Management and Monitoring of Invasive shot hole borers - Fusarium Dieback on Avocado

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5. UCCE Farm Advisor, Ventura County

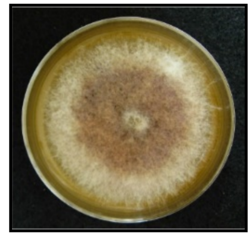
Fungal Pathogens associated with SHB

Euwallacea whitfordiodendrus

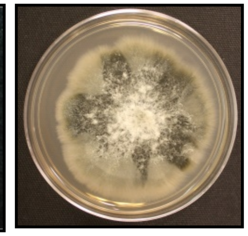
G. Arakelian

Los Angeles Co.
Orange Co.
Ventura Co.
San Bernardino Co.
Riverside Co.

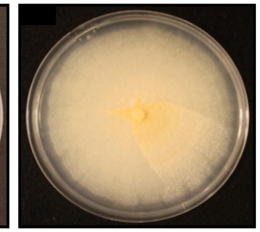
Polyphagous Shot Hole Borer **(PSHB)**



Fusarium euwallaceae



Graphium euwallaceae

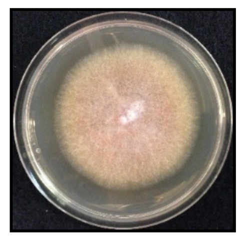


Paracremonium pembeum

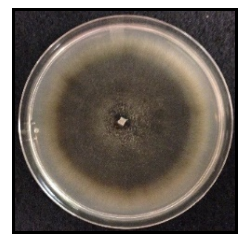
E. kuroshio

San Diego Co.
Orange Co.
Santa Barbara Co.
San Luis Obispo Co.

Kuroshio Shot Hole Borer **(KSHB)**



F. kuroshium

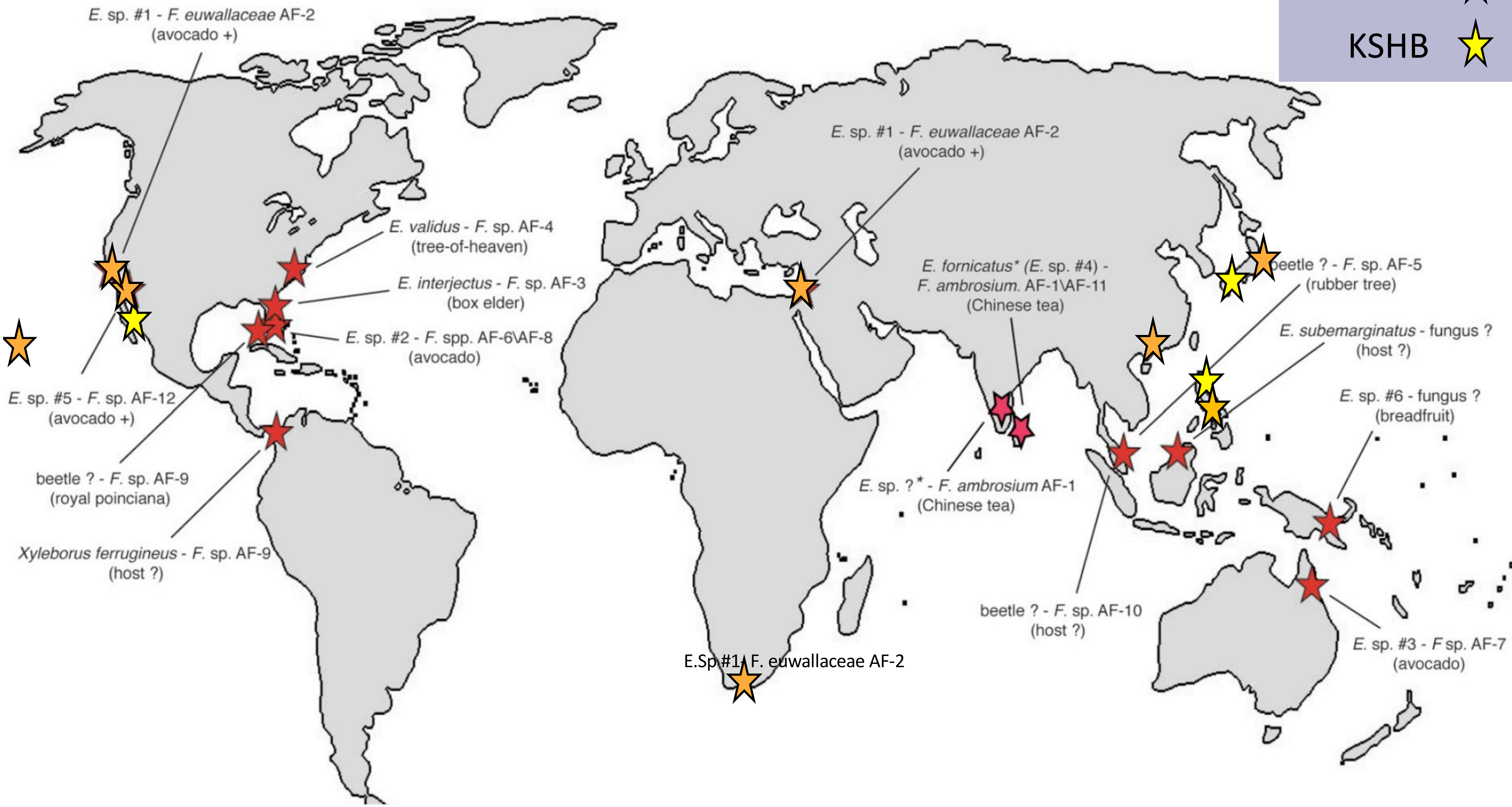


G. kuroshium

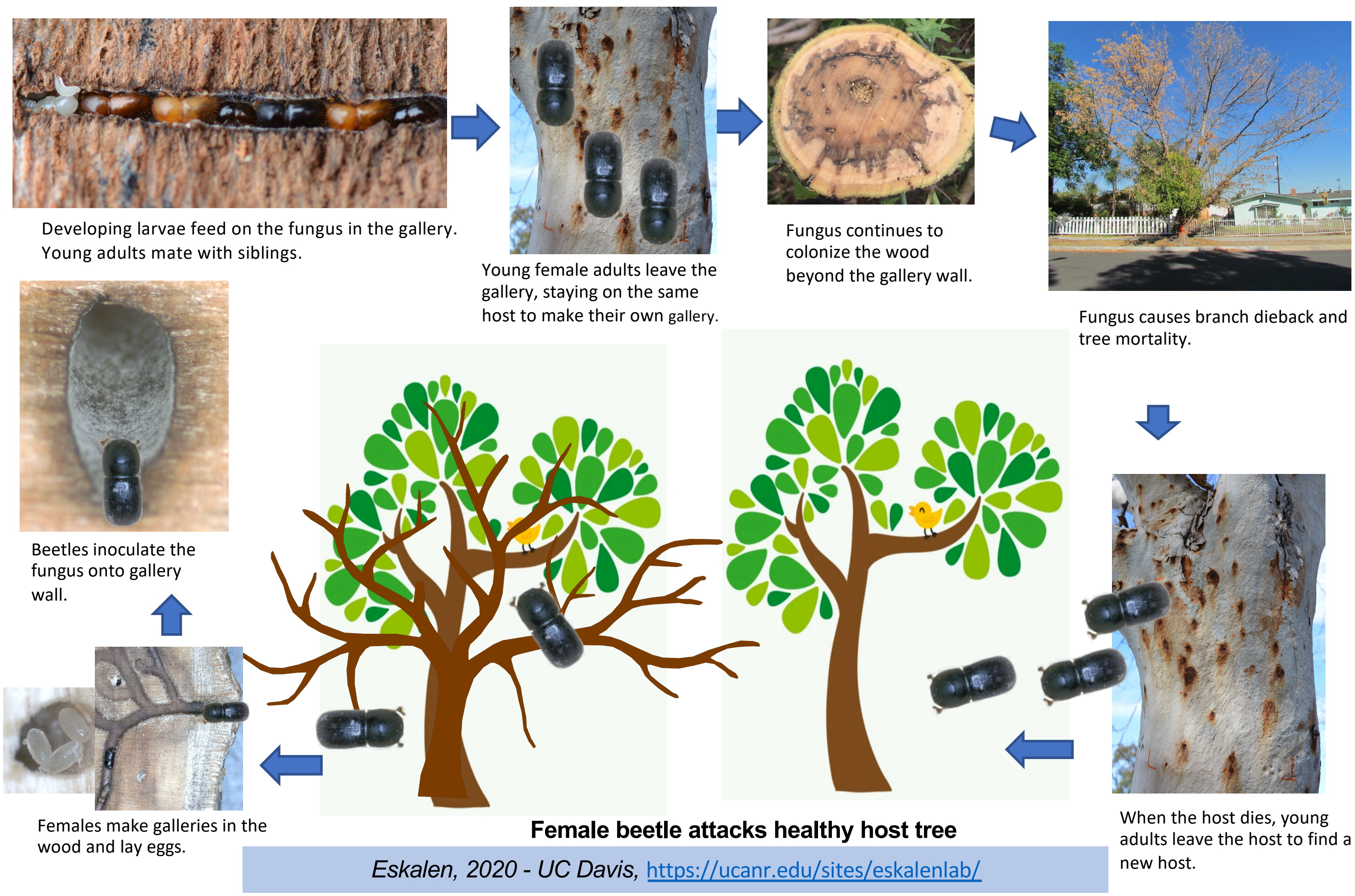
Lynch et all 2016. *Mycologia, 1*08(2), pp.313-329.
Na et all 2018. Plant Disease. 102(6)1154-1164.
Carrillo, et all 2020 Symbiosis. (https://doi.org/10.1007/s13199-019-00652-0).

