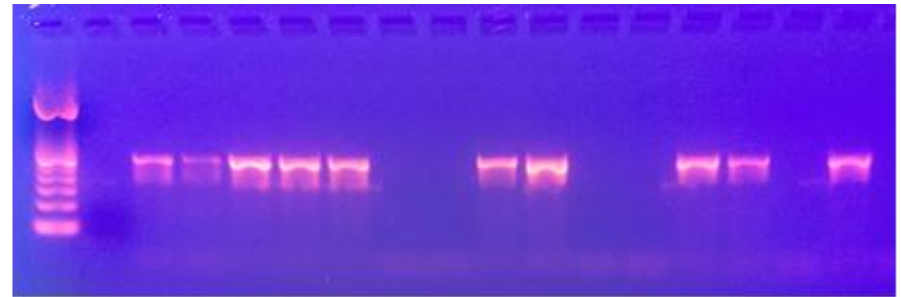
- Orchard planted in 2000
- Nuts
- Rachis
- Young leaves
- Old leaves

| Nut drop orchard | Number of trees sampled | Number of trees positive for Rf |
|--------------------|-------------------------|---------------------------------|
| Asymptomatic Trees | 10 | 0 |
| Nut Drop Trees | 10 | 1 (from leaf) |

Nut drop orchard: testing for *Rhodococcus* spp.



- *Bacillus* sp (16S rRNA, Leveau's lab UCD)



vicA1497/vicA1990 specific primers
(Nikolaeva et al. 2012)

| Nut drop orchard | Number of trees sampled | Number of trees vicA positive with the pink bacterium |
|--------------------|-------------------------|---|
| Asymptomatic Trees | 10 | 0 |
| Nut Drop Trees | 10 | 3 (from leaf + nut) |

Host range of *R. fascians*:

➤ Test results

All trees were asymptomatic...

| Host range | Number of tree sampled | Number of trees positive for Rf |
|-------------------|------------------------|---------------------------------|
| Chinese pistachio | 10 | 0 |
| Walnut | 10 | 1 |
| Peach | 10 | 2 |
| Prune | 10 | 0 |
| White mulberry | 10 | 1 |
| Willow | 10 | 2 |
| Almond | 20 | 2 |

Survey 2015: searching for PBTS

➤ 2015 replant orchards and new plantings



2015 replant orchards and new plantings:

Testing for *Rhodococcus* spp.

➤ Test results

| Replant orchard # | Number of tree sampled | Number of trees positive for Rf |
|--|------------------------|---------------------------------|
| 15-79 (same holes) (asymptomatic) | 6 | 0 |
| 15-57 (between holes) (asymptomatic) | 5 | 0 |
| Total Trees | 11 | 0 |

| New planting orchard # | Number of tree sampled | Number of trees positive for Rf |
|------------------------|------------------------|---------------------------------|
| 15-80 (asymptomatic) | 6 | 0 |
| 15-73 (asymptomatic) | 10 | 1 (from leaf) |
| Total Trees | 16 | 1 |

Survey 2015: searching for PBTS

➤ 2015 replant orchards and new plantings



Survey 2015: searching for PBTS

➤ 2015 replant orchards and new plantings



Only one
bushy tree
was found

Orchard with no pistachio history, UCB-1 Clonal rootstocks (not from Duarte)

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Survey 2015: searching for PBTS

- 2015 replant orchards and new plantings



Bushy rootstock



Normal UCB-1

Survey 2015: testing for *Rhodococcus*

- 2015 replant orchards and new plantings



We sampled:

- Red leaves
- Yellow-green leaves
- Dark green leaves

We proceeded with:

- Leaf print
- Leaf grindate

30 isolations in Petri Dish
with D2 medium

No *Rhodococcus*

Sampling at the nursery:



Sampling at the nursery:



Sampling at the nursery: 2013 UCB-1 Clonal



Sampling at the nursery: 2013 UCB-1 Clonal



Sampling at the nursery: 2013 UCB-1 Clonal



| Nursery field site 1 | Number of trees sampled | Number of trees positive for Rf |
|----------------------|--------------------------|---------------------------------|
| UCB-1 2013 | 5 Bushy + 5 Asymptomatic | 0 |

Sampling at the nursery: 2011 UCB-1 Clonal



| Nursery field site 2 | Number of trees sampled | Number of trees positive for Rf |
|----------------------|--------------------------------------|---------------------------------|
| UCB-1 2011 | 2 with galls/cracks + 2 Asymptomatic | 1 (Asymptomatic tree) |

Sampling at the nursery: 2011 UCB-1 Clonal



UCB-1 clone



Experimental Clone

Sampling at the nursery: greenhouses + benches



All plants were asymptomatic...

| Nursery | Number of plants sampled | Number of plants positive for Rf |
|-------------------------|--------------------------|----------------------------------|
| Almond (bench) | 10 | 0 |
| Pistachio (bench) | 18 | 2 |
| Poinsettia (greenhouse) | 20 | 1 |
| Walnut (greenhouse) | 20 | 4 |

Summary:

- Complex issue
- No (re-)emergence so far of PBTS in 2015 replants and new plantings
- Survey will continue in 2016
- Only *R. fascians* was isolated in our study
- No strict correlation between the “bushy” morphology and the presence of cracks/galls in the bark
- Consistent, uniform morphology among the “bushy” rootstocks
- Low occurrence of *R. fascians* on “bushy” rootstocks
- *R. fascians* was recovered from 22.5 % of trees with crack/galls
- What caused the galls and bark cracks?
- *R. fascians* was found at low levels on plants
- *R. fascians* can be found on asymptomatic plants
- Potential broad host range for *R. fascians* in CA

Thank you!

- Mohamed Nouri (KARE)
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