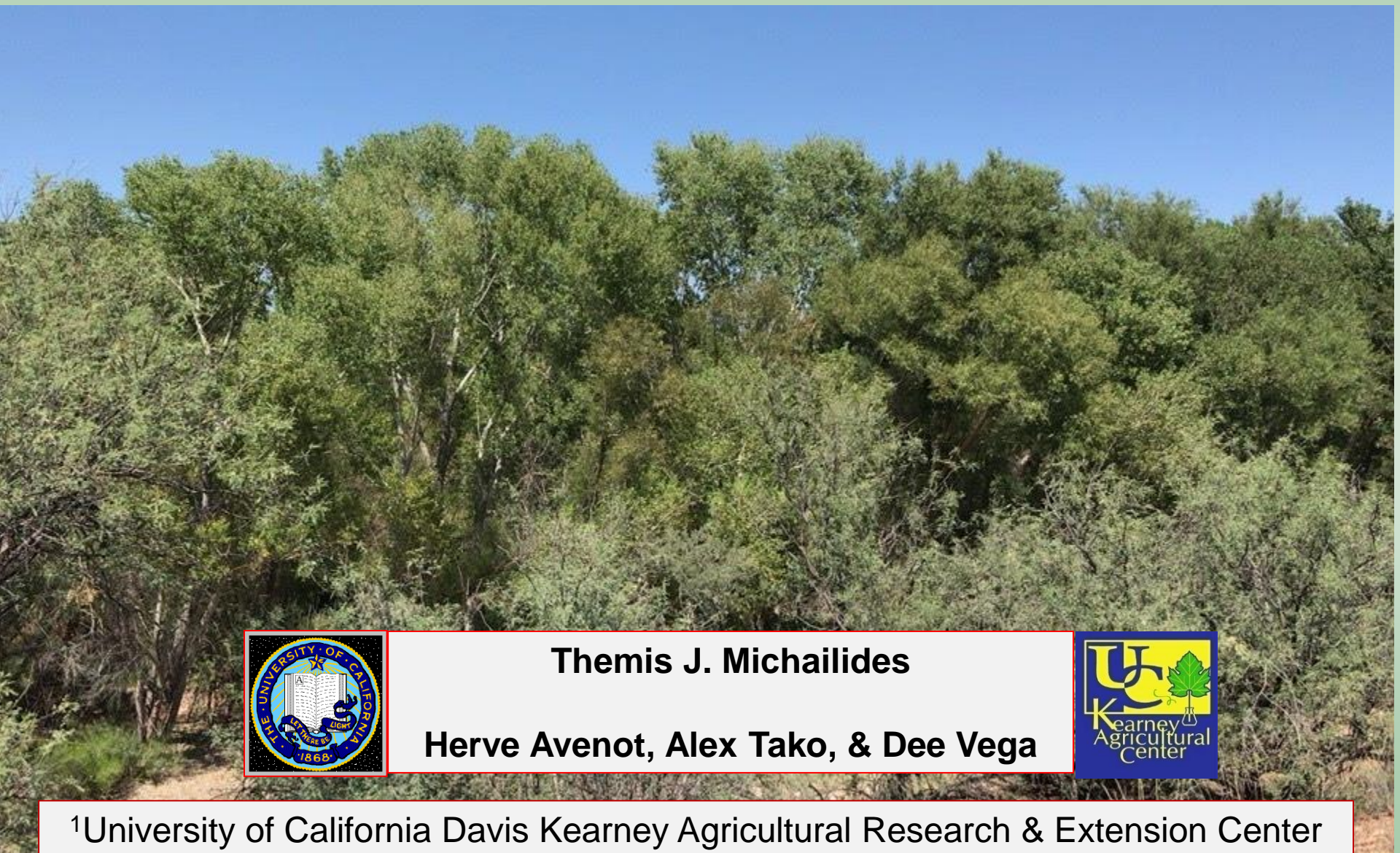


Branch Canker and Dieback and Anthracnose Blight of Avocado



Themis J. Michailides

Herve Avenot, Alex Tako, & Dee Vega



¹University of California Davis Kearney Agricultural Research & Extension Center

Dothiorella rot of avocado fruit caused by
Botryosphaeria ribis

CALIFORNIA
AGRICULTURAL EXTENSION SERVICE
CIRCULAR 120
January, 1941

DISEASES OF FRUITS
AND NUTS

RALPH E. SMITH

Cooperative Extension work in Agriculture and Home Economics, College of Agriculture,
University of California, and United States Department of Agriculture cooperating.
Distributed in furtherance of the Acts of Congress of May 8, and June 30, 1914.
R. H. Crocker, Director, California Agricultural Extension Service.

THE COLLEGE OF AGRICULTURE
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

covered controls dothiorella rot of avocado fruit. Tipburn, removal of dead twigs, early picking, and prompt utilization of fruit are considered the most promising measures for this disease. Low temperatures retard the ripening process and also the growth of most of the molds which cause decay, but Fuertes and most other varieties should not be kept colder than 45° Fahrenheit. The calyx or stem scar is the most vulnerable point for entrance of decay organisms; it may be disinfected and waxed.

Sun-blotch.—Affected green stems show yellowish depressed streaks, leaves are sometimes variegated and misshaped, and the fruits are deformed by depressed broad streaks or spots. Older stems are abnormal, with dead areas; they age prematurely and hang down. Severely affected trees are worthless, but many are only slightly affected and appear to recover. This disease is poorly named, for it is not caused by sunburn. It is a virus disease transmitted to healthy scions by diseased stocks or to healthy stocks by diseased scions. It is not propagated by seed.

Select mother trees from which scions are to be taken by carefully observing the twigs and fruits, and by observing the growth of scions from the individual trees under trial. Trees which show any sun-blotch symptoms or which give sun-blotched propagations should not be used.

Tipburn.—This trouble gets its name from the fact that the mature leaves dry back from the tips and edges, the trouble increasing as the time for leaf shedding in the spring approaches. The new leaves are for a time normal. The disease is due to an accumulation of salt (common salt) in the leaves, and is brought about by an excess of this substance in the soil or irrigation water. Drought, wind, and lack of organic matter and nitrogen in the soil contribute to the trouble. Avocados will tolerate somewhat less salt than citrus fruits. Fungi like *Botryosphaeria* and *Colletotrichum*, which cause decay in the softening fruit, propagate in tipburned areas, particularly in the coastal districts.

To prevent this trouble, make sure that the drainage is adequate, use water of low salt content for irrigation, and water freely to wash down accumulated salt and keep soil moisture fairly high.

Unfruitfulness.—Many large seedling avocado trees do not blossom. Other trees blossom but set no fruit; still others set fruit, but it falls before maturity. Avocado flowers are perfect, but have an unusual

Circular 120
January 1941

UNIVERSITY OF CALIFORNIA
COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
BERKELEY, CALIFORNIA

Melaxuma of the Walnut, "*Juglans regia*"
(A PRELIMINARY REPORT)

**Bot of walnut caused
by *Botryosphaeria ribis***

By HOWARD S. FAWCETT

BULLETIN No. 261

Berkeley, Cal., November, 1915

1915

**Branch wilt: *Neoscytalidium dimitiatum*
(member of Botryosphaeriaceae family)**



Botryosphaeria canker and blight of walnut



cankers



Fruit blight

Band canker of almond (1950s)



Botryosphaeria panicle and shoot blight of pistachio (discovered in 1984)



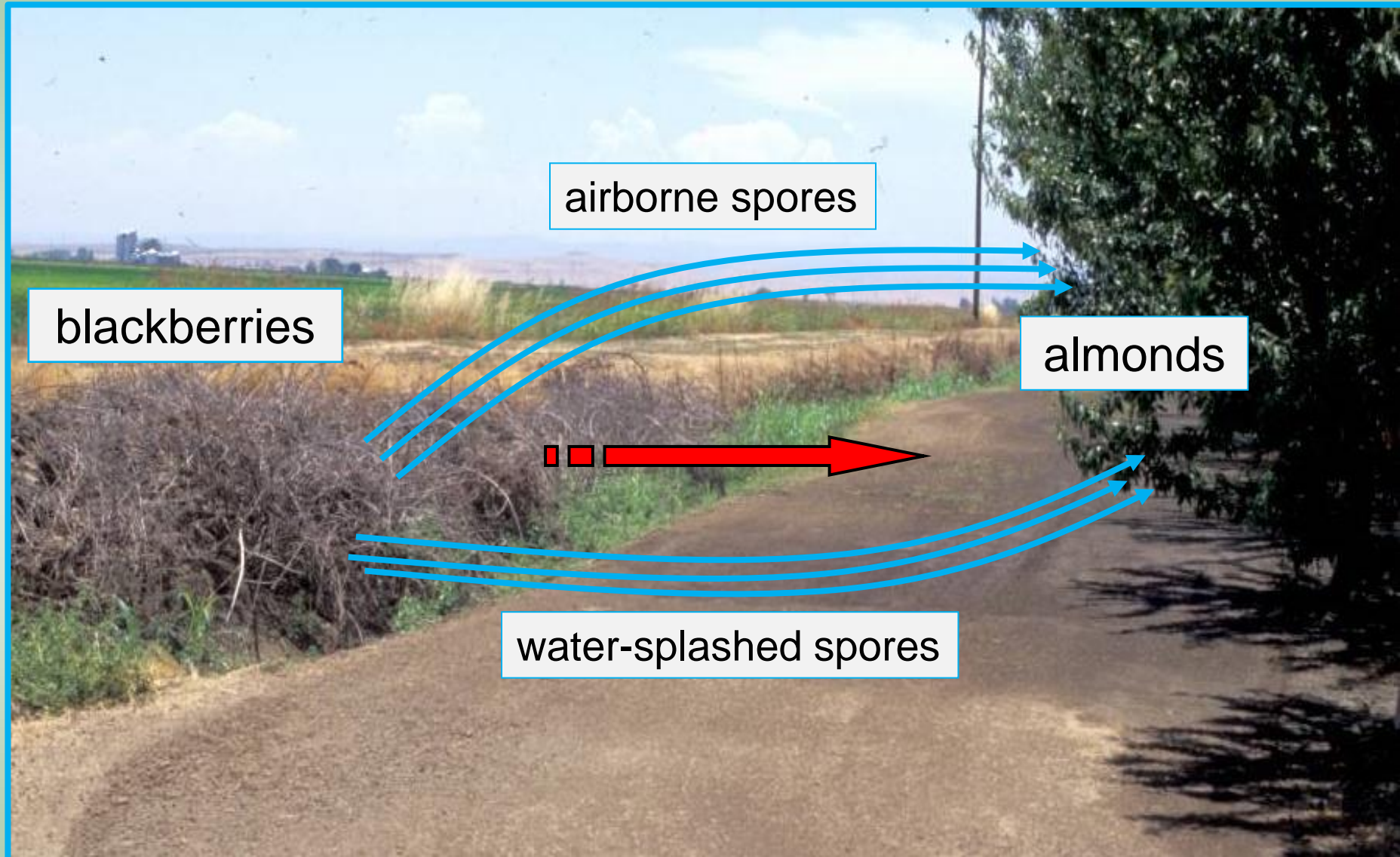
... following the *Botryosphaeria* epidemic in 1996-1998