Current Distribution of *Euwallaceae* spp

TSHB
PSHB
KSHB



Life cycle of Fungal Pathogens associated with shot hole borers



Beetle Life Cycle and Reproduction

- Sex ratio of offspring female biased
- Brothers mate with sisters in galleries
- Mated females leave the galleries to create their own galleries
- Female can fly, male usually stay in the gallery

Beetle attack locations with different hosts

Sycamore

Avocado

Beetle attack locations on avocado

Beetle colonization on branch collar of avocado

Branch dieback on Avocado

Beetle colonization on branch collar of avocado

Branch dieback on Avocado

Beetle attack locations on avocado

Beetle attack attempt on avocado trunk

Black Streak disease of avocado caused by *Botryosphaeriaece*

Beetle attack on young avocado trees in high-density groves

Host Range:

	2012
Tree Species Attacked by Beetle	286
Tree Species Infected by Fungus	117
Agricultural Crops	13
California Native Tree Species	11
Number of Tree Families	56
Number of Reproductive Hosts	19

Eskalen, A., Stouthamer, R., Lynch, S.C., Rugman-Jones, P., Twizeyimana, M., Gonzalez, A., Thibault, T. 2013. Host Range of Fusarium Dieback and its Ambrosia Beetle (Coleoptera: Scolytinae) Vector in Southern California. [Plant Disease.In 97:7, 938-951]

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1. Box Elder (*Acer negundo*)*
2. Big leaf maple (*Acer macrophyllum*)*
3. Evergreen Maple (*Acer paxii*)
4. Trident maple (*Acer buergerianum*)
5. Japanese maple (*Acer palmatum*)
6. Castorbean (*Ricinus communis*)
7. California Sycamore (*Platanus racemosa*)*
8. Mexican sycamore (*Platanus mexicana*)
9. Red Willow (*Salix laevigata*)*
10. Arroyo willow (*Salix lasolepis*)*
11. Avocado (*Persea americana*)
12. Mimosa (*Albizia julibrissin*)
13. English Oak (*Quercus robur*)
14. Coast live oak (*Quercus agrifolia*)*
15. London plane (*Platanus x acerifolia*)
16. Cottonwood (*Populus fremontii*)*
17. Black cottonwood (*Populus trichocarpa*)*
18. White Alder (*Alnus rhombifolia*)*
19. Titoki (*Alectryon excelsus*)
20. Engelmann Oak (*Quercus engelmannii*)*
21. Cork Oak (*Quercus suber*)
22. Valley oak (*Quercus lobata*)*
23. Coral tree (*Erythrina corallodendon*)
24. Blue palo verde (*Cercidium floridum*)*
25. Palo verde (*Parkinsonia aculeata*)
26. Moreton Bay Chestnut (*Castanospermum australe*)
27. Brea (*Cercidium sonorae*)
28. Mesquite (*Prosopis articulata*)*
29. Weeping willow (*Salix babylonica*)
30. Chinese holly (*Ilex cornuta*)
31. Camelia (*Camellia semiserrata*)
32. Acacia (*Acacia spp.*)
33. Japanese wisteria (*Wisteria floribunda*)
34. Black willow (*Salix gooddingii*)*
35. Tree of heaven (*Ailanthus altissima*)
36. Kurrajong (*Brachychiton populneus*)**
37. Black mission fig (*Ficus carica*)**
38. Japanese beech (*Fagus crenata*)
39. Dense logwood (*Xylosma congestum*)
40. Mule Fat (*Baccharis salicifolia*)*
41. Black Poplar (*Populus nigra*)*
42. Carrotwood (*Cupaniopsis anacardioides*)
43. California buckeye (*Aesculus californica*)*
44. Canyon Live oak (*Quercus chrysolepis*)*
45. Kentia Palm (*Howea forsteriana*)
46. King Palm (*Archontophoenix cunninghamiana*)
47. Tamarix (*Tamarix ramosissima*)
48- Red Flowering Gum (*Eucalyptus ficifolia*)**
49- American Sweetgum (*Liquidambar styraciflua*)
50- Honey Locust (*Gleditsia triacanthos*)
51. Brazilian Coral Tree (*Erythrina falcata*)
52. Purple Orchid Tree (*Bauhinia variegata*)**
53. Council Tree (*Ficus* altissima)**
54. Tulip Wood (*Harpullia pendula*)
55. Chinese Flame Tree (*Koelreuteria bipinnata*)**
56. Laurel-leaf Snailseed tree (*Cocculus laurifolius*)**
57. Southern Magnolia (*Magnolia grandiflora*)**
58. Jacaranda (*Jacaranda mimosifolia*)**
59. Coast coral tree (*Erythrina caffra*)**
60. Australian blackwood (*Acacia melanoxylon*) **
61. Sweet Bay (*Magnolia virginiana*)**
62. African Tulip Tree (*Spathodea campanulata*)**
63. Strawberry snowball tree (*Dombeye cacuminum*)**
64. Chinese Wingnut (*Pterocarya stenoptera*)**

1. California box elder (*Acer negundo*)
2. Coast live oak (*Quercus agrifolia*)
3. California Sycamore (*Platanus racemosa*)
4. Big leaf maple (*Acer macrophyllum*)
5. Red Willow (*Salix laevigata*)
6. Valley oak (*Quercus lobata*)
7. Blue palo verde (*Cercidium floridum*)
8. Fremont Engelmann oak (*Quercus engelmannii*)
9. White Alder (*Alnus rhombifolia*)
10. Canyon live oak (*Quercus chrysolepis*)
11. California bay laurel (*Umbellularia californica*)
12. Desert fan palm (*Washingtonia filirera*)
13. California buckeye (*Aesculus californica*)
14. Coffee berry (*Ramnus californica*)
15. Velvet ash (*Fraxinus velutina*)
16. Fremont cottonwood (*Populus fremontii*)
17. California ash (Fraxinus dipetala)
18. Gooding's black willow (Salix gooddingii)
19. Canyon Live Oak (*Quercus chrysolepis*)

Avocado (*Persea americana*)*
Persimmon (*Diospyros spp.*)
Olive (*Olea europea*)
Macadamia (*Macadamia integrifolia*)
Eastern mulberry (*Broussonetia papyrifera*)
Hazelnut (*Corylus colurna*)
Loquat (*Eriobotrya japonica*)
Peach (*Prunus persica*)
Grapevine (*Vitis viticola*)
10. Citrus (*Citrus cinensis*)
11. Cassava (*Manihot esculenta*)
12. Japanese Persimmon (*Diospyros lycidioides*)
13. Japanese crabapple (*Malus floribunda*)
14. Prunes (*Prunus spp.*)

Susceptible hosts to ISHB-FD (may cause tree death)

1. *Acer buergerianum* - Trident maple
2. *Acer macrophyllum* - Big leaf maple*
3. *Acer negundo* - Box elder*
4. *Parkinsonia aculeata* - Palo verde
5. *Platanus racemosa* - California sycamore*
6. *Platanus* x *hispanica* - London plane
7. *Populus fremontii* - Fremont cottonwood*
8. *Populus nigra* - Black poplar*
9. *Populus trichocarpa* - Black cottonwood*
10. *Quercus lobata* - Valley oak*
11. *Quercus robur* - English oak
12. *Ricinus communis* - Castorbean
13. *Salix gooddingii* - Black willow*
14. *Salix laevigata* - Red willow*
15. *Salix lasiolepis* - Arroyo willow*

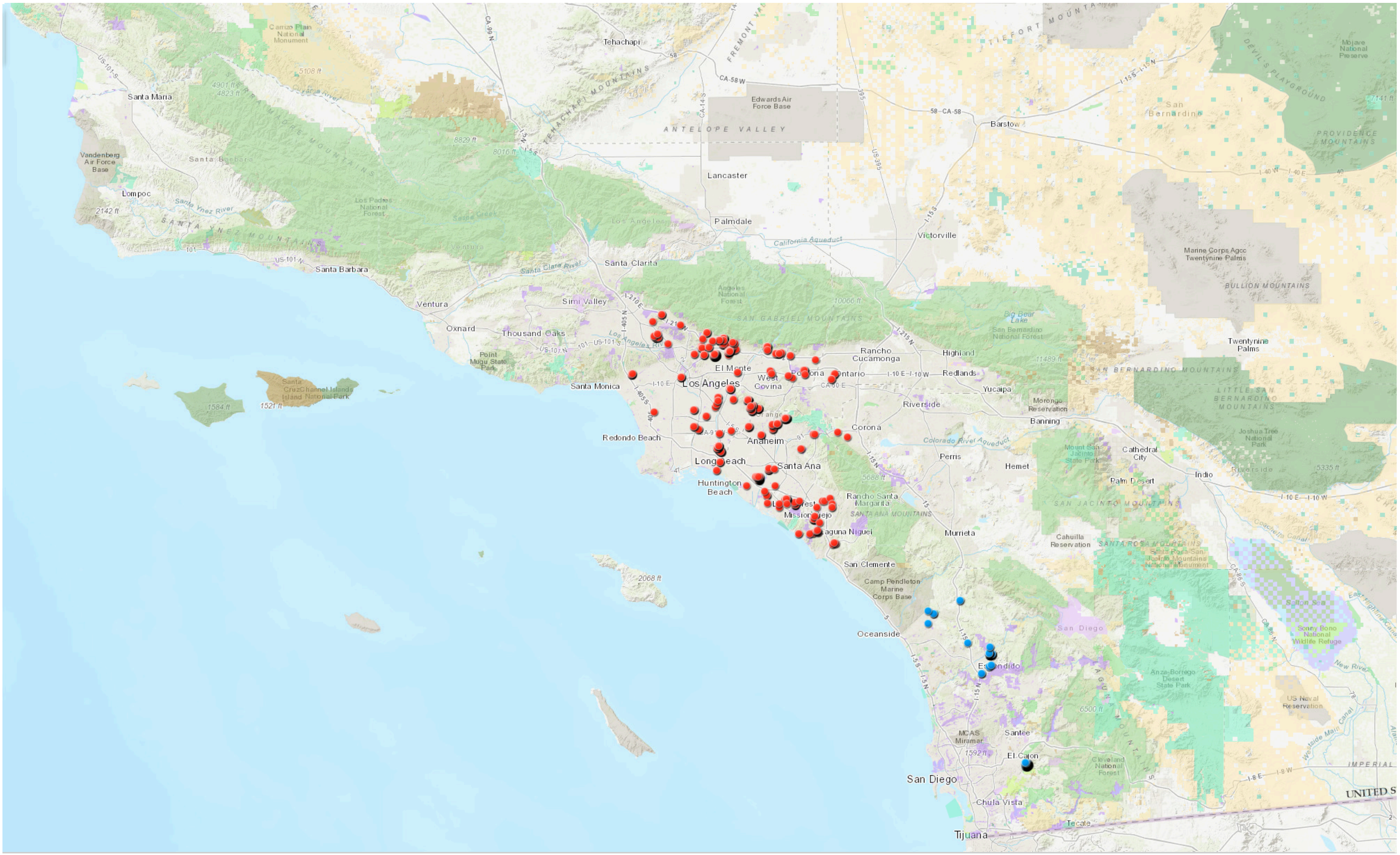
Less Susceptible hosts to ISHB-FD (cause branch dieback) and/or the beetle commonly colonizes at the margin of canker diseases.

