Early Detection and Management of Band Canker

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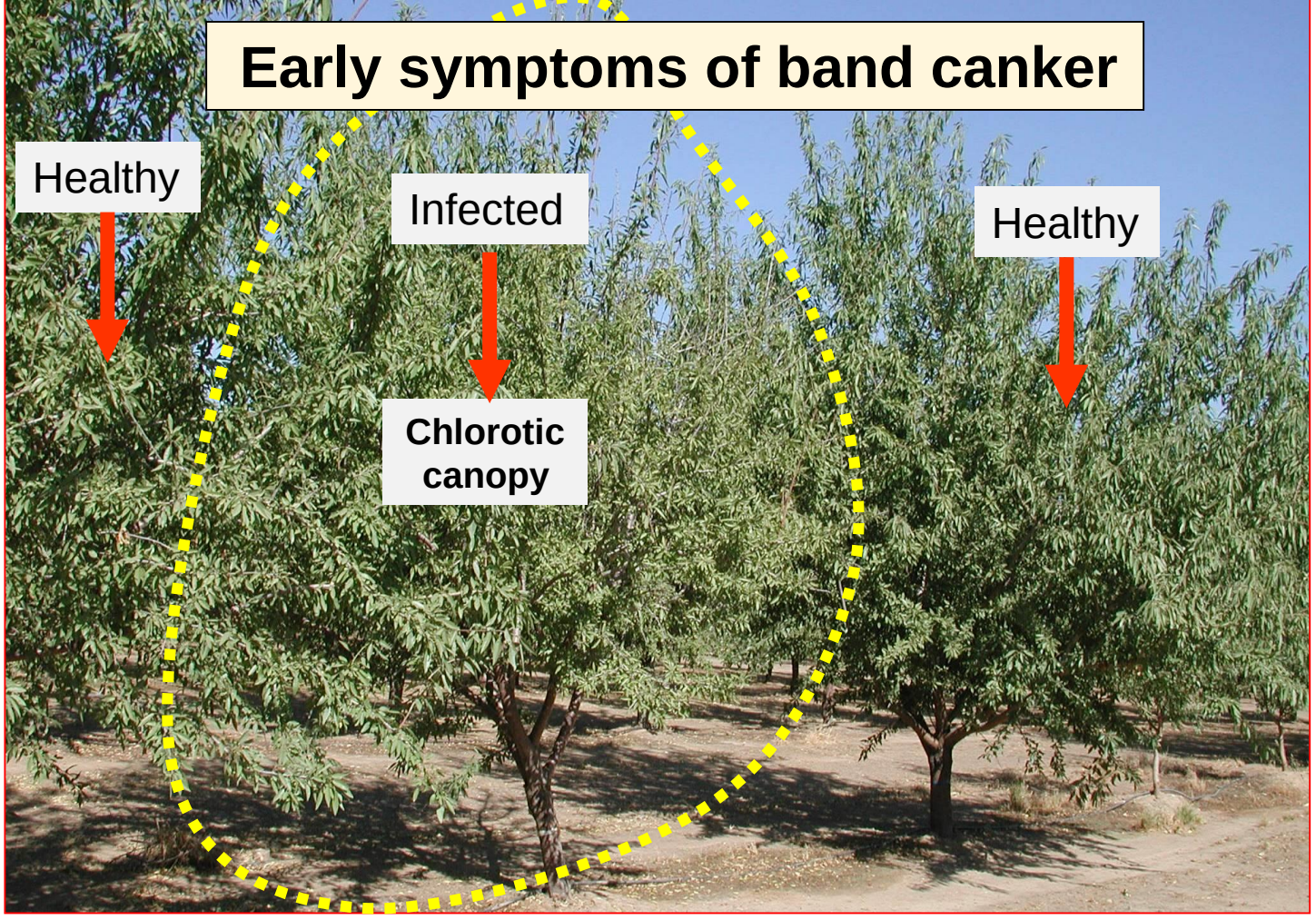
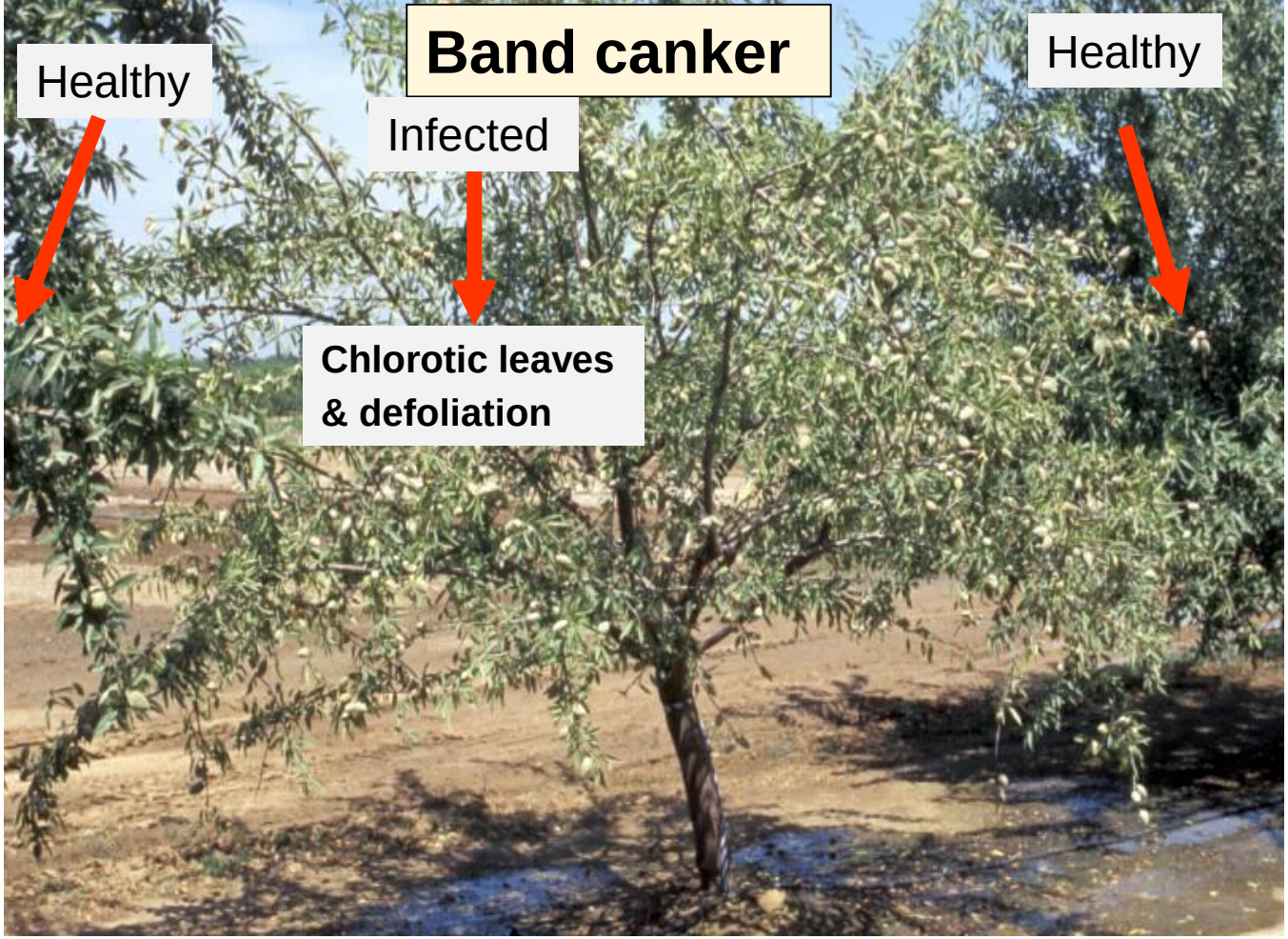
OUTLINE:

- Symptoms
- Causes
- Factors affecting disease
- Disease management
- Early detection and implication in management

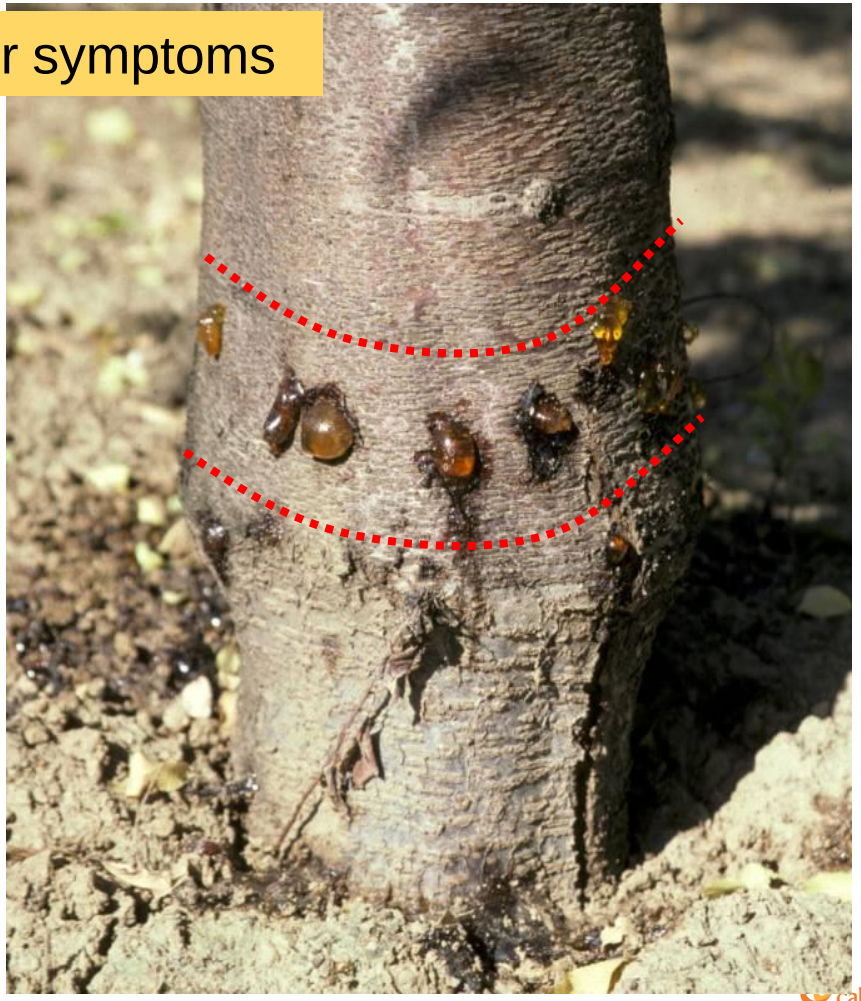
Early symptoms of band canker