Elderberry along Highway 5 with severe Bot blight (Colusa)



Botryosphaeria from blackberries to almonds



Severe Botryosphaeria blight on sequoia trees (in Sacramento)



Cedar & Redwood trees with
Bot blight (Fresno)



Cedar trees in a public park
with Bot blight (Madera)



Bot blight in trees of a public park (Fresno)



Hosts from which *Botryosphaeria dothidea* was frequently isolated in California

Host	Scientific name	Family
Almond	<i>Prunus dulcis</i>	Rosaceae
Apple	<i>Malus domestica</i>	Rosaceae
Avocado*	<i>Persea americana</i>	Lauraceae
Blackberry*	<i>Rubus ursinus</i>	Rosaceae
Black walnut	<i>Juglans hinsii</i>	Juglandaceae
Carob seed tree	<i>Ceratonia siliqua</i>	Leguminosae
Incense cedar	<i>Cedrus libani</i>	Pinaceae
Deodar cedar	<i>Cedrus deodara</i>	Pinaceae
Chinese hackberry	<i>Celtis sinensis</i>	Ulmaceae
California redwood*	<i>Sequoia sempervirens</i>	Taxodiaceae
Cotoneaster	<i>Cotoneaster frigidus</i>	Rosaceae
Cottonwood	<i>Populus deltoides</i>	Populaceae
English walnut	<i>Juglans regia</i>	Juglandaceae
Eucalyptus	<i>Eucalyptus coccifera</i>	Myrtaceae
Euonymus	<i>Euonymus fortunei</i>	Celestraceae
Silver dollar Eucalyptus	<i>Eucalyptus orbifolia</i>	Myrtaceae
Feijoa	<i>Feijoa sellowiana</i>	Myrtaceae
Fig	<i>Ficus carica</i>	Fagaceae

Hosts from which *Botryosphaeria dothidea* was frequently isolated in California

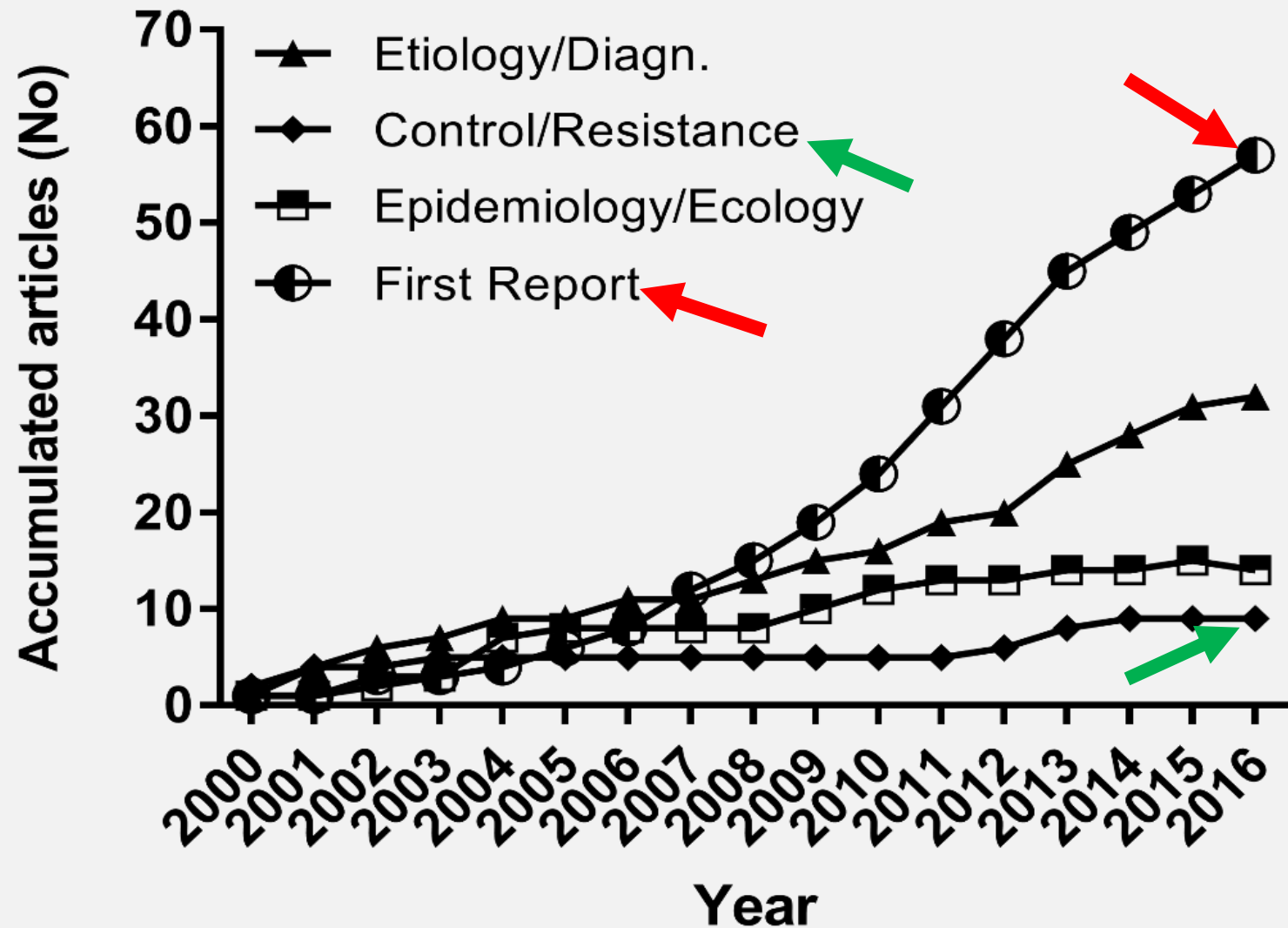
Host	Scientific name	Family
Giant sequoia*	<i>Sequoiadendron giganteum</i>	Taxodiaceae
Juniper	<i>Juniperus occidentalis</i>	Cypressaceae
Jasmine	<i>Jasminum officinale</i>	Jasminaceae
Lemon	<i>Citrus × limon</i>	Citraceae
Sweet gum	<i>Liquidambar styraciflua</i>	Mamamelidaceae
Maple	<i>Acer sp.</i>	Aceraceae
Oak	<i>Quercus sp.</i>	Fagaceae
Olive*	<i>Olea europea</i>	Olivaceae
Orange	<i>Citrus × auranteum</i>	Citraceae
Pistachio	<i>Pistacia vera</i>	Anacardiaceae
Pear	<i>Pyrus communis</i>	Rosaceae
Pecan	<i>Carya illinoensis</i>	Junglandaceae
Persimmon	<i>Diospyros kaki</i>	Ebenaceae
Pine	<i>Pinus radiata</i>	Pinaceae
Prune	<i>Prunus domestica</i>	Rosaceae
Firethorn*	<i>Pyracantha coccinea</i>	Rosaceae
Raymond ash	<i>Fraxinus augustifolia</i> <i>augustifolia</i> subsp. <i>oxycarpa</i>	Oleaceae

Hosts from which *Botryosphaeria dothidea* was frequently isolated in California

Host	Scientific name	Botanical family
Sycamore maple	<i>Acer pseudoplatanus</i>	Aceraceae
Wax leaf Privet	<i>Ligustrum japonicum</i>	Oleaceae
Western redbud	<i>Cedris canadensis</i>	Leguminosae
Wild rose	<i>Rosa sp.</i>	Rosaceae
White willow	<i>Salix alba</i>	Salicaceae
Arroyo willow	<i>Salix lasiolepis</i>	Salicaceae
Weeping willow	<i>Salix babylonica</i>	Salicaceae

* This Table includes fruit and nut trees, vines, ornamentals, and wild forest trees and bushes

Dynamic of articles on **Botryosphaeriaceae spp.** published in the American Phytopathological Society Journals from 2000 to 2016



Disease cause: *Botryosphaeria* spp.

saprophytes – endophytes – parasites

DISEASES

- Leaf spots
- Fruit rots
- Dieback back
- Perennial Cankers
 - Avocado, Citrus
 - Pistachio, Walnut
 - Almond
 - Grapevine, Apple ...
 - Oak
 - Giant Sequoia ...

HOSTS

- Woody perennial crops
- Ornamental plants
- Native and introduced forest trees
- Common in riparian areas

Some species can survive as “endophytes” (latent infections) in symptomless tissues.

Avocado branch canker and dieback



Killing of major branches:

- Fruit loss
- Production wood loss

Avocado branch canker and dieback disease (Dothiorella canker)

- Increased incidence in recent years in California avocados.
- Caused by a complex of Botryosphaeriaceae species (McDonald & Eskalen, 2011).



Project funded by CAC:

Overall objective:

- To establish a comprehensive picture on the identity, prevalence, distribution, and impact of *Botryosphaeria* and *Colletotrichum* species in California avocados;
 - Year 1: Emphasis on old avocado orchards
 - Year 2: Emphasis on nurseries and young orchards
- To understand the infection process by these two diseases (*Botryosphaeria* branch canker and dieback and Anthracnose) in order to develop management approaches.