1.*Acacia melanoxylon* - Australian blackwood
2.*Acacia spp.* - Acacia
3.*Acer paxii* - Evergreen maple
4.*Aesculus californica* - California buckeye*
5.*Ailanthus altissima* - Tree of heaven
6.*Albizia julibrissin* - Mimosa
7.*Alectryon excelsus* - Titoki
8.*Alnus rhombifolia* - White alder*
9.*Archontophoenix cunninghamiana* - King palm
10.*Baccharis salicifolia* - Mule fat*
11.*Bauhinia variegata* - Purple orchid tree
12.*Brachychiton populneus* - Kurrajong
13.*Camellia semiserrata* - Camellia
14.*Castanospermum australe* - Moreton Bay chestnut
15.*Casuarina equisetifolia* - Australian pine tree
16.*Parkinsonia florida* - Blue palo verde*
17.*Parkinsonia x sonorae* - Sonoran palo verde
18.*Cocculus laurifolius* - Laurel leaf snailseed tree
19.*Cupaniopsis anacardioides* - Carrotwood
20.*Dombeya cacuminum* - Strawberry tree
21.*Erythrina caffra* - Coast coral tree
22.*Erythrina coralloides* - Coral tree
23.*Erythrina falcata* - Brazilian coral tree
24.*Corymbia ficifolia* - Red flowering gum
25.*Fagus crenata* - Japanese beech
26. *Ficus altissima* - Council tree
27. *Ficus carica* - Black mission fig
28. *Gleditsia triacanthos* - Honey locust
29. *Harpullia pendula* - Tulip wood
30. *Howea forsteriana* - Kentia palm
31. *Ilex cornuta* - Chinese holly
32. *Jacaranda mimosifolia* - Jacaranda
33. *Acer palmatum* - Japanese maple
34. *Koelreuteria bipinnata* - Chinese flame tree
35. *Liquidambar styriciflua* - American sweet gum
36. *Magnolia grandiflora* - Southern magnolia
37. *Magnolia virginiana* - Sweet bay
38. *Persea americana* - Avocado
39. *Platanus mexicana* - Mexican sycamore
40. *Prosopis articulata* - Mesquite*
41. *Pterocarya stenoptera* - Chinese wingnut
42. *Quercus agrifolia* - Coast live oak*
43. *Quercus chrysolepis* - Canyon live oak*
44. *Quercus engelmannii* - Englemann oak*
45. *Quercus suber* - Cork oak
46. *Salix babylonica* - Weeping willow
47. *Spathodea campanulata* - African tulip tree
48. *Tamarix ramosissima* - Tamarisk
49. *Wisteria floribunda* - Japanese wisteria
50. *Xylosma congesta* - Dense logwood / Shiny *Xylosma*

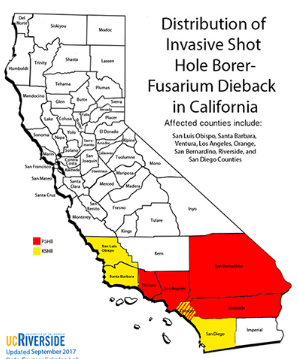
www.Ishb.org

Spread of Fusarium Dieback-SHB

2003
2012
2013
2014



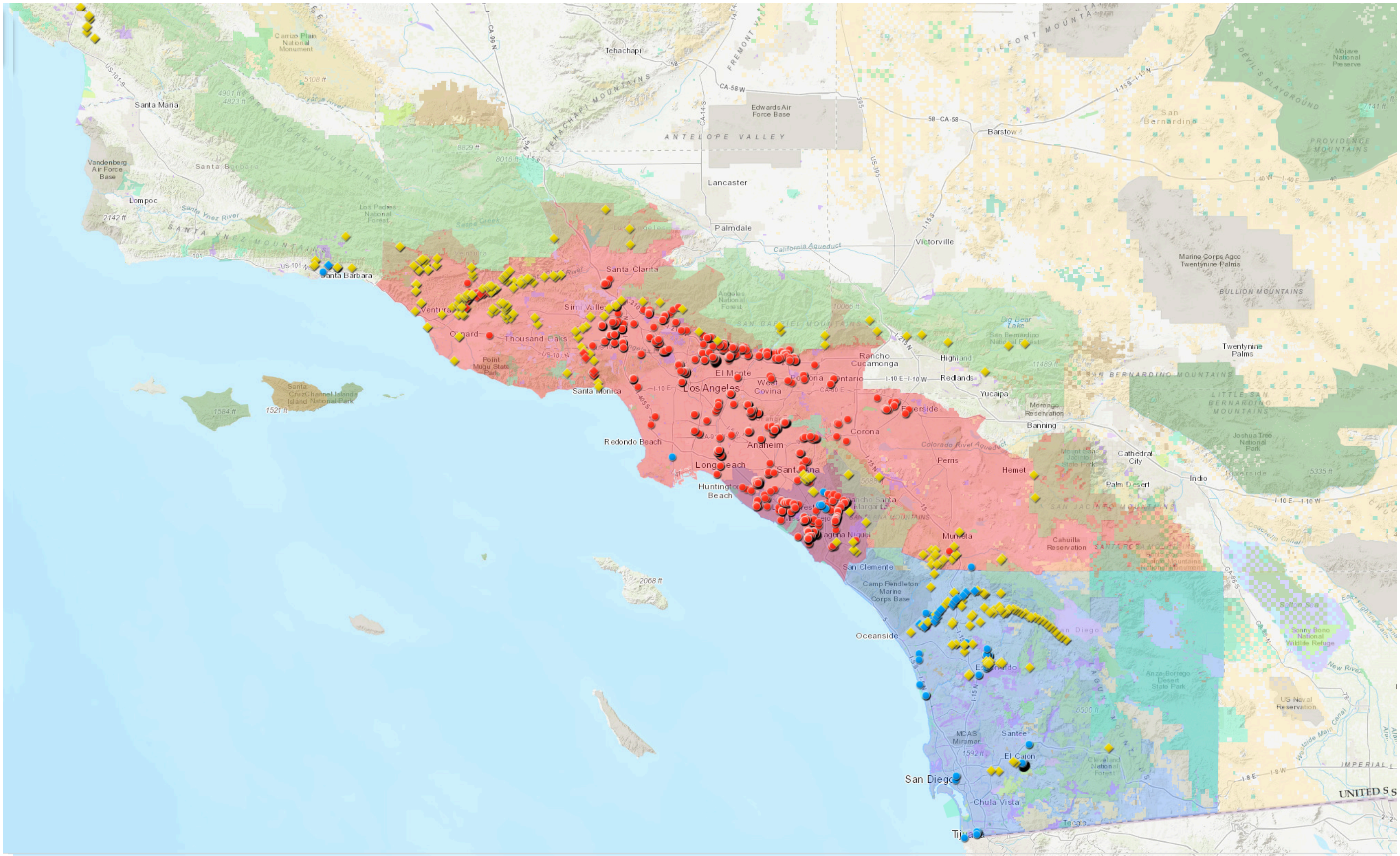
● **PSHB**
● **KSHB**



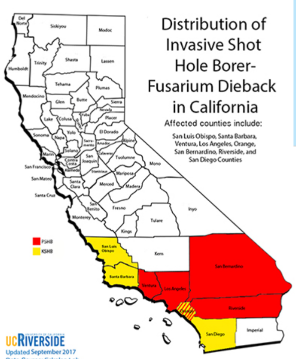
http://eskalenlab.ucr.edu/distribution

Spread of Fusarium Dieback-SHB

2003
2012
2013
2014
2016
2018
2019



PSHB
KSHB
TRAP



http://eskalenlab.ucr.edu/distribution

Objectives

- Probe-based multiplex real-time PCR as a diagnostic tool to distinguish distinct fungal symbionts associated with *Euwallacea* spp. in California

- Determine the rate of spread of PSHB and KSHB on avocado trees, between groves, and within groves

- Identify potential environmental factors that contribute to the distribution, establishment, and spread of PSHB and KSHB

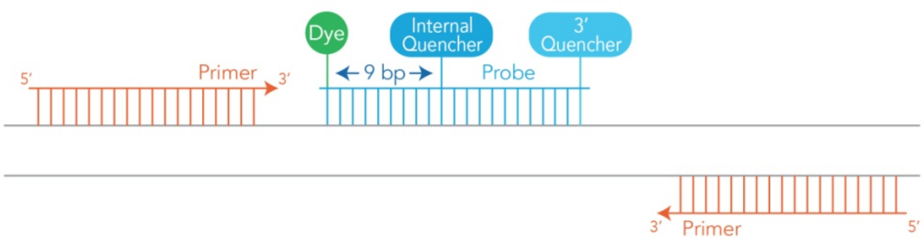
- Provide management guidelines in light of ongoing experiments and monitoring studies

Real-time PCR Workflow



Unknown Sample

DNA Extraction



- Primer and probe design

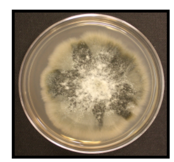
Run PCR

CFX96 (Biorad®)

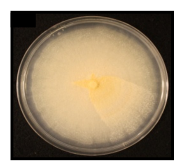
Analyze Results and Identify
- Biorad® CFX Manager