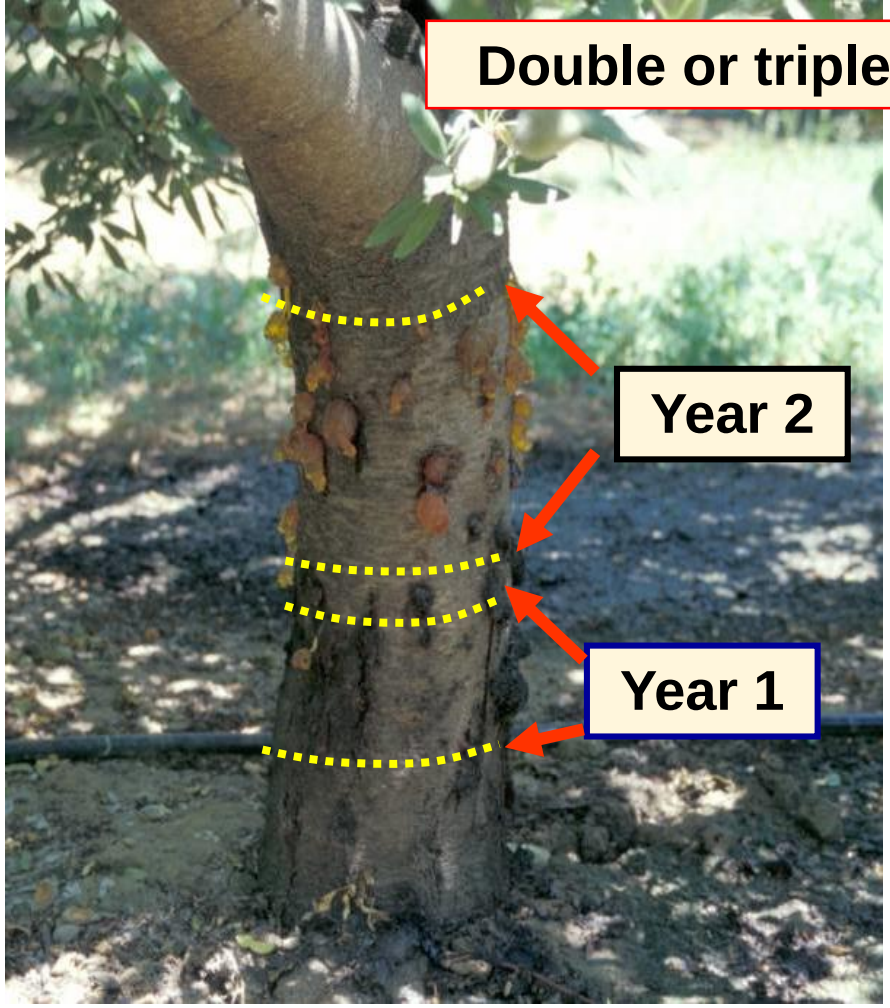

Band canker
Healthy
Infected
Healthy
Chlorotic leaves
& defoliation

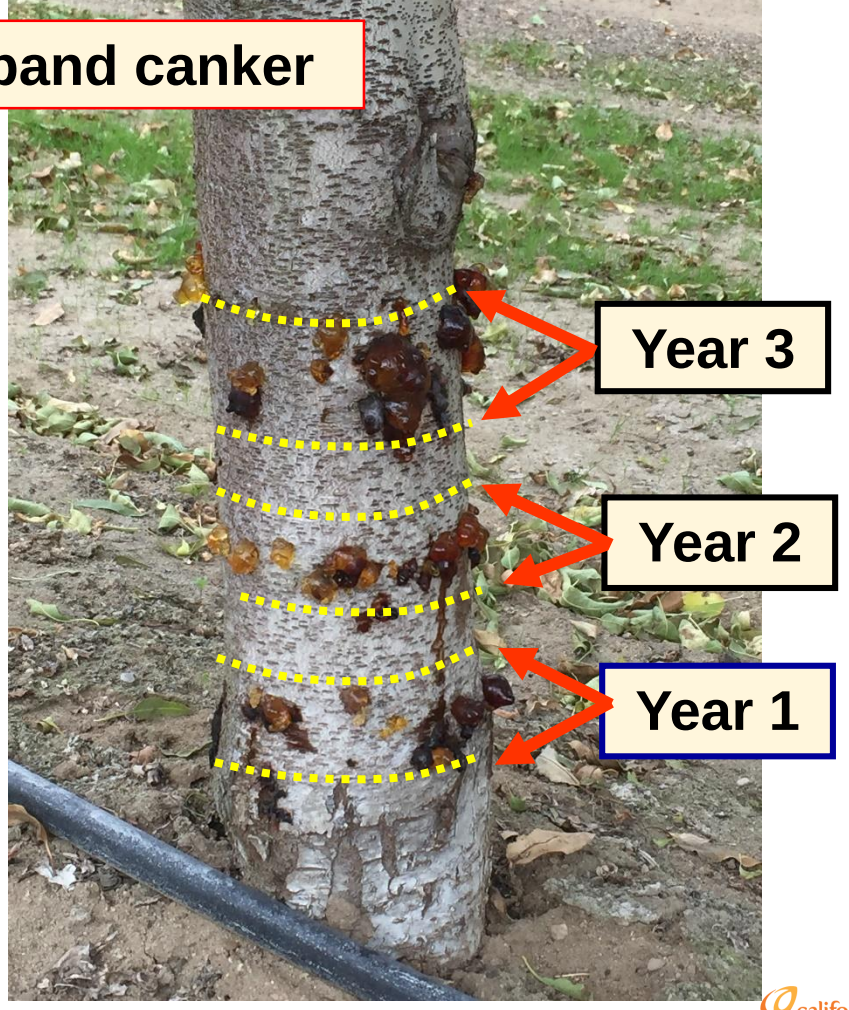
Tree death (note excess suckering)