Specific objectives:

- 1. Determine the extent of Botryosphaeria/Anthracnose presence in avocado groves.**
- 2. Identify the most common and aggressive species of Botryosphaeriaceae and *Colletotrichum*.**
- 3. Study the life/ disease cycle of these pathogens in avocado groves and factors affecting these diseases.**
- 4. Develop disease control measures.**

Botryosphaeria canker and dieback invades sunburned tissues and pruning wounds



Pruning wound with infection & canker

Sunburn damage



Death (canker) of graft union



Courts of infection of avocado by *Botryosphaeria*

- Pruning wounds → cankers
- Grafting wounds → death
- Wounds from hail, freeze, sunburn → cankers
- Leaf tip burn due to drought, salt, or lack of organic matter → leaf blight
- Cracks caused by drought or wind → cankers
- Mechanical wounds → cankers
- Latent infections → twig and leaf lesions, & fruit rot

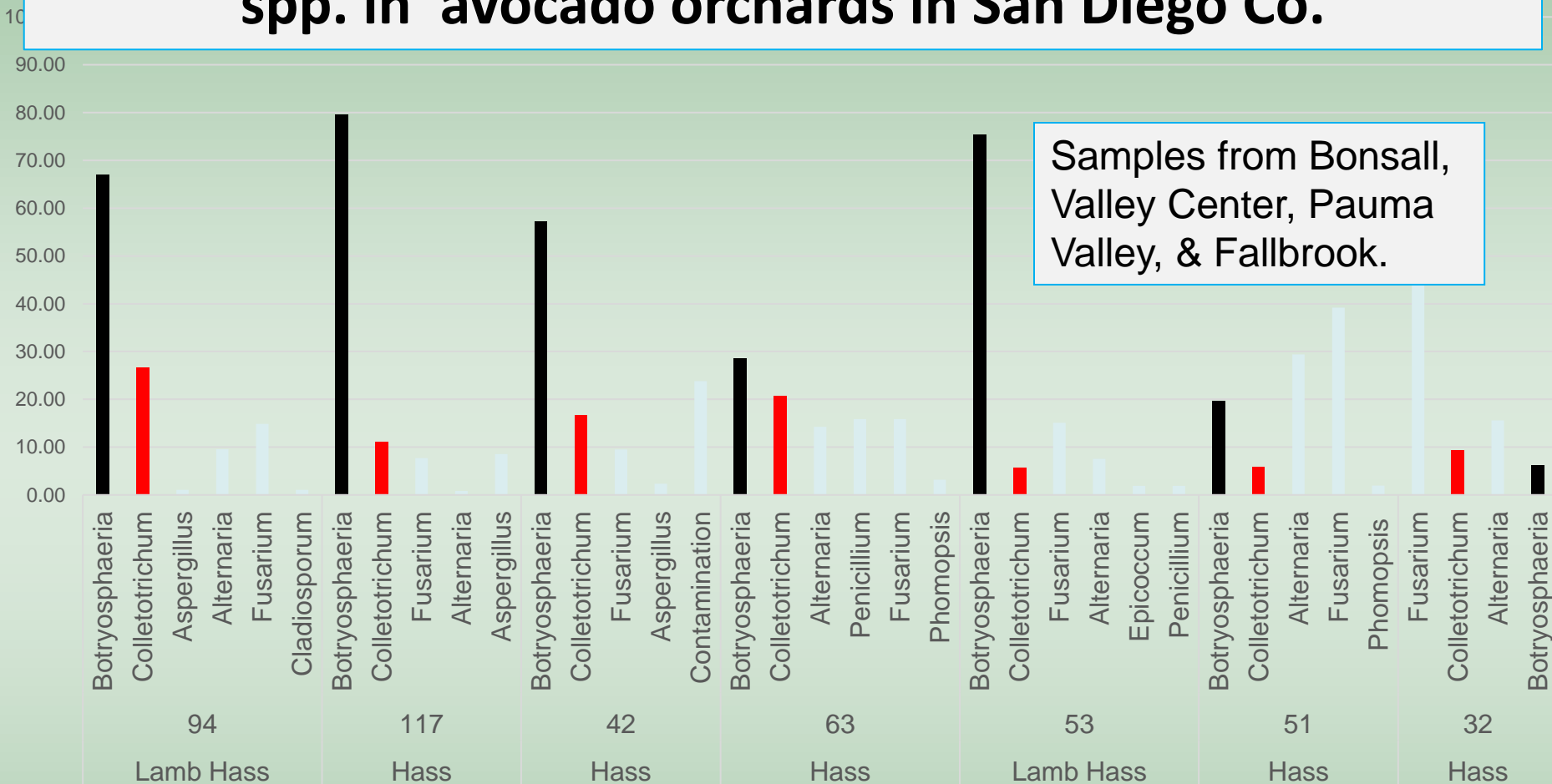
Survey of old avocado orchards in main Counties of production

Sampling and plating of various avocado tissues



San Luis obispo; Ventura; Riverside; & San Diego

Survey: Incidence of *Botryosphaeria* and *Colletotrichum* spp. in avocado orchards in San Diego Co.



Samples include mainly twigs and branches

Grower 1

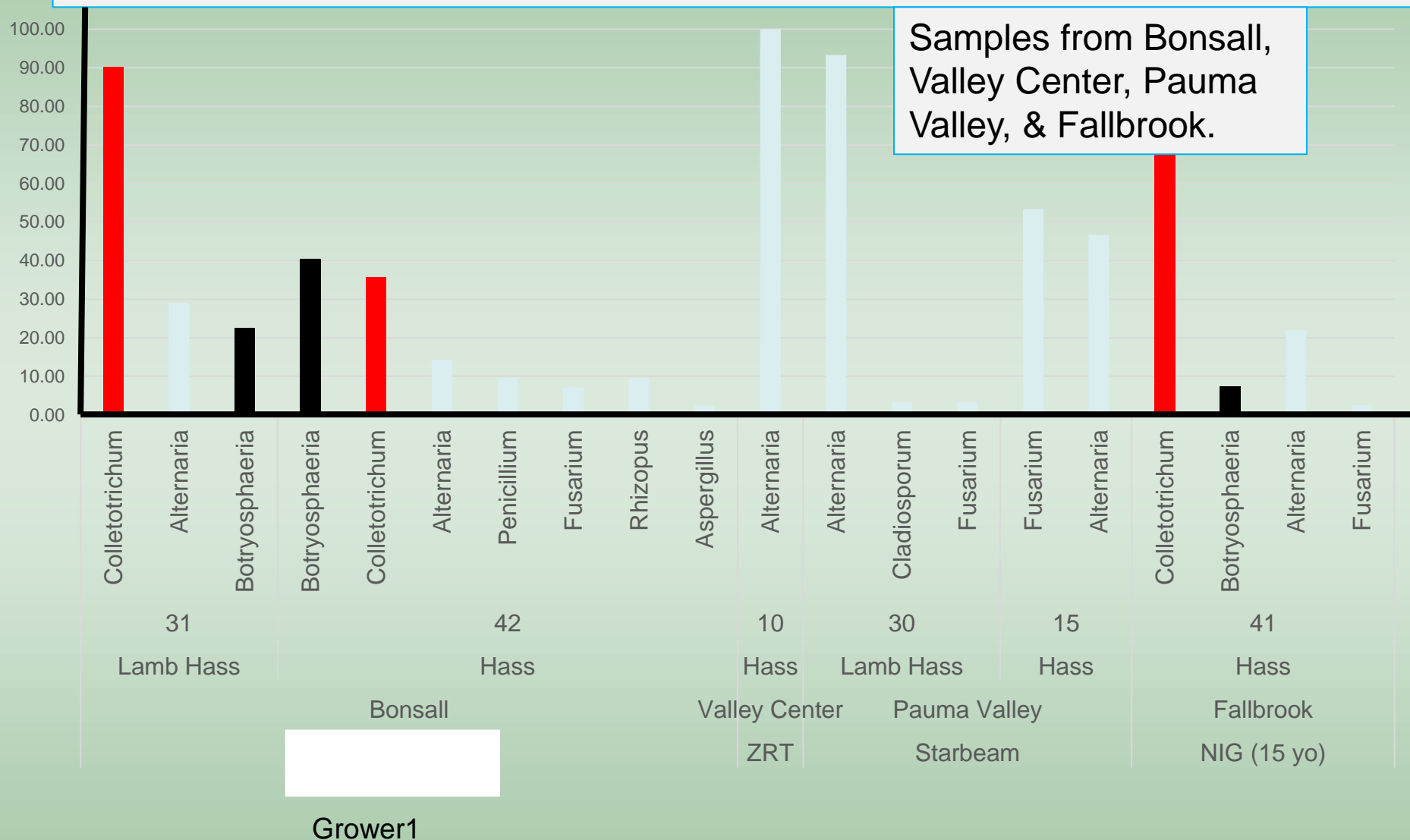
Grower 2

Grower 3

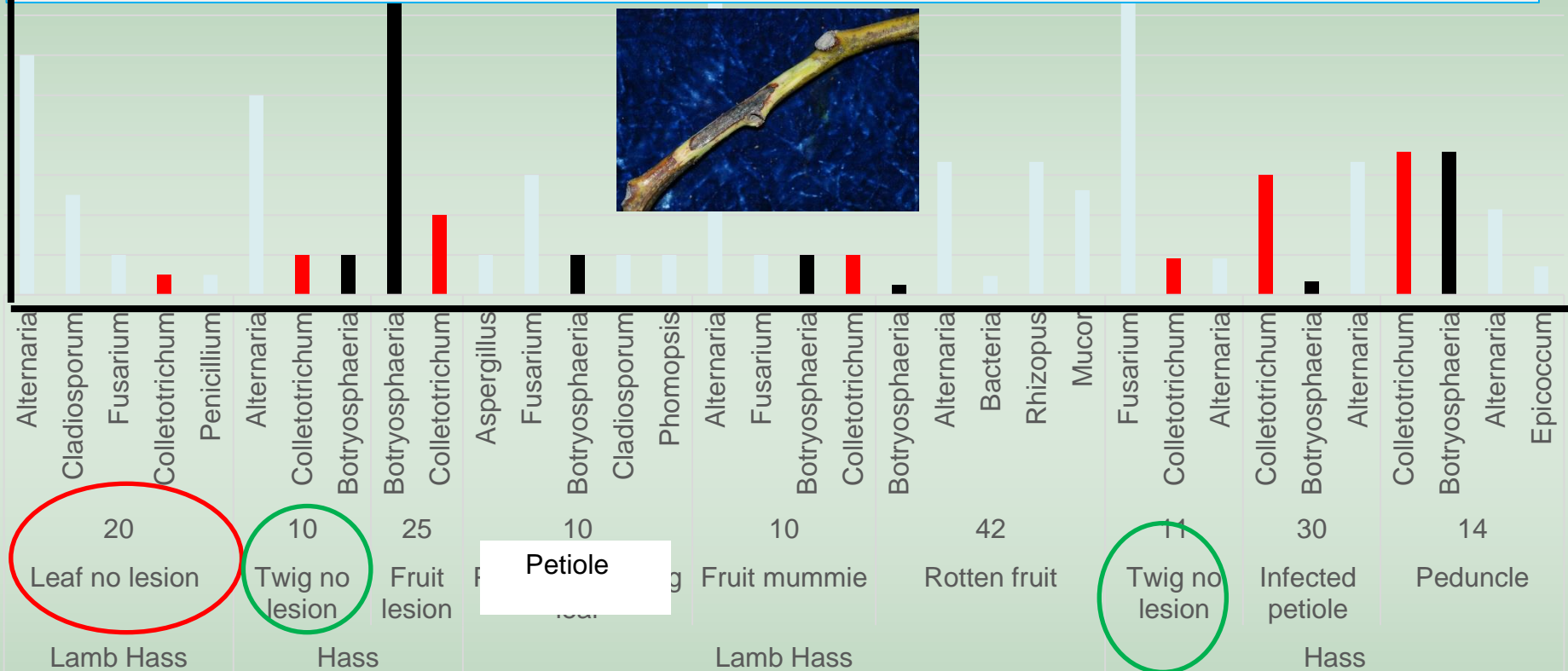
Grower 4

Grower 5

Incidence of *Botryosphaeria* spp. and *Colletotrichum* spp. & other fungi isolated from **avocado leaves in San Diego Co.**