PSHB symbionts

F. euwallaceae

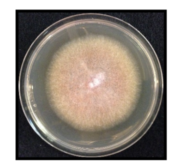


G. euwallaceae

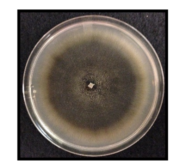


P. pembeum

KSHB symbionts

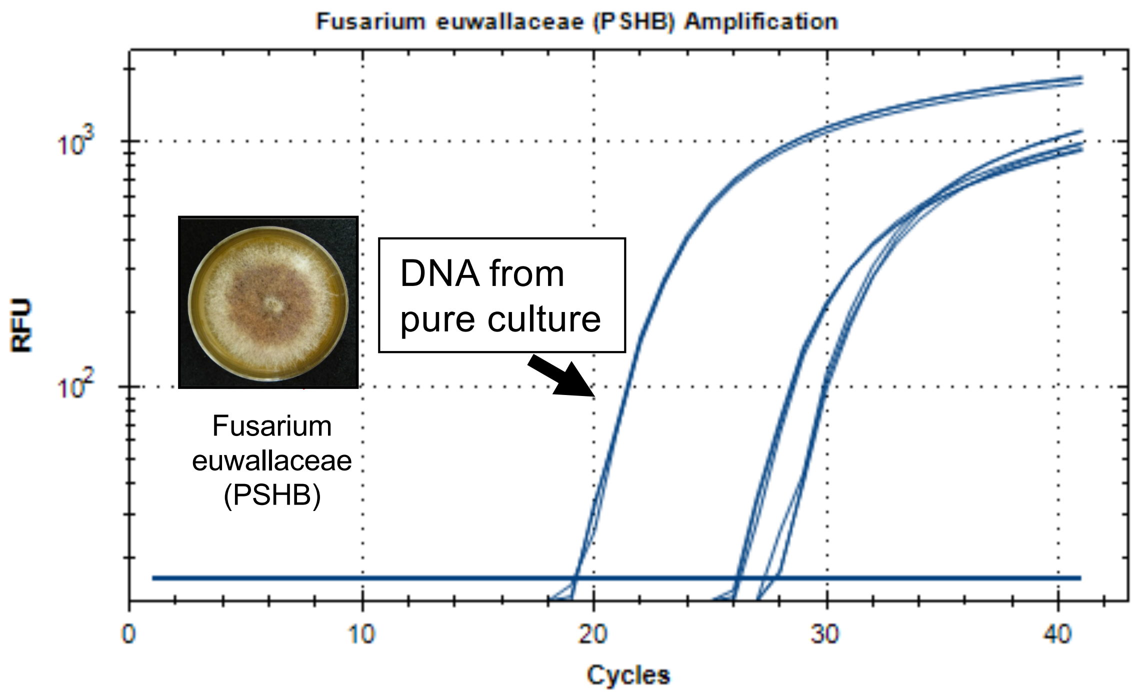


F. kuroshium



G. kuroshium

Early Detection Tools



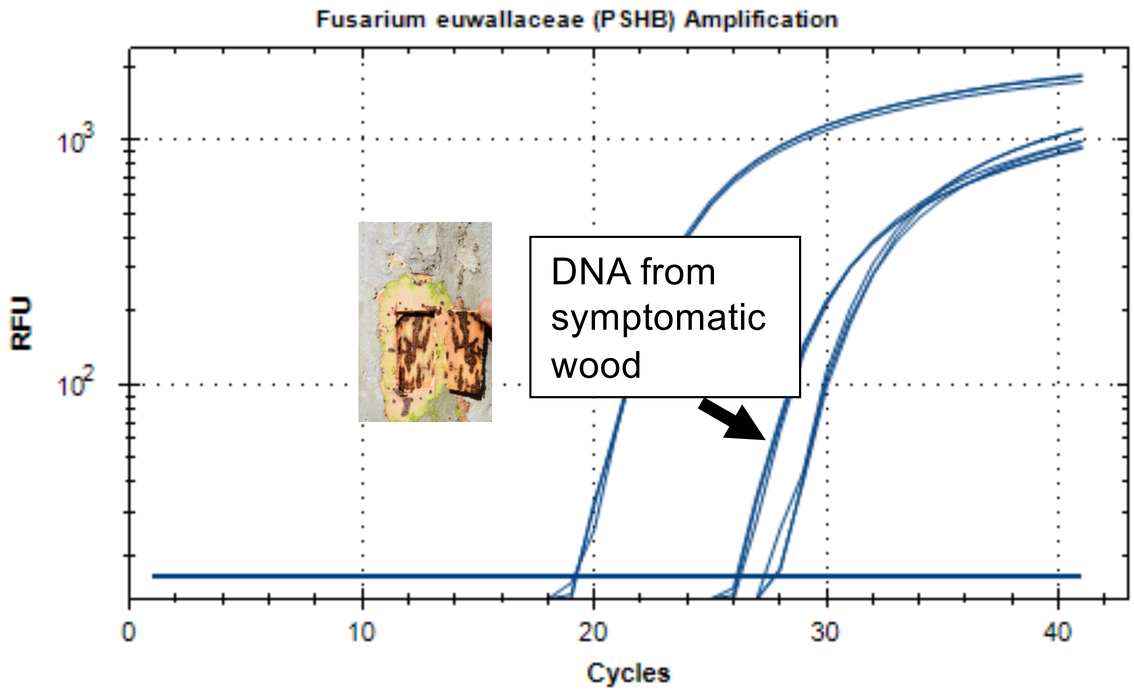
Controls:

- Asymptomatic Wood (-)
- Fusarium solani (-)
- NTC (-)

Legend:
- *F. euwallaceae* (PSHB)(FAM)
- *F. kuroshium* (KSHB) (HEX)

Ref: Carrillo et all. , J.D., Mayorquin, J.S., Stajich, E.S., Eskalen, A. 2019. Probe-based multiplex real-time PCR as a diagnostic tool to distinguish distinct fungal symbionts associated with *Euwallacea kuroshio* and *E. whitfordiodendrus* in California. Plant Disease. https://doi.org/10.1094/PDIS-01-19-0201-RE

Early Detection Tools



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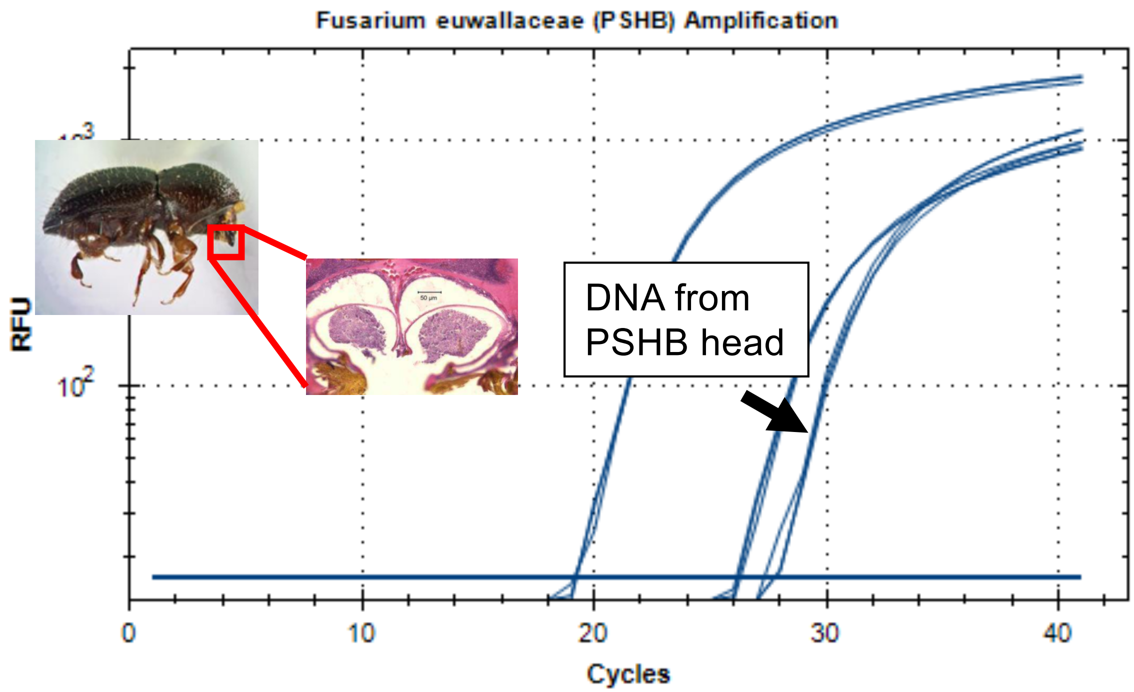

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- Determine the rate of spread of PSHB and KSHB on avocado trees, between groves, and within groves

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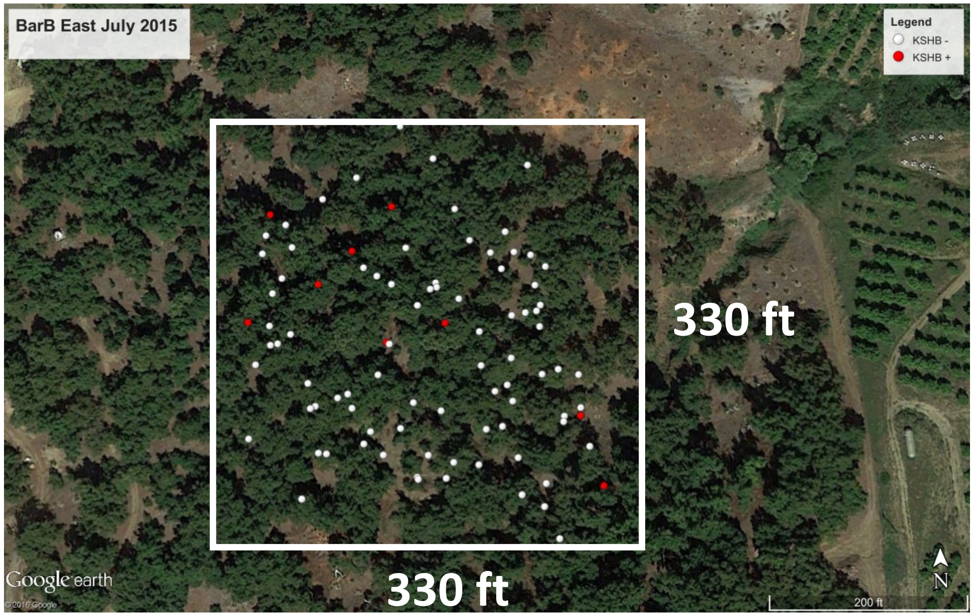
Determine the rate of spread of ISHB on Avocado trees, between groves, and within groves



1 ha monitoring plot

Microclimate
Beetle Activity

Permanent plot establishment for long term monitoring

Assessment:

- Health,
- Overall PSHB and KSHB severity,
- Attack severity by plant part,
- Recorded the presence of other pests;