Band canker symptoms

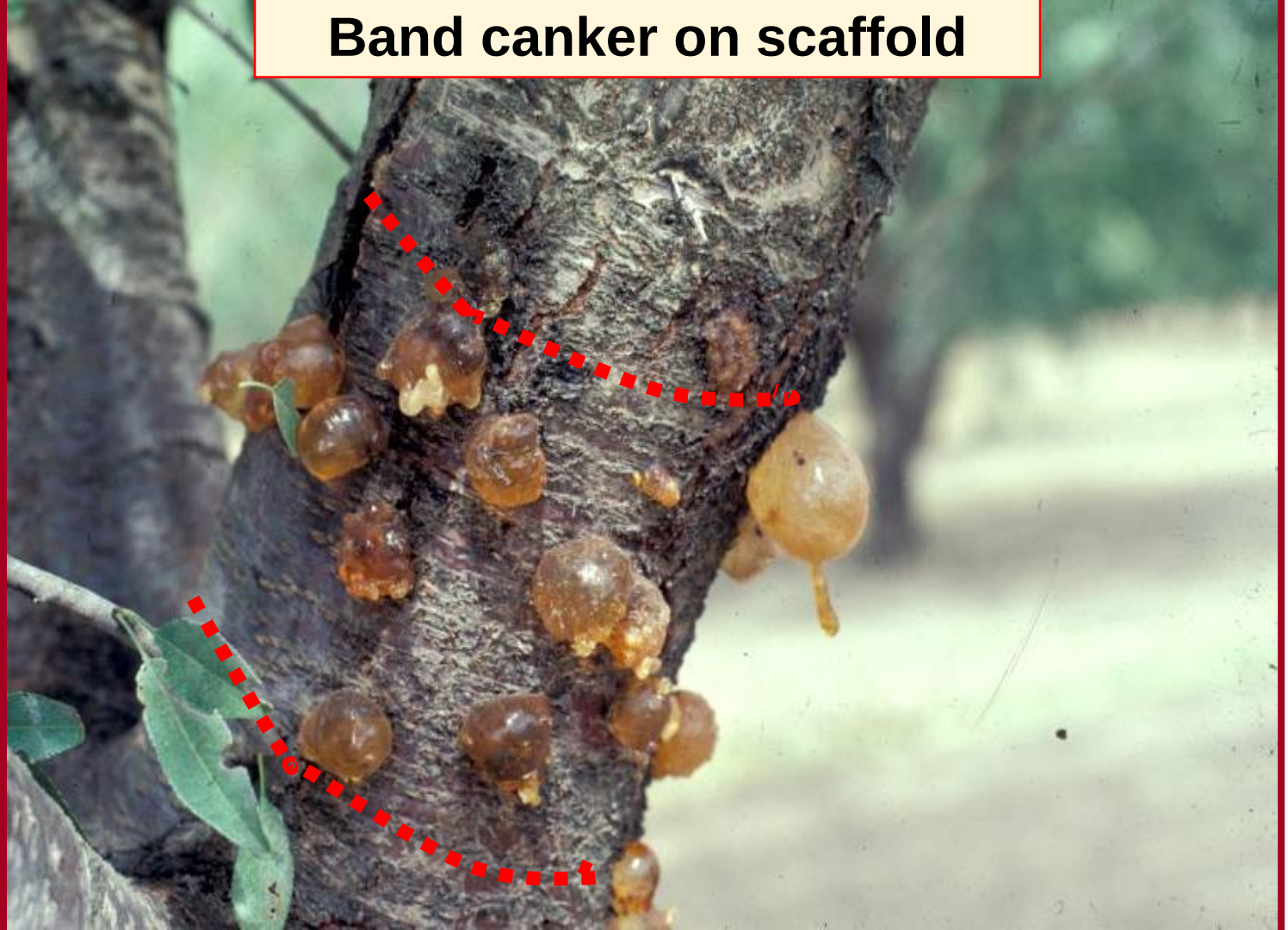


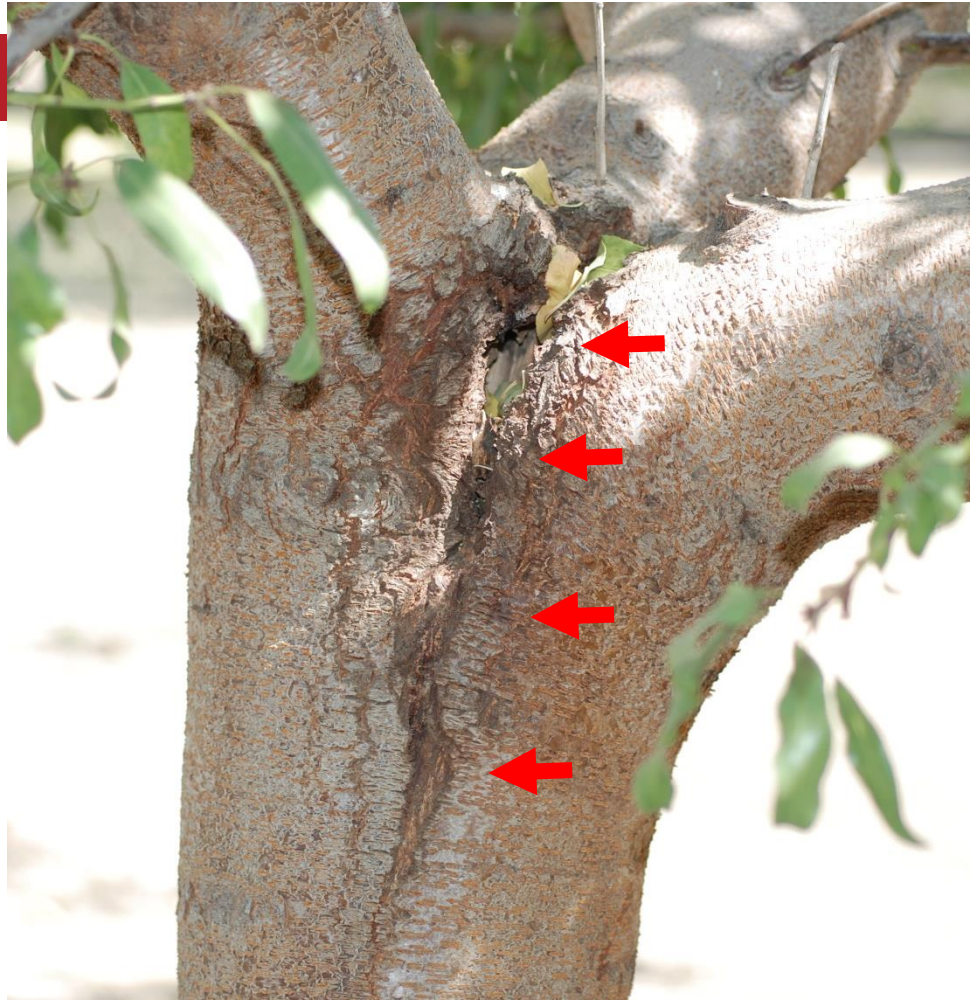
Double or triple band canker





Band canker on scaffold

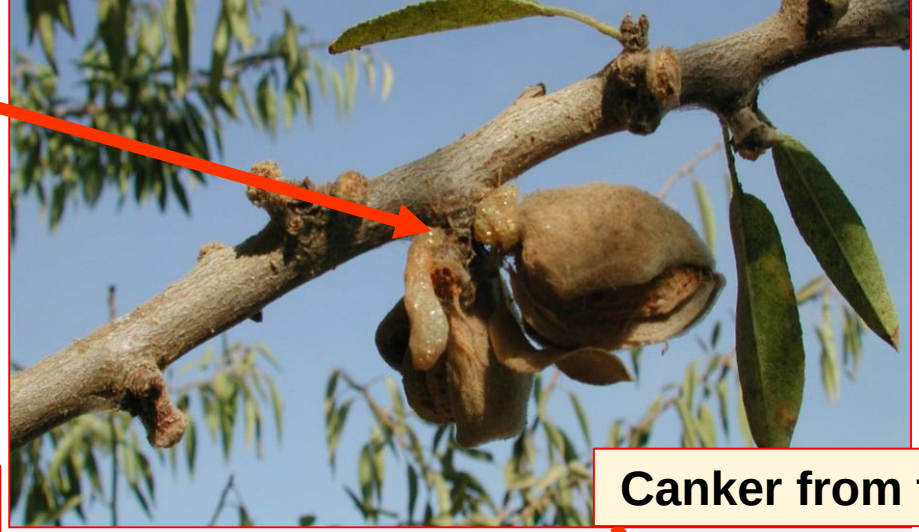




Infection via growth cracks