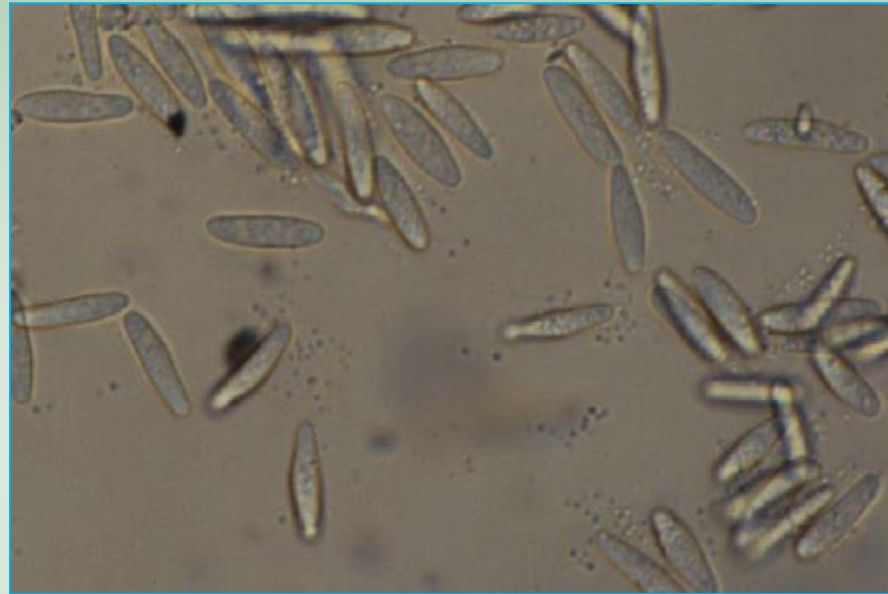
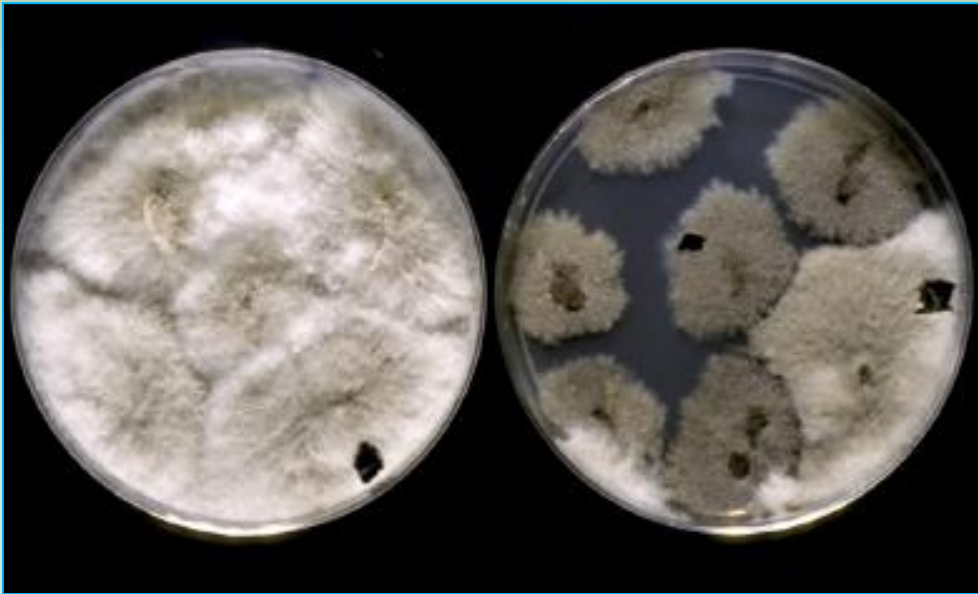


Incidence of Botryosphaeria spp. and Colletotrichum spp. & other fungi isolated from other organs of avocado in San Diego Co.

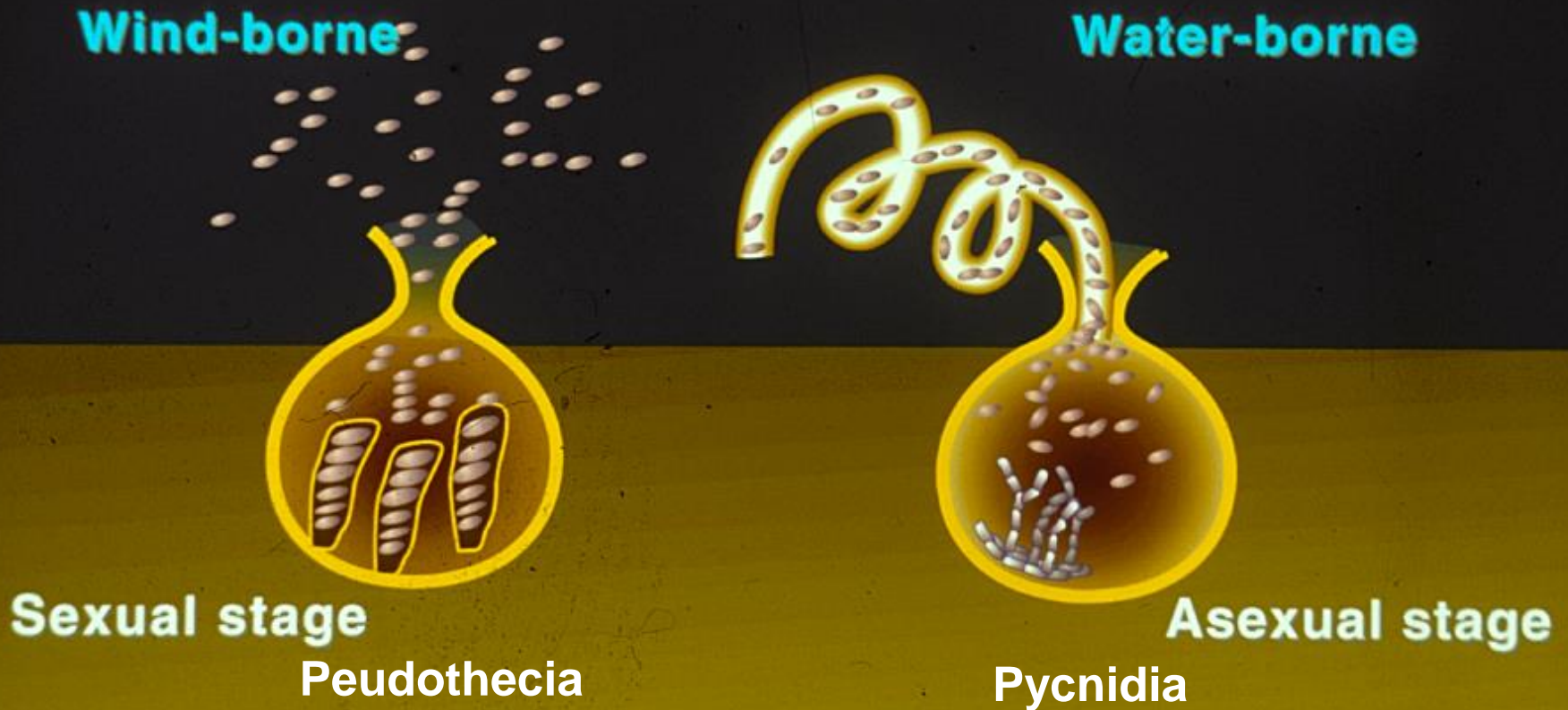


Samples from Bonsall, Valley Center, Pauma Valley, & Fallbrook.

Pathogen morphological identification at the genus level

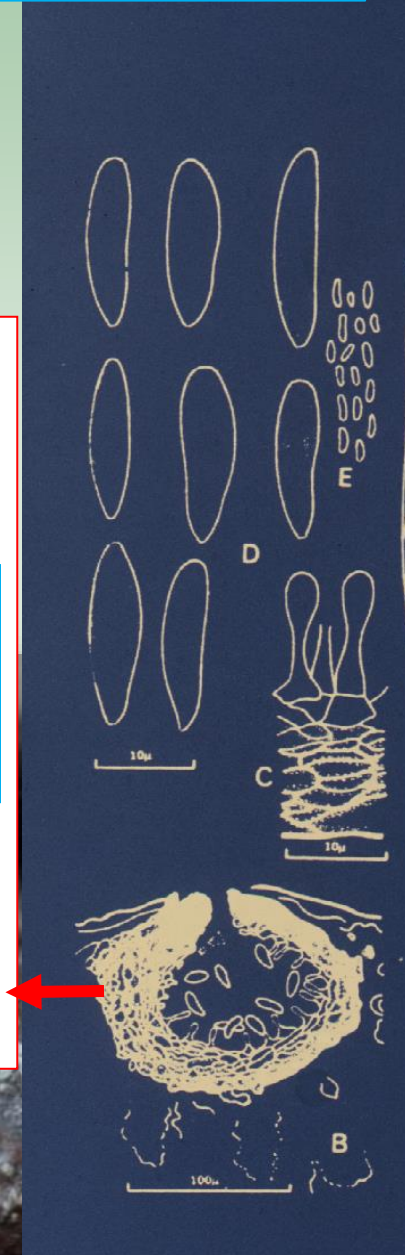
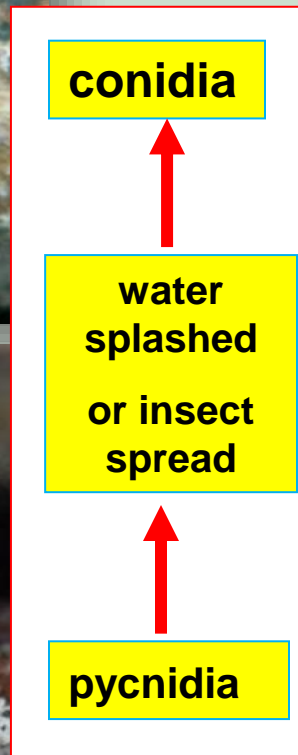
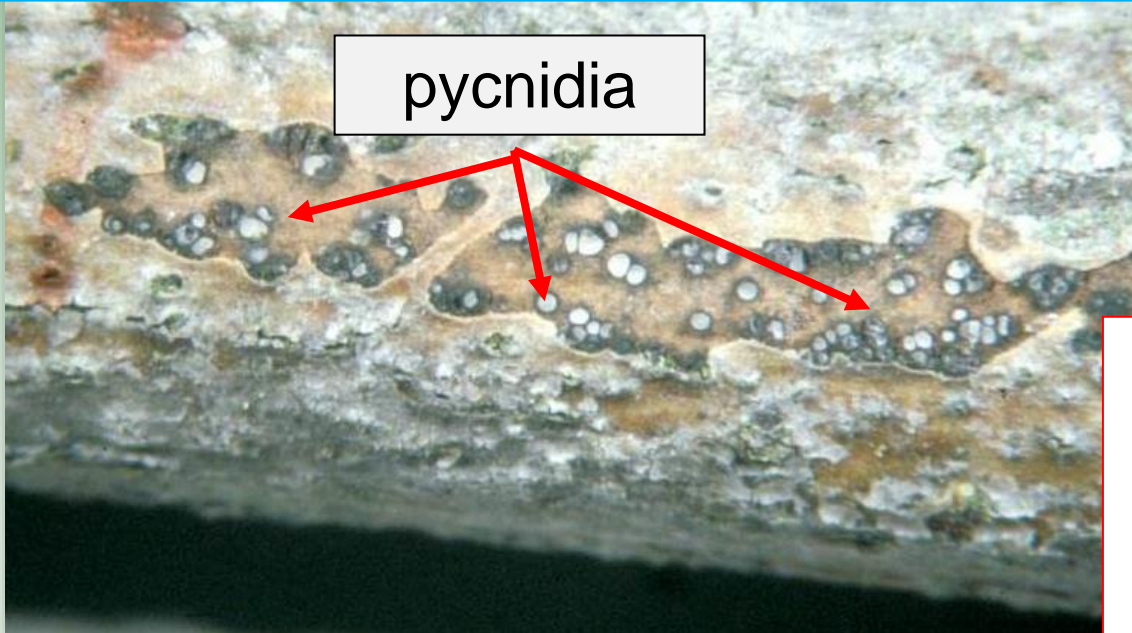


***Botryosphaeria* spp. reproductive structures**



Both pycnidia and pseudothecia in avocado tissues

Water-splashed inoculum in pycnidia (very common)





Leaf tip burn



ascospores



pseudothecia

We also found pseudothecia in avocado tissues (branches, shoots, and leaves)

Selection of representative *Botryosphaeria* isolates for Identification **at the species** level using molecular methods



Botryosphaeria dothidea

***Neofusicoccum* spp.**

(*N. mediterraneum*, *N. luteum*, *N. parvum*...)