  -bacterial canker,

  -bot canker,

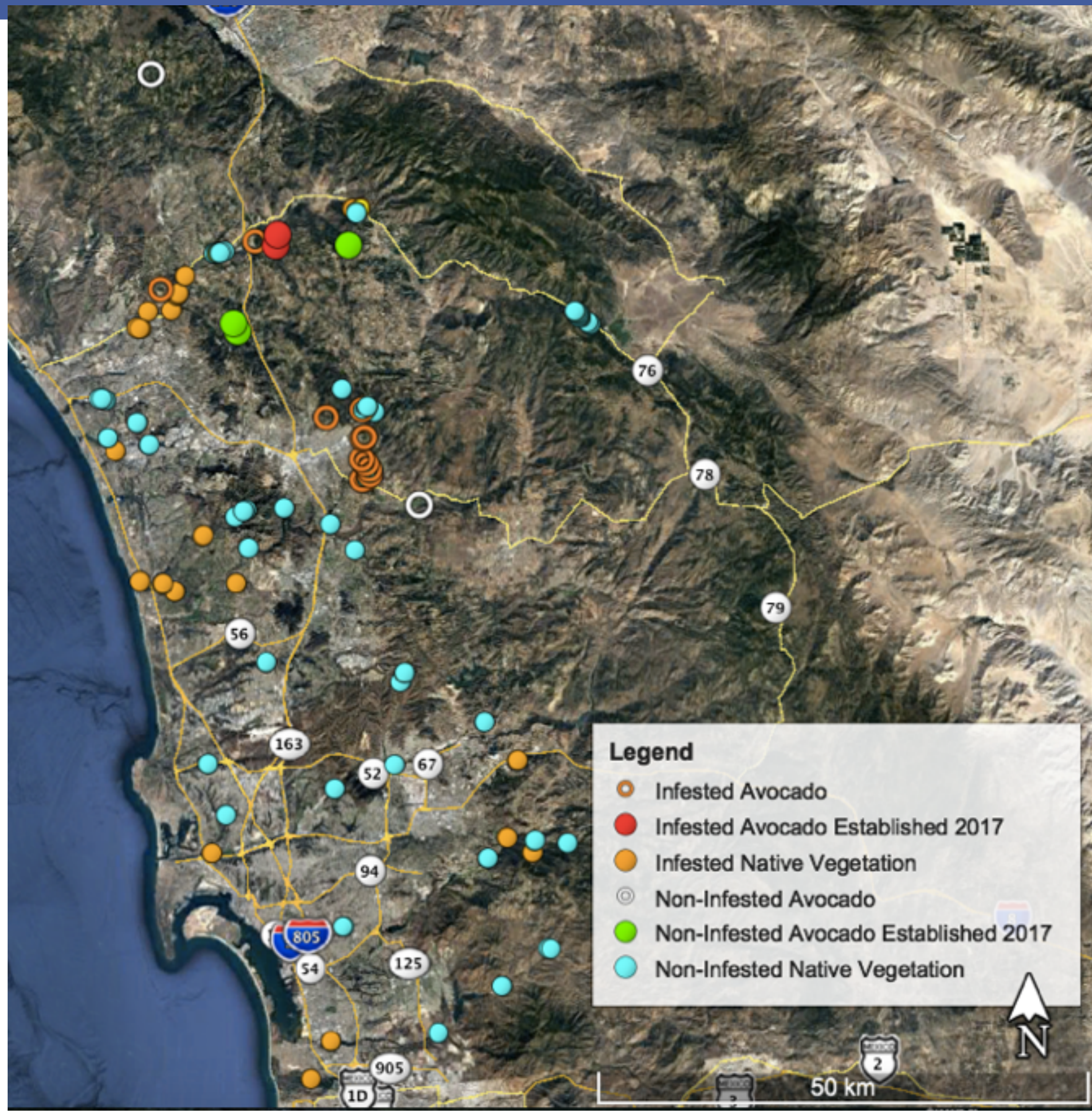
  -black streak,

  -phytophthora root rot

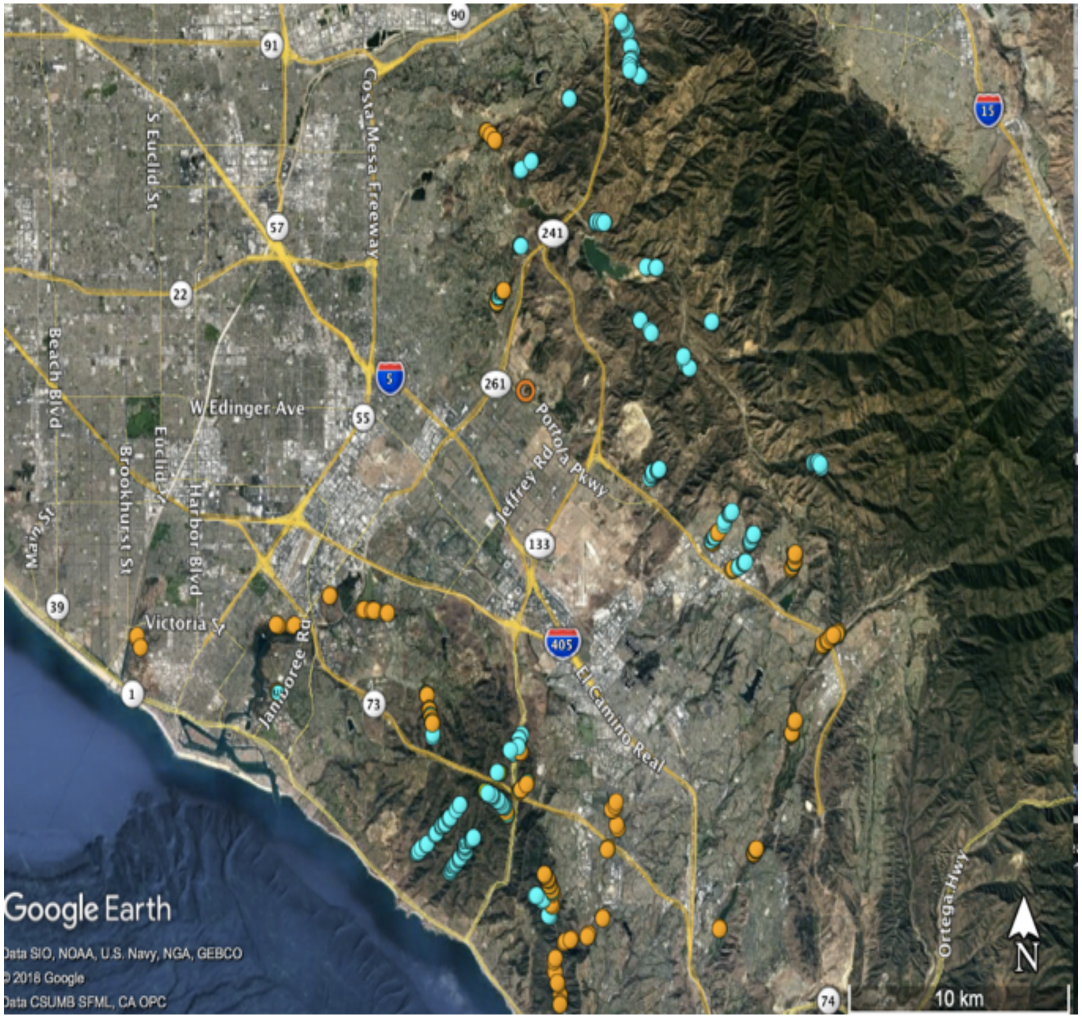
Monitoring ISHB infested Avocado Groves

- 2015 -Initial number of avocado groves surveyed: 19
- 2017 -Additional avocado groves: 41
- 2017 -265 plots in native vegetation

Monitoring Plots in San Diego County



Monitoring Plots in Orange County



Monitoring P/KSHB Infested Avocado Groves

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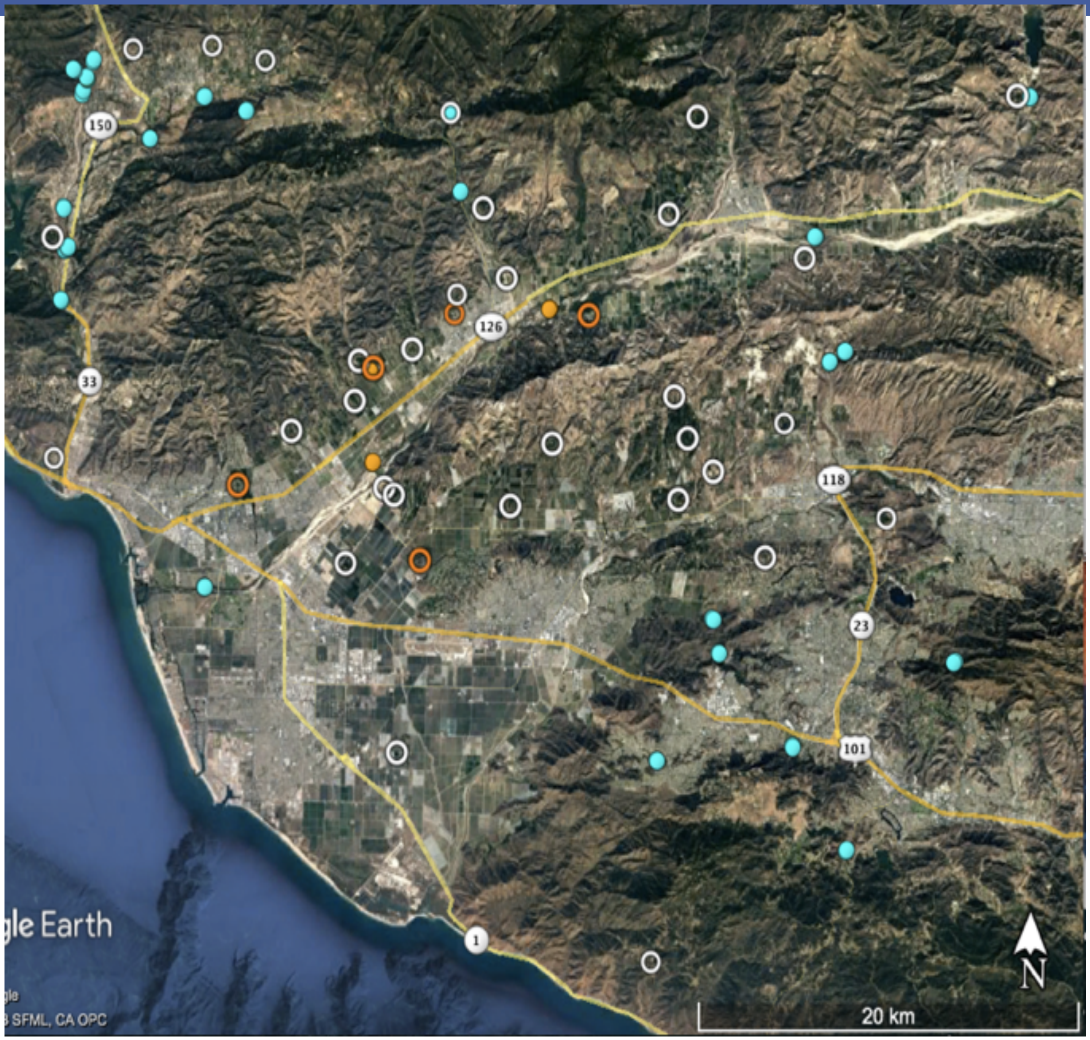
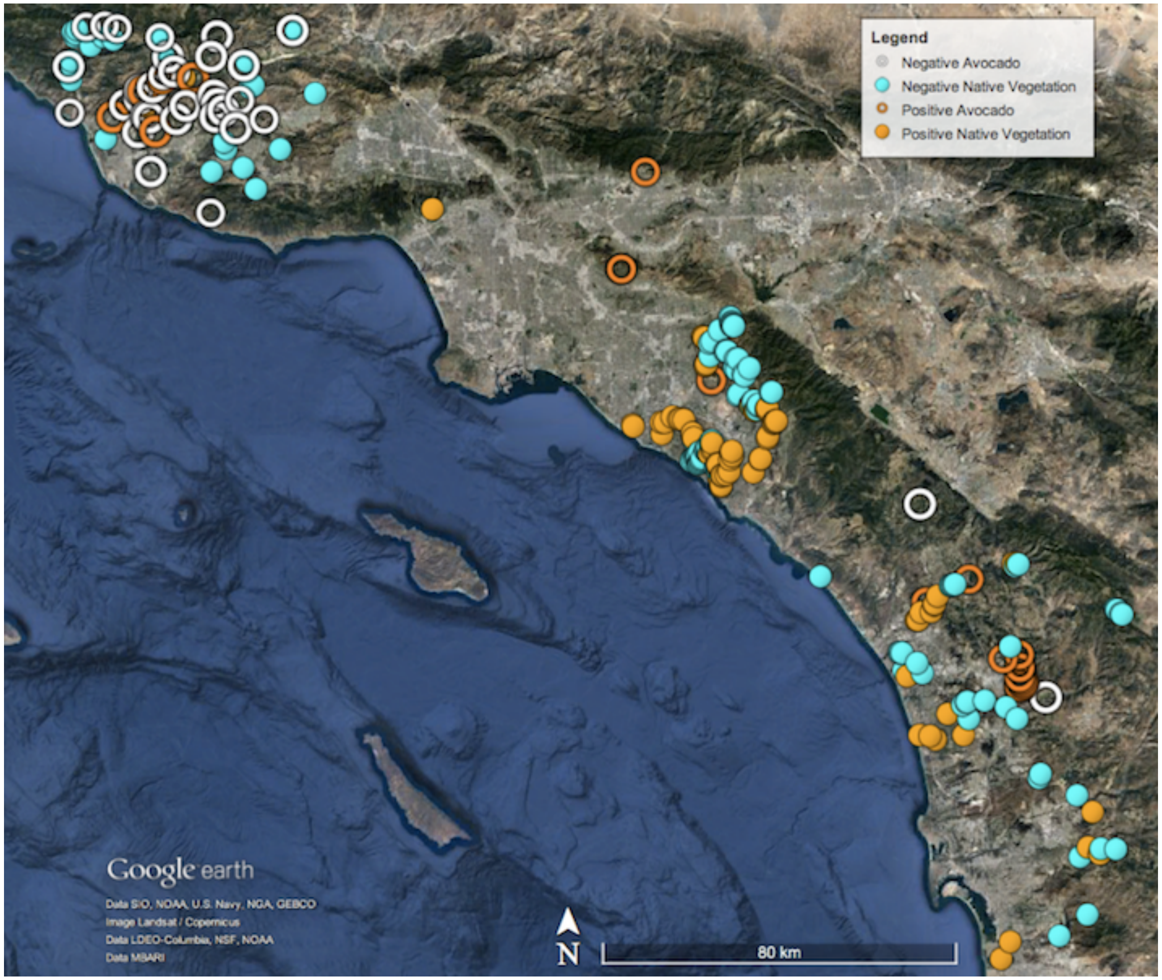
- 2017 -Additional avocado groves: 41
- 2017 -265 plots in native vegetation

Ventura County



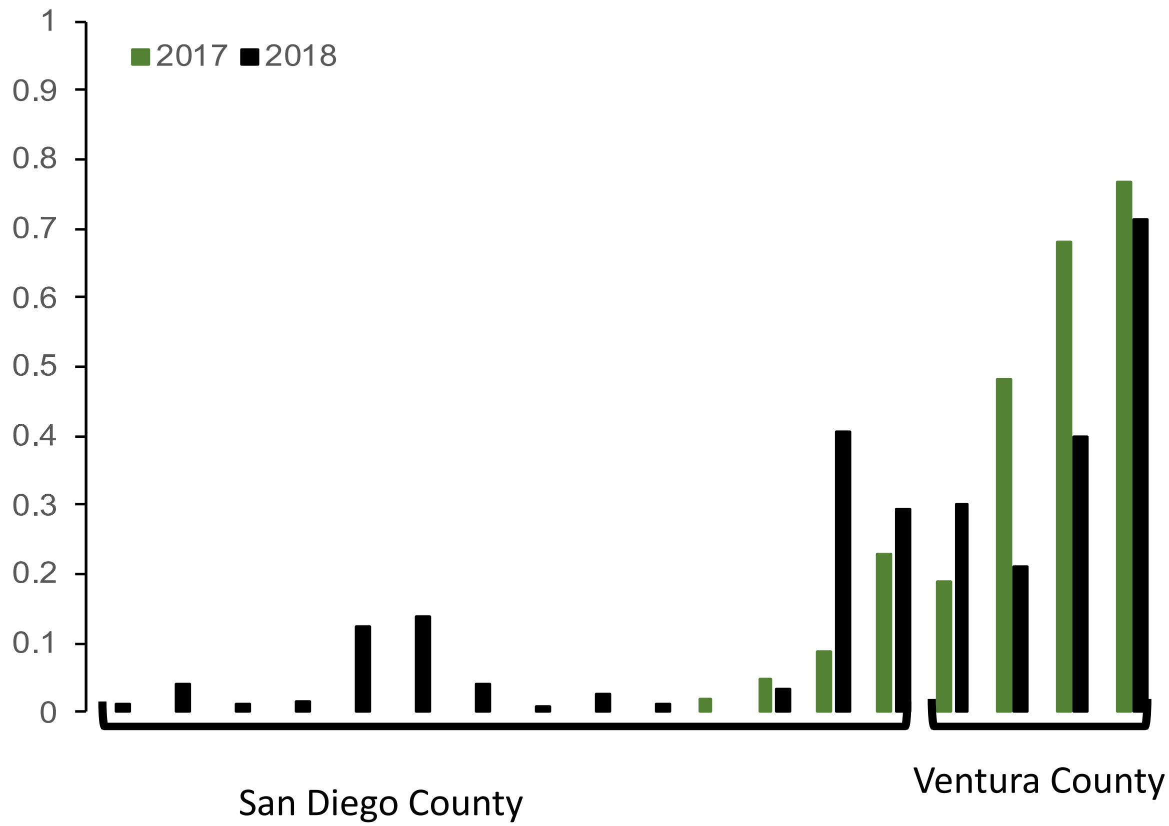
Monitoring plots in in southern California



Diversity
Microclimate

Predicting risk in heterogeneous landscapes

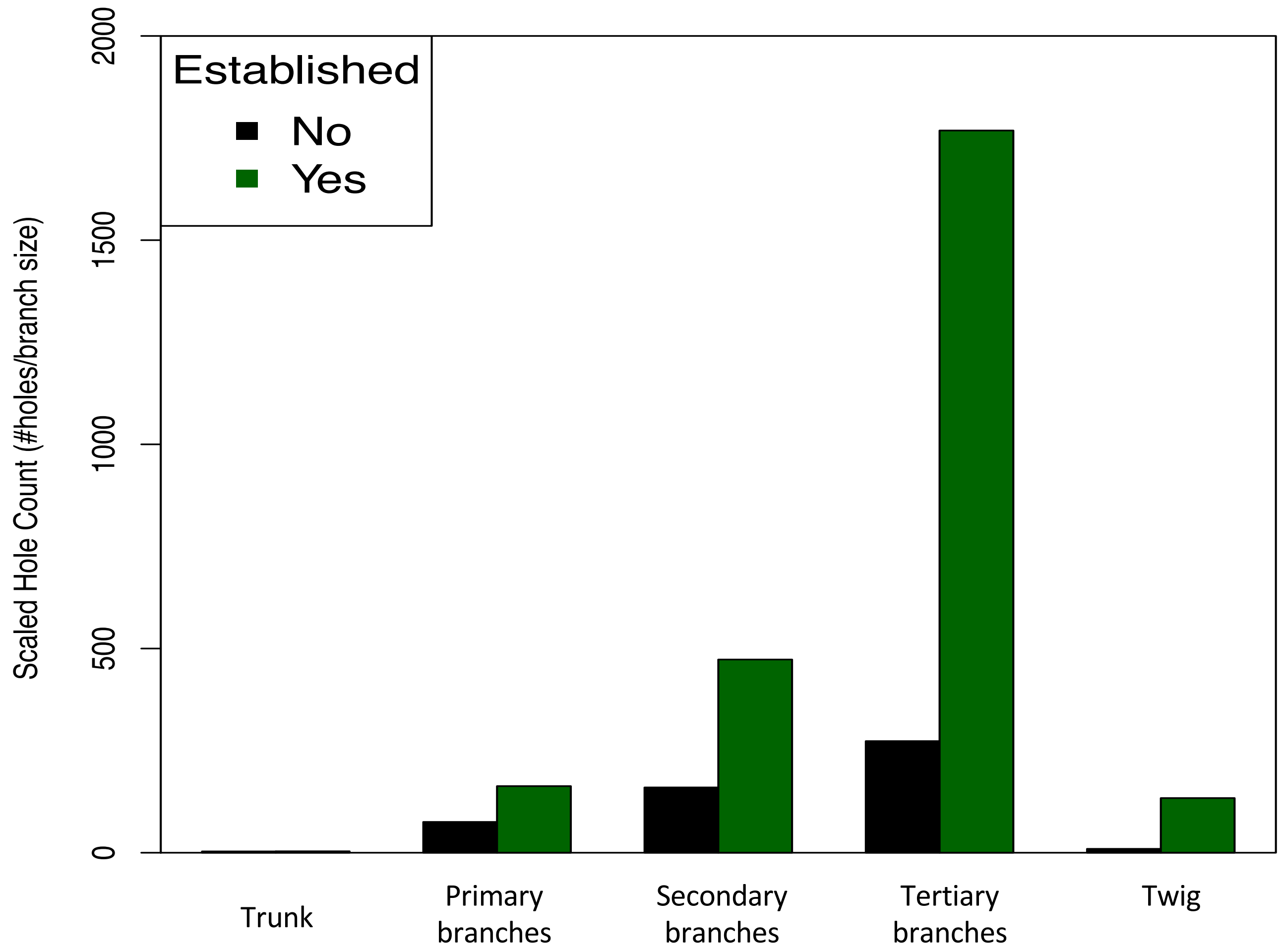
SHB incidence in San Diego and Ventura Counties