***Lasiodiplodia* spp.**

Identification of *Botryosphaeria* species through DNA sequencing and Distribution in avocados

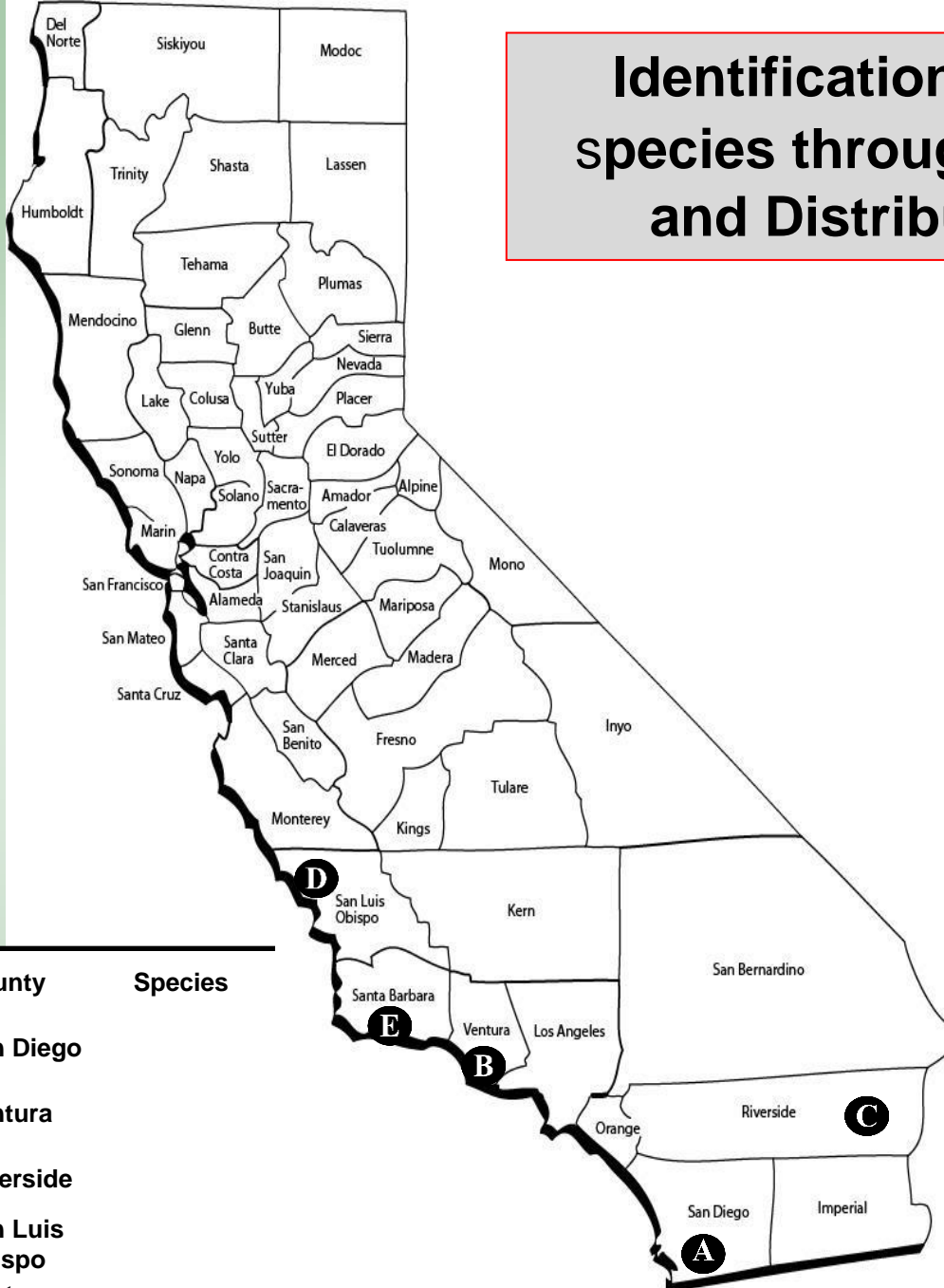
Botryosphaeriaceae isolates

1. *Neofusicoccum luteum*
2. *N. nonquaesitum*
3. *N. australe*
4. *N. cryptoaustrale*
5. *Botryosphaeria dothidea*
6. *N. mediterraneum*
7. *Lasiodiplodia theobromae*
8. *N. parvum*
9. *Diplodia pseudoseriata*
10. *D. corticola*
11. *D. africana*

Diaporthaceae



County	Species
A San Diego	
B Ventura	
C Riverside	
D San Luis Obispo	
E Santa Barbara	



Similar examples in other crops

Summary of Botryosphaeriaceae in nut crops – California

Fungal species	Walnut	Pistachio	Almond
<i>Botryosphaeria dothidea</i>	+	+	+
<i>Neofusicoccum parvum</i>	+	+	+
<i>Neofusicoccum mediterraneum</i>	+	+	+
<i>Diplodia mutila</i>	+	---	---
<i>Neofusicoccum nonquaesitum</i>	+	---	+
<i>Neofusicoccum vitifusiforme</i>	+	+	---
<i>Diplodia seriata</i>	+	+	+
<i>Dothiorella iberica</i>	+	+	+
<i>Lasiodiplodia citricola</i>	+	+	+
<i>Neoscytalidium dimitiatum</i> (= <i>Hendersonula toruloidea</i>)	+	+	+
<i>Diaporthe rhusicola</i> (<i>Phomopsis</i>)	+	+	+
<i>Diaporthe neitheicola</i> (<i>Phomopsis</i>)	+	---	---

Anthracnose symptoms on avocados:

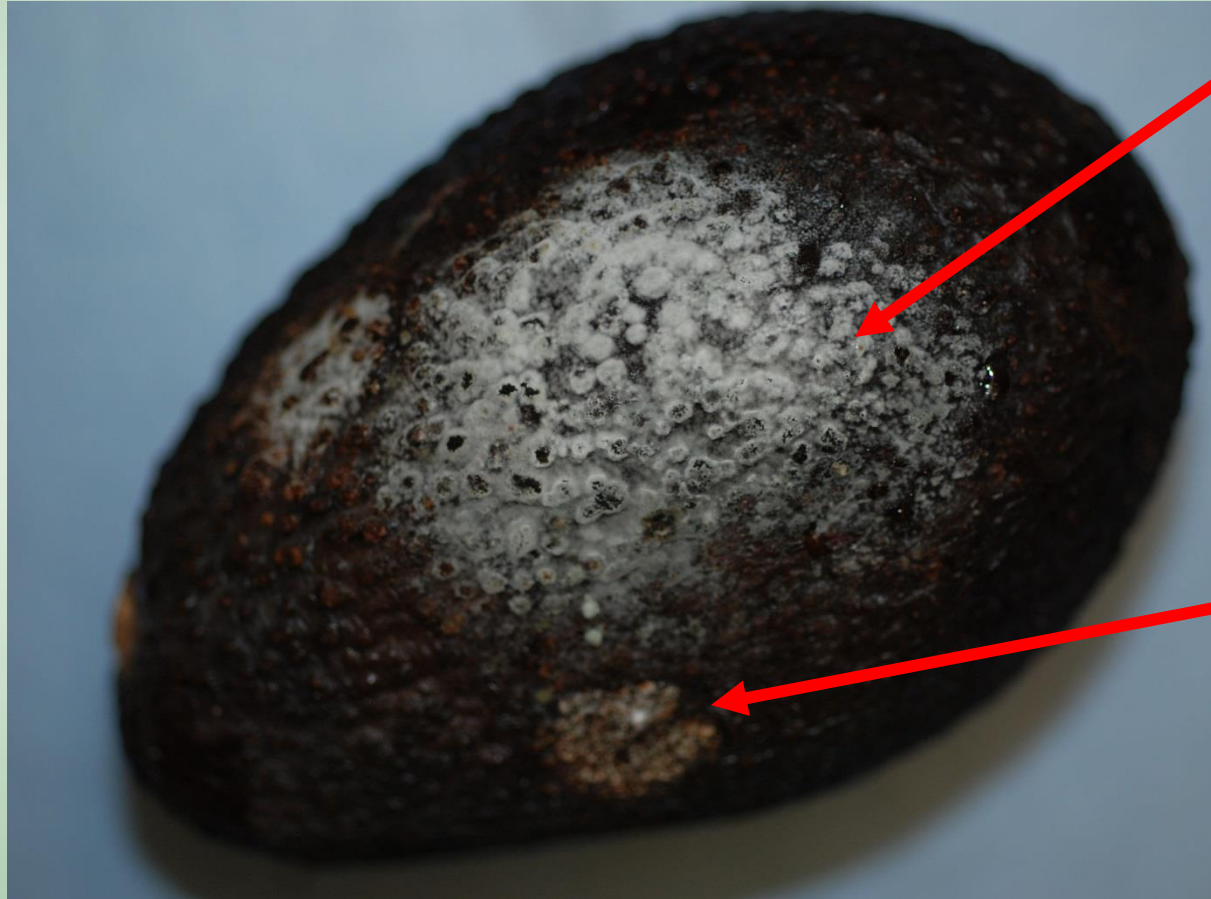
Mainly a postharvest issue; it is also found in cankers and leaf lesions and the litter

- Avocado
- Citrus
- Mango
- Papaya ...



Survey: 70% *Botryosphaeria* spp. and 30% *Colletotrichum* spp. (2017)

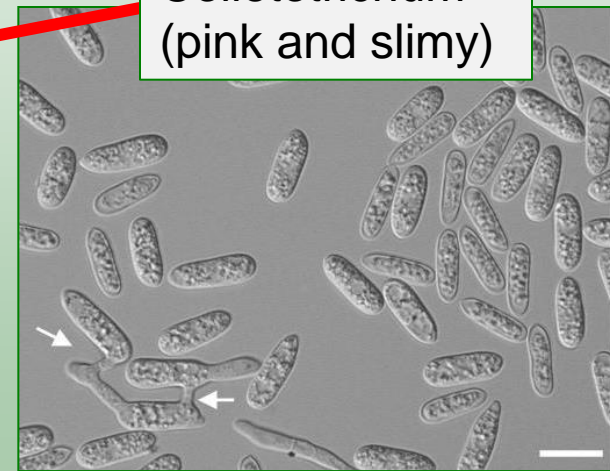
Botryosphaeria and *Colletotrichum* lesions on mature fruit



Botryosphaeria
(mouse gray)



Colletotrichum
(pink and slimy)



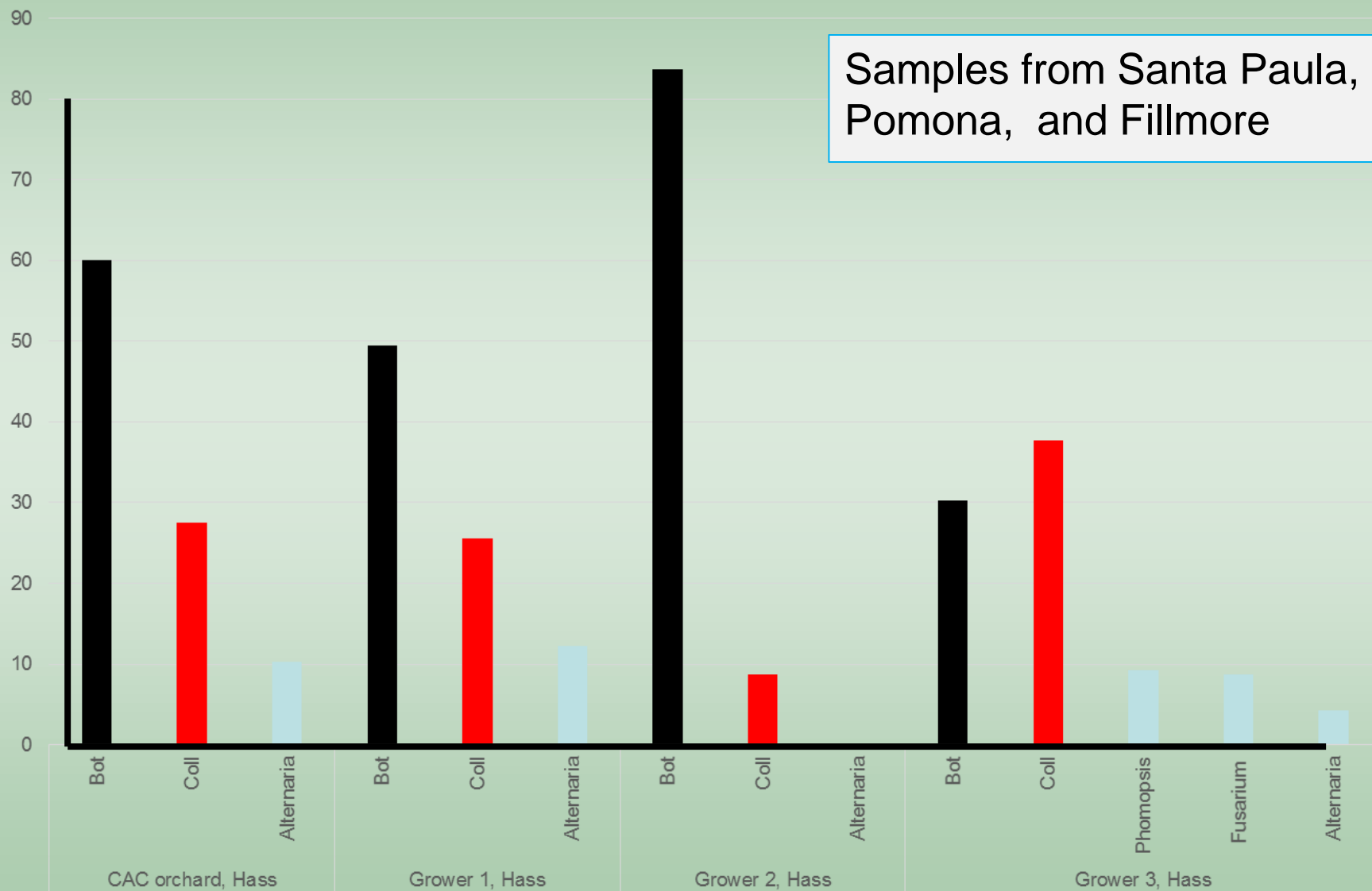
Tip burn due to salt damage:
Colonized by *Bot* and *Colletotrichum* pathogens



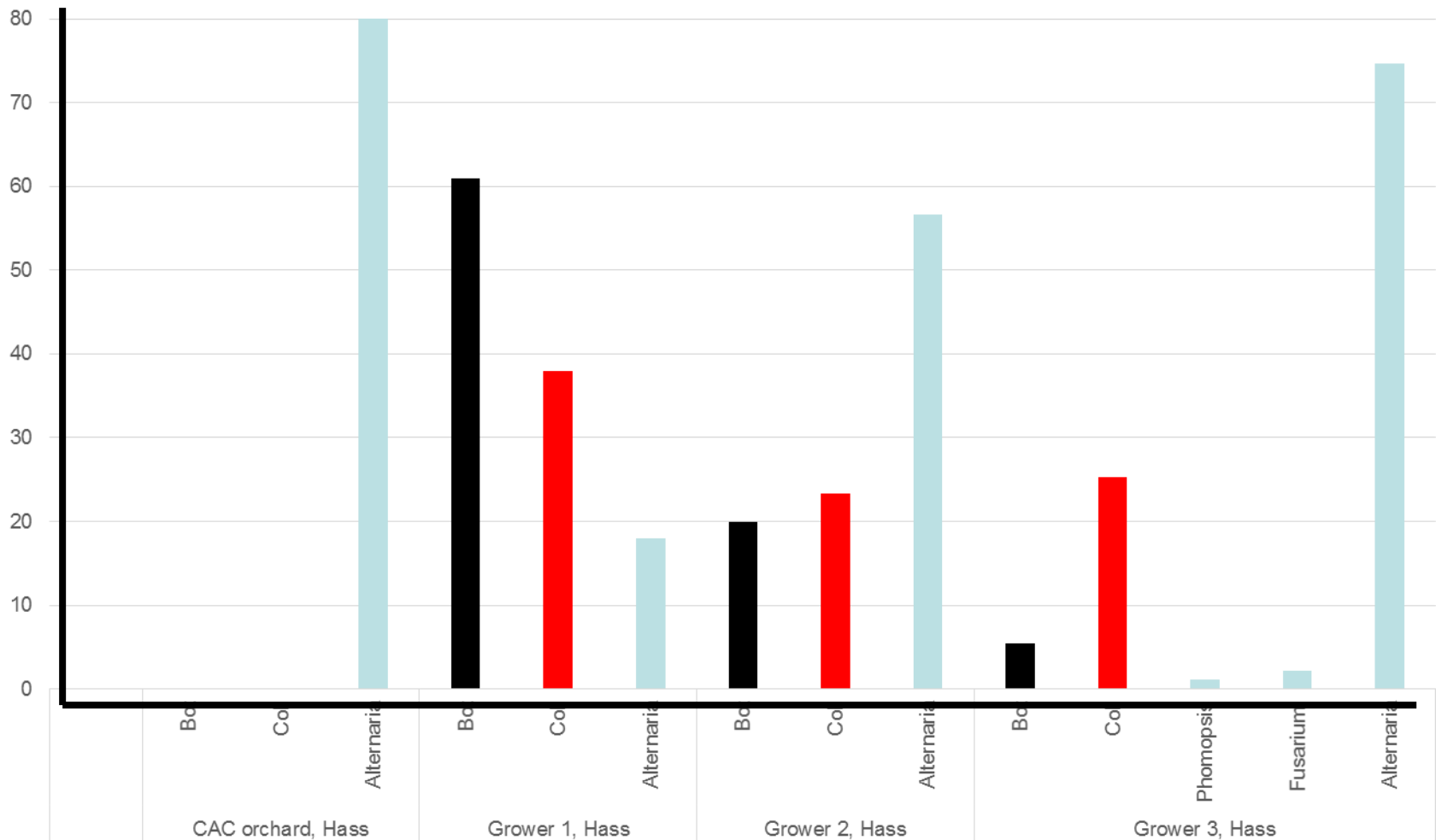
Causal agent of Anthracnose

- Historically known as *Colletotrichum gloeosporioides*
- Life cycle of *Colletotrichum* spp. comprises:
 - * conidia produced in open structures (acervulli) (spread by water)
 - * ascospores produced in closed structures (perithecial) (spread by air)