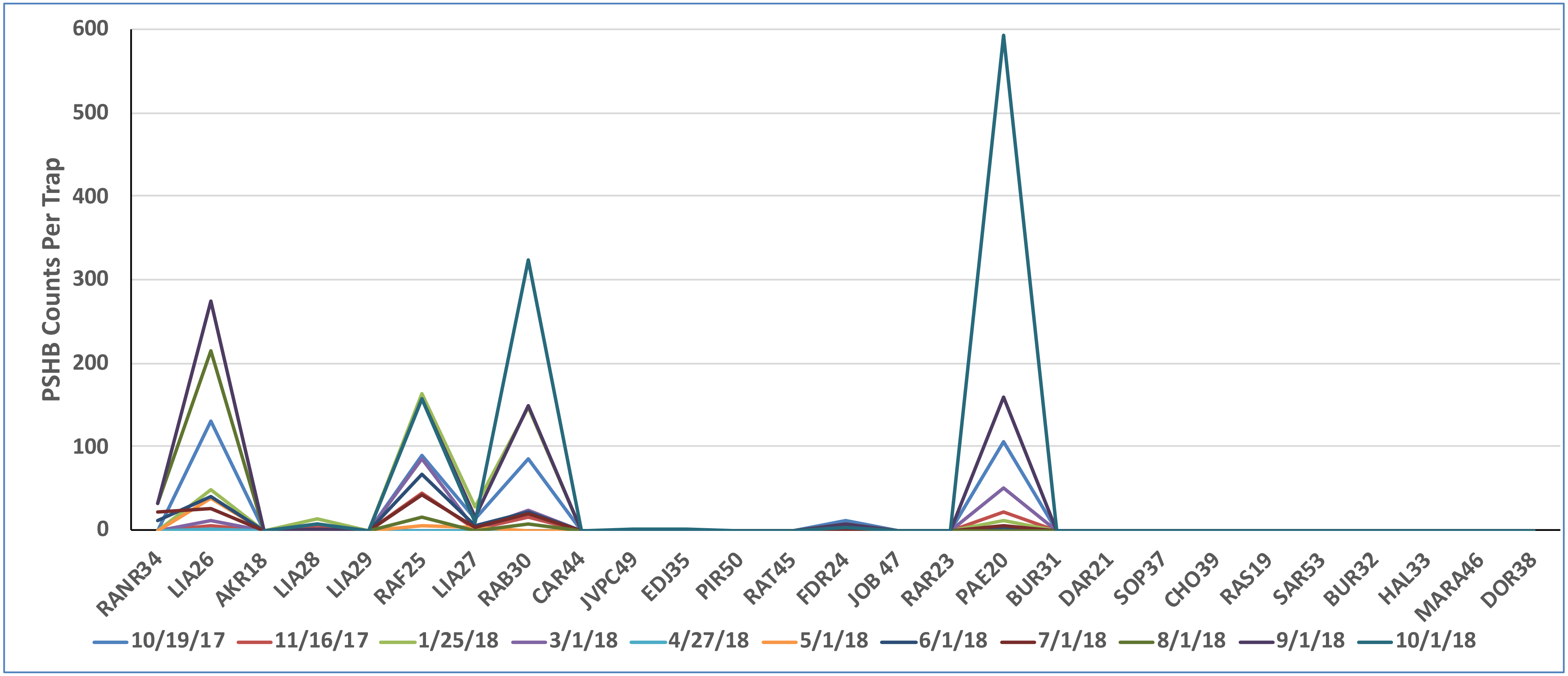
SHB incidence (the fraction of trees infested in each plot, between (0 – 1) for 14 of the 36 avocado groves that were infested in Ventura County and four of the five groves that were infested in San Diego County in 2017 and 2018 (green and black respectively).

Lynch, S.C., Carrillo, D.J., Stouthamer, R., Eskalen, A. 2018. Severity of Fusarium Dieback-Shot Hole Borers Analyzed. [From the Grove, Vol 8, No 4. pp.46-50.]

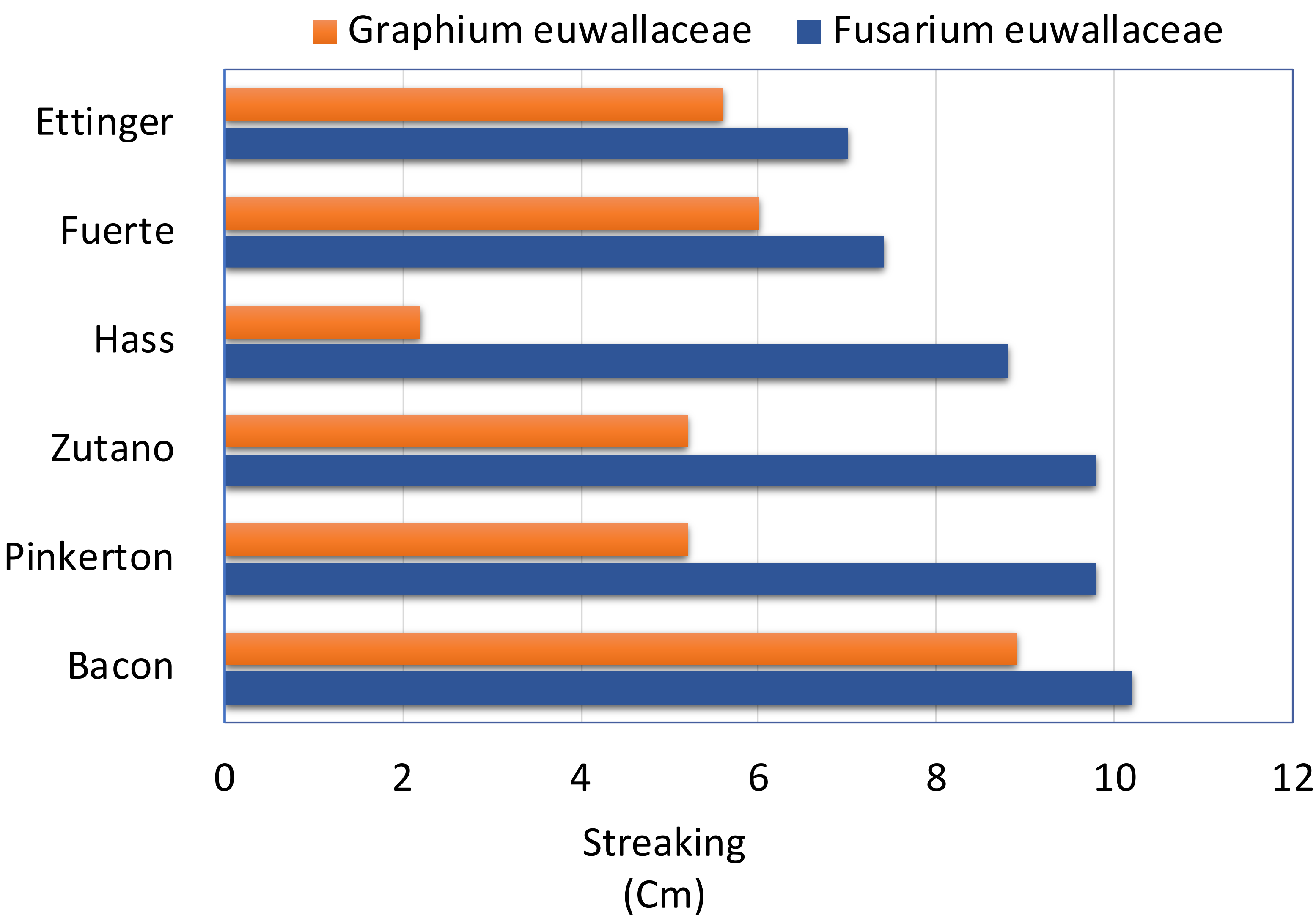
Attack frequency id ISHB on infested trees by plant part



Number of beetles trapped over time in Ventura County avocado groves



Pathogenicity test with different avocado varieties



Beetle infestation in riparian areas near avocado groves

Conclusion

- Monitoring and experimental results show that SHB-FD is causing less damage to avocado trees compared to other hosts in native vegetation (e.g., box elder, sycamores and willows).

- The beetle seems to prefer the branches of avocado — in particular the tertiary branches.

- Hence, removal of infested branches and orchard sanitation is essential to control the population of the beetle.

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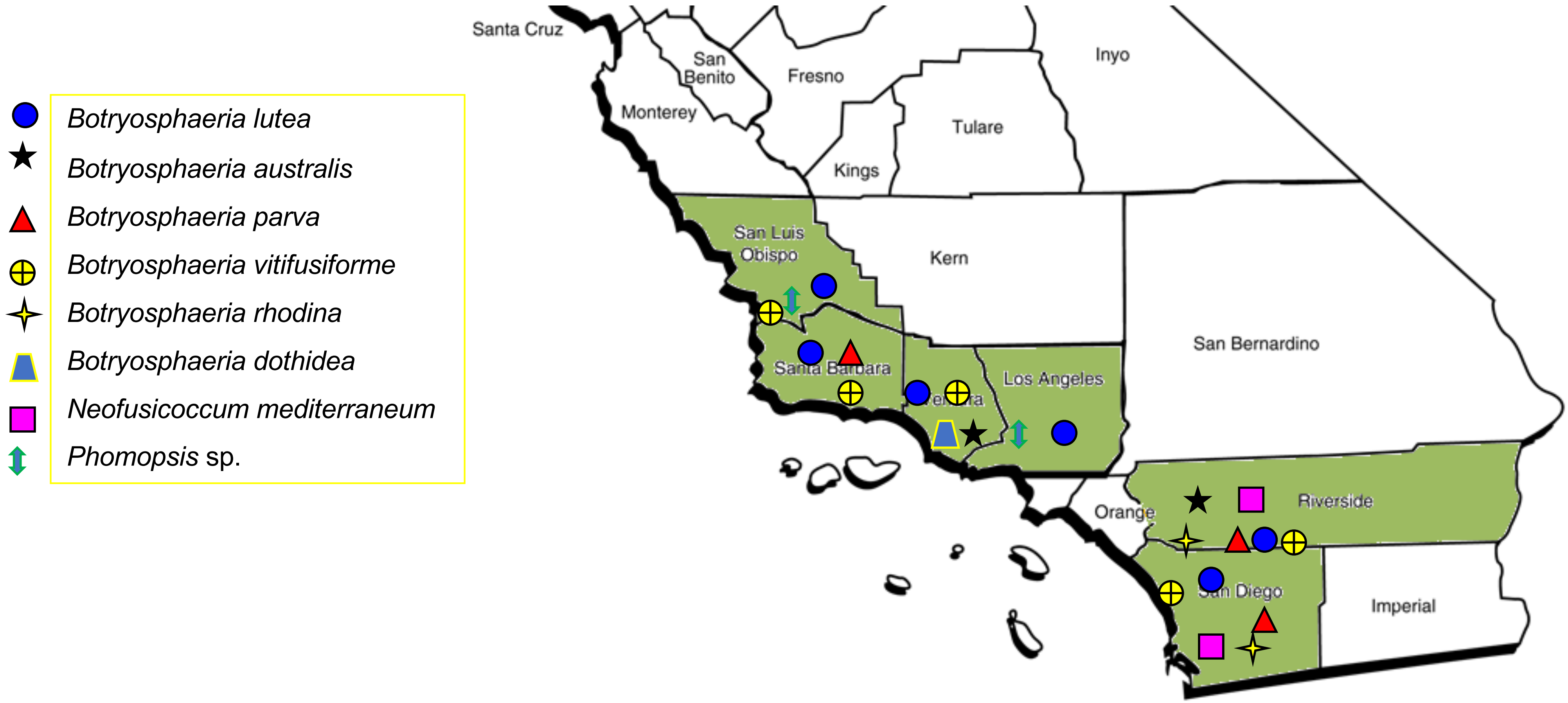
Wood canker pathogens contribute to beetle colonization on branches of avocado