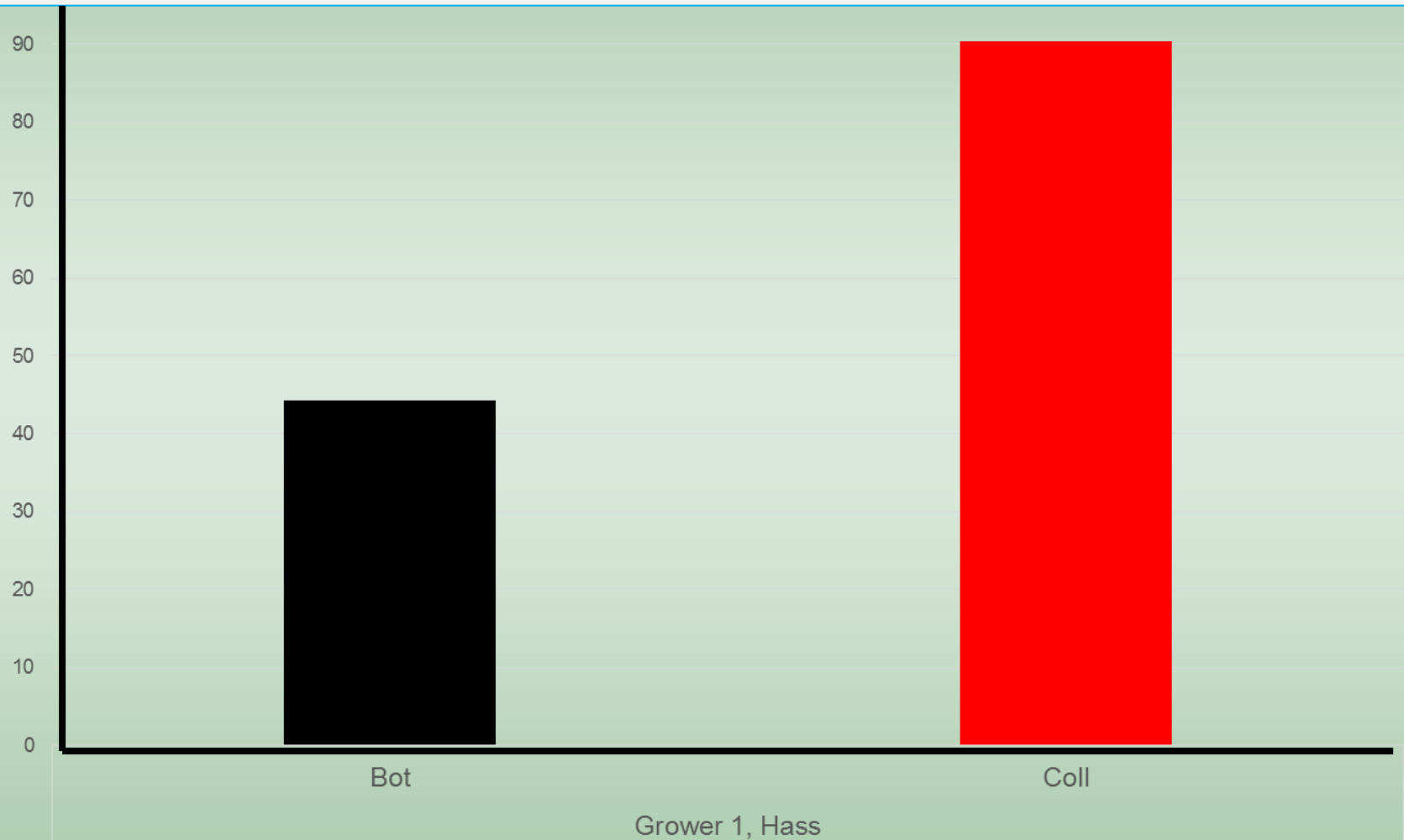
Incidence of *Botryosphaeria* spp. and *Colletotrichum* spp. isolated from avocado branches in **Ventura Co.**



Incidence of *Botryosphaeria* spp. and *Colletotrichum* spp. isolated from avocado leaf lesions in Ventura Co.



Incidence of Botryosphaeria and Colletotrichum spp. isolated from avocado leaf necrotic lesions in San Luis Obispo Co.



Identification of *Colletotrichum* species through molecular procedures

Several *Colletotrichum* species in the

C. gloeosporioides complex

- 1 *C. perseae*
- 2 *C. fructicola*
- 3 *C. siamense*
- 4 *C. gloeosporioides*
- 5 *C. aotearoa*

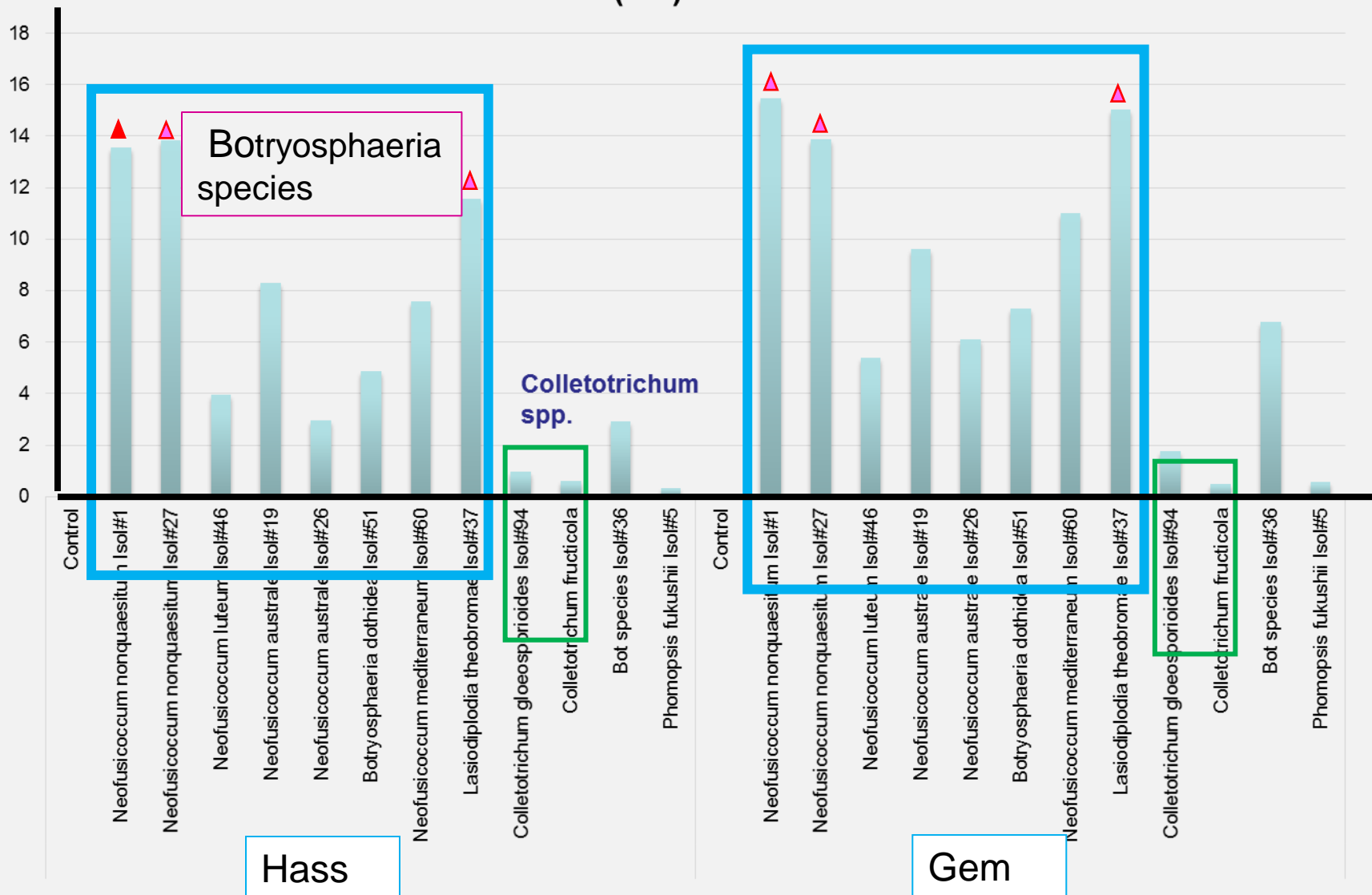
County	Species
A San Diego	
B Ventura	
C Riverside	
D San Luis Obispo	
E Santa Barbara	



Pathogenicity of *Botryosphaeria* spp. from avocado orchards in laboratory experiments



Lesion size of cankers (cm) on Hass and Gem avocado cultivars



▲ Significant differences in lesion sizes

Pathogenicity of *Botryosphaeria* spp. in potted trees



Accumulation of persitol

Periodic inoculations of avocado organs:

Lasio. theobromae;
Neof. nonquaesitum;
Coll. gloeosporioides

- Started on 1 May 2019
- Spore suspensions (no wounding)
- Mycelial plugs (+ wounding)



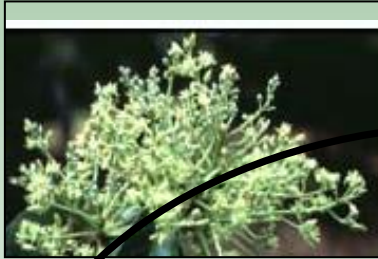
Monthly monitoring
/recording of disease

**Site: Pine Tree Ranch of Avocado
experimental orchard in Pomona, CA
(Ventura Co.)**



Proposed Botryosphaeria canker and dieback disease cycle

flowering



fruit set



pruning wounds



... fruit maturation



... shoot growth



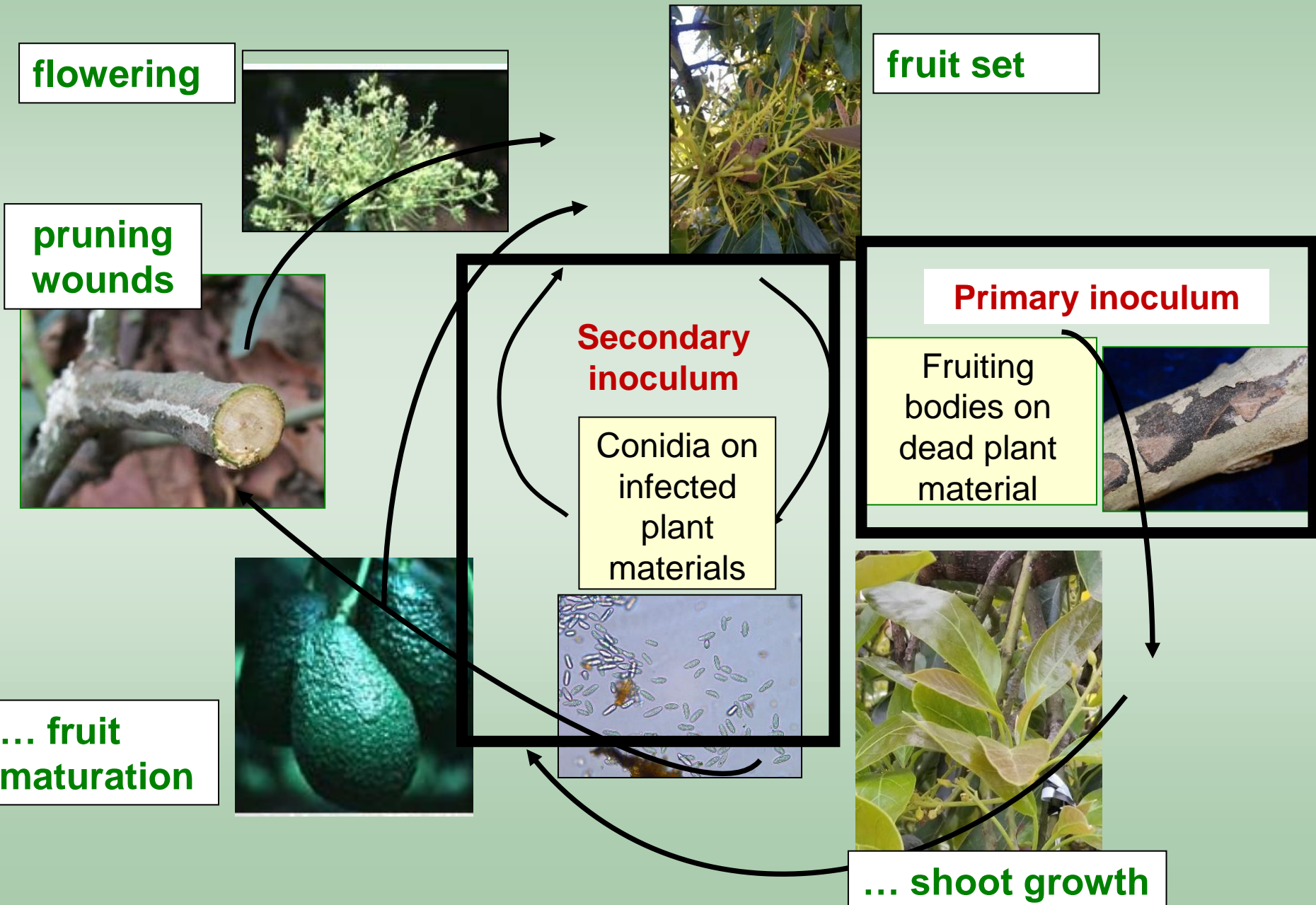
Secondary inoculum

Conidia on infected plant materials



Primary inoculum

Fruiting bodies on dead plant material



2nd-leaf almond orchard with gaps due to band (Botryosphaeria) canker

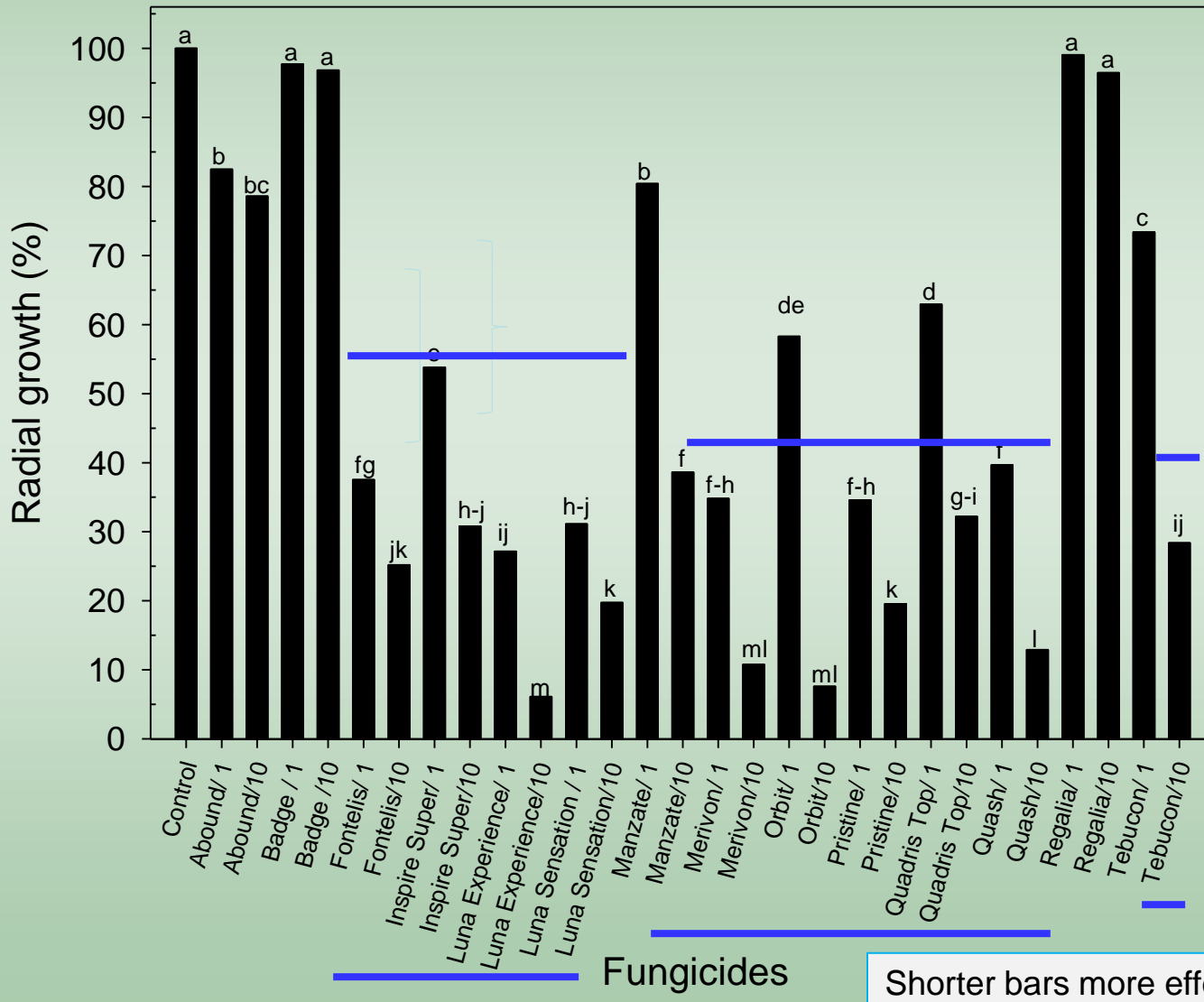


In progress

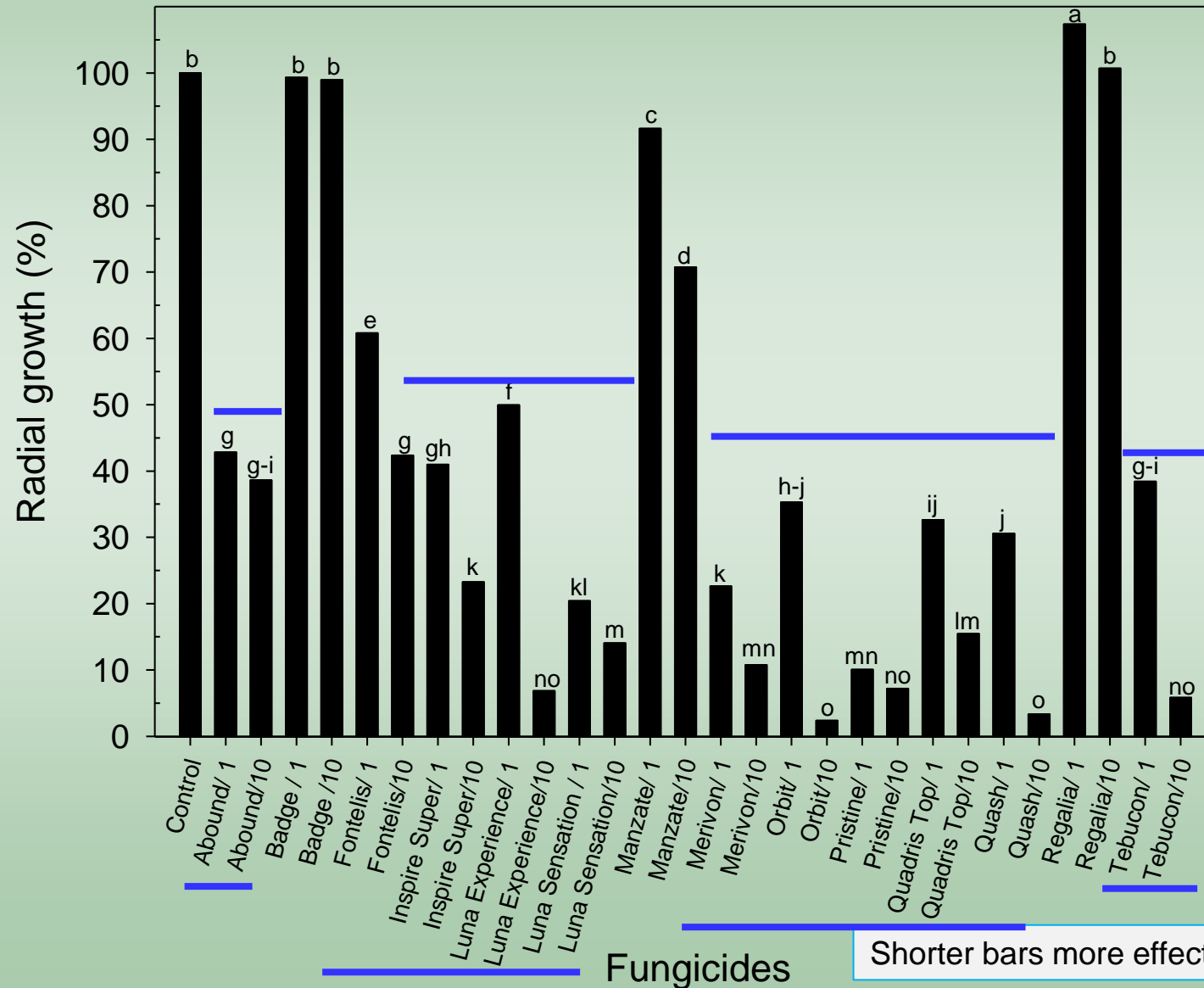
1. ID of Botryosphaeriaceae and *Colletotrichum* to species in nurseries and young orchards using DNA sequencing and phylogenetic analyses.
2. Determine the genetic relatedness between isolates and sources of inoculum.
3. Develop PCR diagnostic tools for fast and reliable identification and monitoring of the causal agents (*Botryosphaeria*, *Phomopsis*, *Fusarium* and *Colletotrichum* spp. in asymptomatic tissues.
4. Develop IPM strategies for the two diseases and outreach activities.

Methods: a) In vitro-chemical efficacy; b) Chemical assay with detached shoots; c) Field trials with fungicides; d) Kaolin field trial(s).

Effect of various fungicides on growth of *Lasiodiplodia theobromae*



Effect of various fungicides on growth of *Botryosphaeria dothidea*



Possible reasons for increased incidence of **Bot canker and dieback** in the last decade

- Very wide host range!
- Increase of inoculum in riparian habitats.
- Increased of inoculum in non-cleared forests (fires ... !)
- Many avocado groves next to riparian areas.
- Disease may start at early age and inoculum built up unnoticed.
- Pushing the trees for more production may stress the trees.
- Drought stressed trees become more susceptible (exp. Is in progress).

Conclusions

Source of disease	Causal agent on Hass, Lamb Hass, Gem (Botryosphaeria & Colletotrichum spp.)	Other factors
Dead twigs	Primary	---
Infected branch	Primary	---
Dead leaves	Primary	---
Green leaves (latent infections)	Primary	Secondary
Infected fruit (on the tree)	Primary	Secondary
Fruit mummies	Primary	---

Thank
you

tjmichailides@ucanr.edu

- **California Avocado Commission**
- **Avocado growers** for allowing sampling.
- **Mary Lu Arpaia** (UC KARE),
- **Ben Faber** (UCCE, Ventura Co.),
- **James Davis** (PCA, San Diego Co.),
Christopher Greer, UCCE, SLO Co.)
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- The cooperation and help by: **Marcelo Bustamante** (PhD student, UC Davis,
John Lake (Master student candidate,
CSU, Fresno), and **Mirella Zaccheo**
(Visiting student, University of Bari, Italy),
- Special thanks to **Dee Vega** (graduate
student at CA State University Pomona)
is truly appreciated.
- **Brokaw nursery** for donating trees to
this research.