Wood canker symptoms on old pruning wounds

Avocado Black Streak Disease

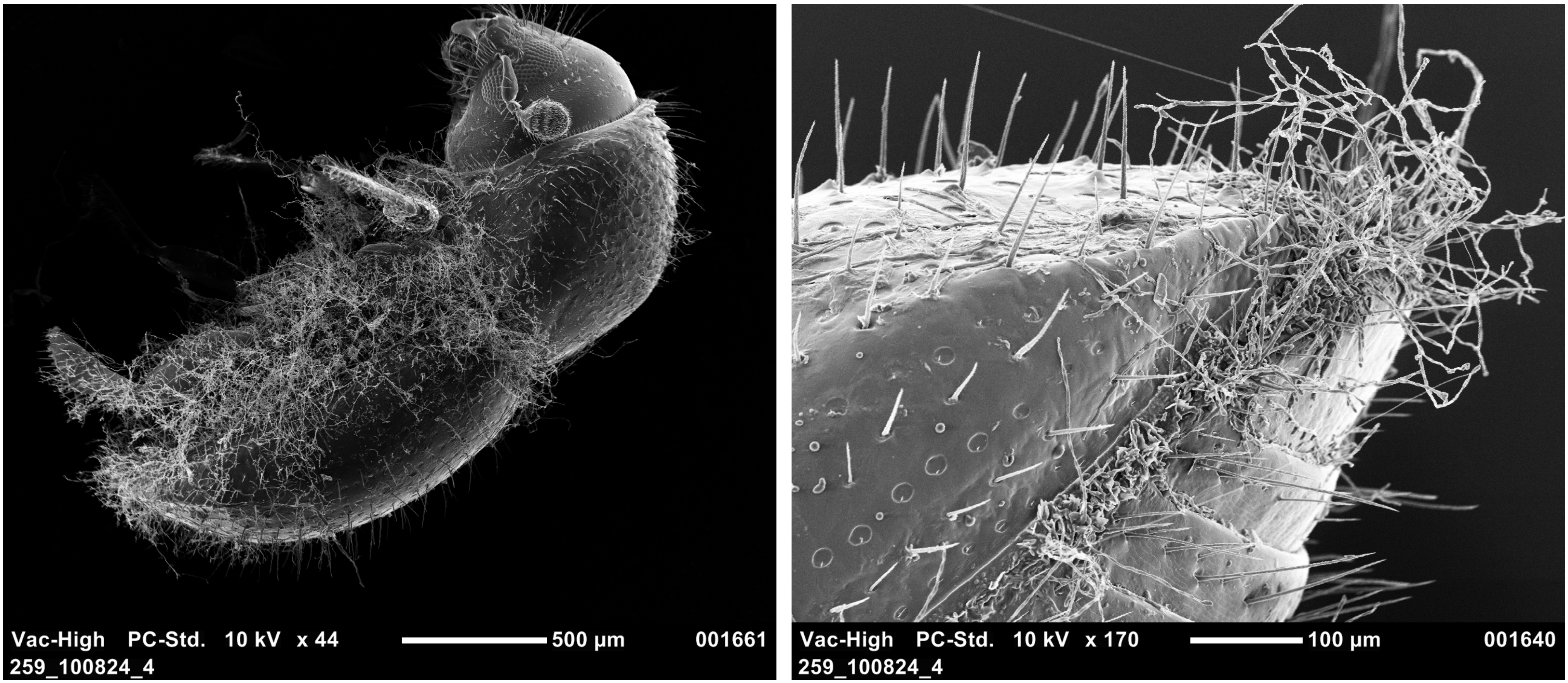
Geographical distribution of wood canker disease of avocado



Twizeyimana, M., Forster, H., McDonald, V., Wang, D.H., Adaskaveg, J.E., Eskalen, A., **2013**. Identification and pathogenicity of fungal pathogens associated with stem-end rot of avocado in California. *Plant Disease*. 97:12, 1580-1584.

Twizeyimam, M., McDonald, V., Mayorquin, J.S., Wang, D.H., Na, F., Akgul, D.S., Eskalen, A. **2013**. Effect of Fungicide Applications on the Management of Avocado Branch Canker (Formerly Dothiorella Canker) in California. *Plant Disease*. 97:7, 897-902.

Fungal contamination on the beetle surface



Picture Credit: Matt Kasson

Beetle transfers secondary pathogenic fungi by contamination on their surface

Secondary fungal pathogen infestation on SHB infested Avocado

Branch canker

Black streak disease

Look for symptoms at the margin of wood canker on pruned branches

Old pruning wounds

Wood canker

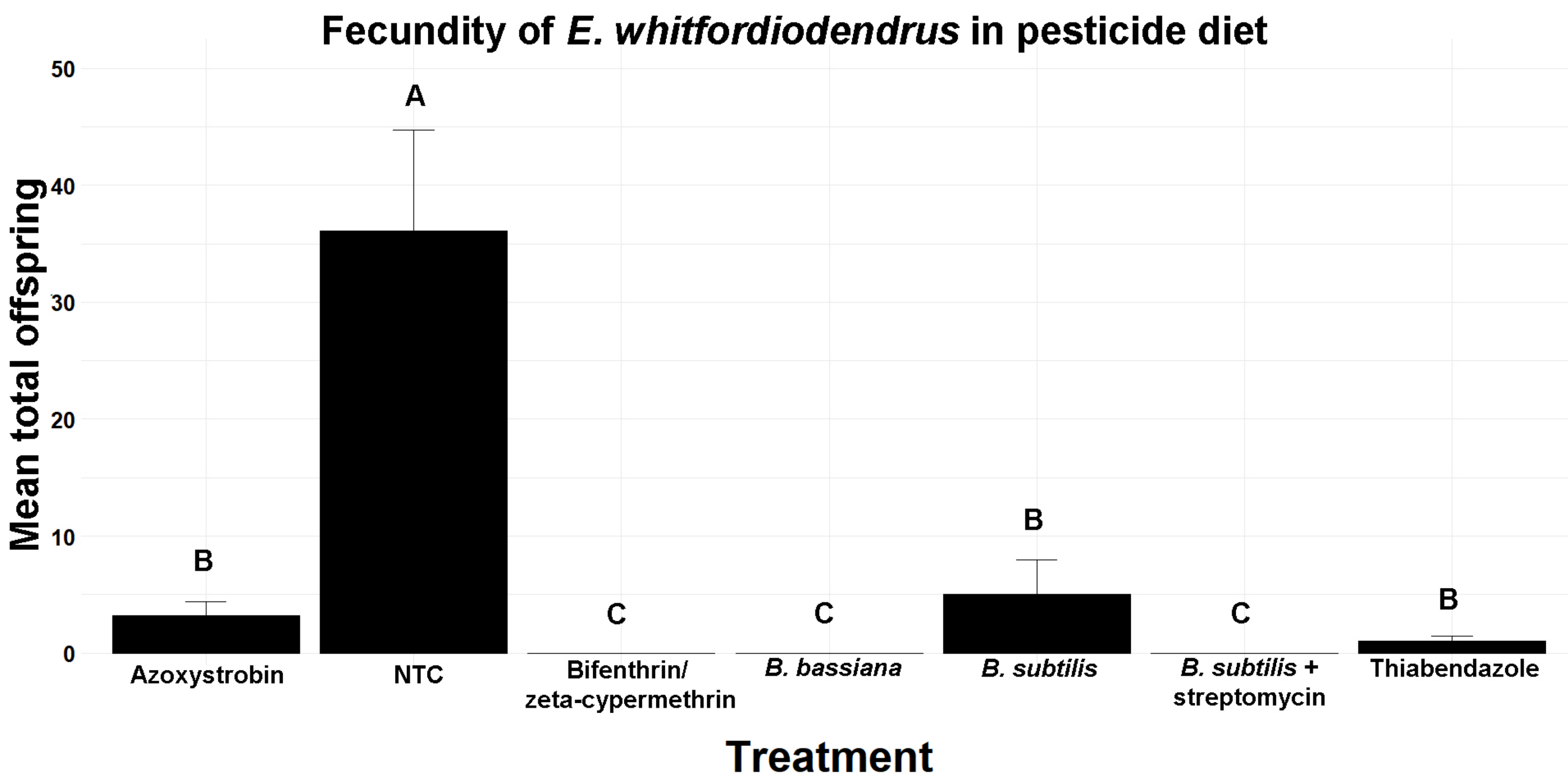
Entry hole

Black Streak disease of avocado caused by *Botryosphaeriaece*

Strategies for management of ISHB-FD in avocado

- Four treatment options shown to be effective *in vitro*:
  - Azoxystrobin (Abound® (Syngenta))
  - Thiabendazole (Arbortect® 20S (Arborjet); *in vitro* only)
  - Bifenthrin & Zeta Cypermethrin (Hero® EW (FMC))
  - *Bacillus subtilis* (Serenade® ASO (Bayer))
  - *Beauvaria bassiana* (Mycotrol® WPO (Bioworks)
- *In vitro*
  - *Fungicides and B. subtilis* products amended to PDA
  - Sawdust media amended with treatments
  - Testing *in vitro* on treated bolts in mason jars
- Field trial
  - Lab reared beetles via 3D printed traps 24hr post pesticide application

Sawdust media amended with treatments *in vitro*



Pairwise comparison (EMM) $P < 0.05$

What to do in an ISHB infested grove

- Inspect branches and branch collar for signs of SHBs and FD symptoms in avocado.
- If the beetle has not colonized (produced a gallery), there is no need to make pruning cuts.
- If the beetle/fungus has colonized on the branch collar, remove the infested branch, including the branch collar.
- Spray pruning wound with *Bacillus subtilis* (1.5 percent Serenade ASO®)
- Do not move infested wood, chip and solarize pruned wood using a clear plastic tarp for several months.

Thank You

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