Infection via a pruning wound

Fruit blight

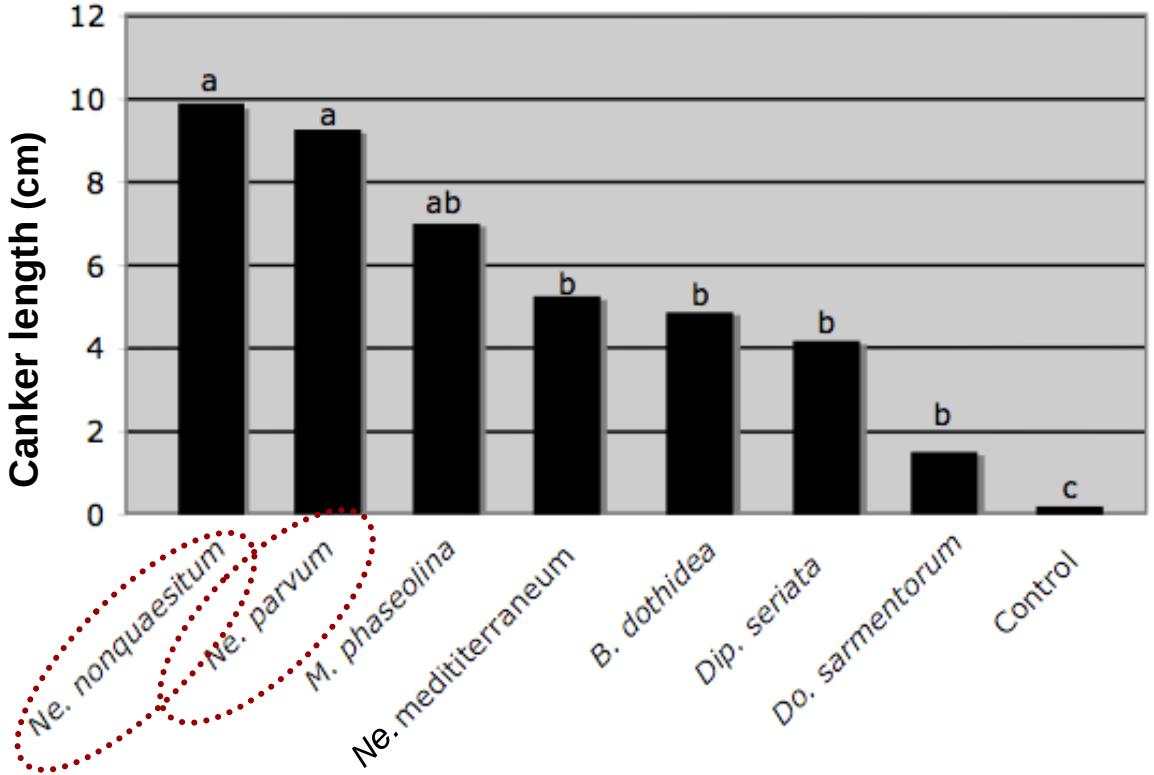


Canker from fruit infection

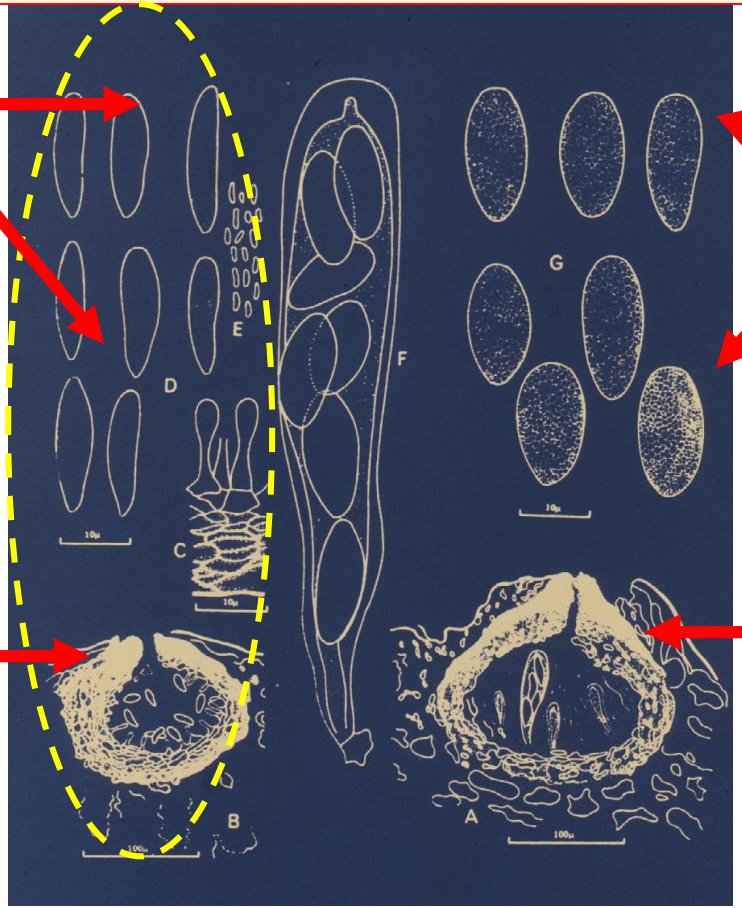
Summary of Botryosphaeriaceae in nut crops – California

Fungal species	**Almond**	Pistachio	Walnut
Botryosphaeria dothidea	+	+	+
Neofusicoccum parvum	+	+	+
Neofusicoccum mediterraneum	+	+	+
Diplodia mutila	---	---	+
Neofusicoccum nonquaesitum	+	---	+
Neofusicoccum vitifusiforme	---	+	+
Diplodia seriata	+	+	+
Dothiorella iberica	+	+	+
Lasiodiplodia citricola	+	+	+
Neoscytalidium dimitiatum (=*Hendersonula toruloidea*)	+	+	+

Pathogenicity of various *Botryosphaeriaceae* on almond cv. Padre



Botryosphaeria dothidea reproductive structures in almond



conidia

water splashed

or insect spread

pycnidia

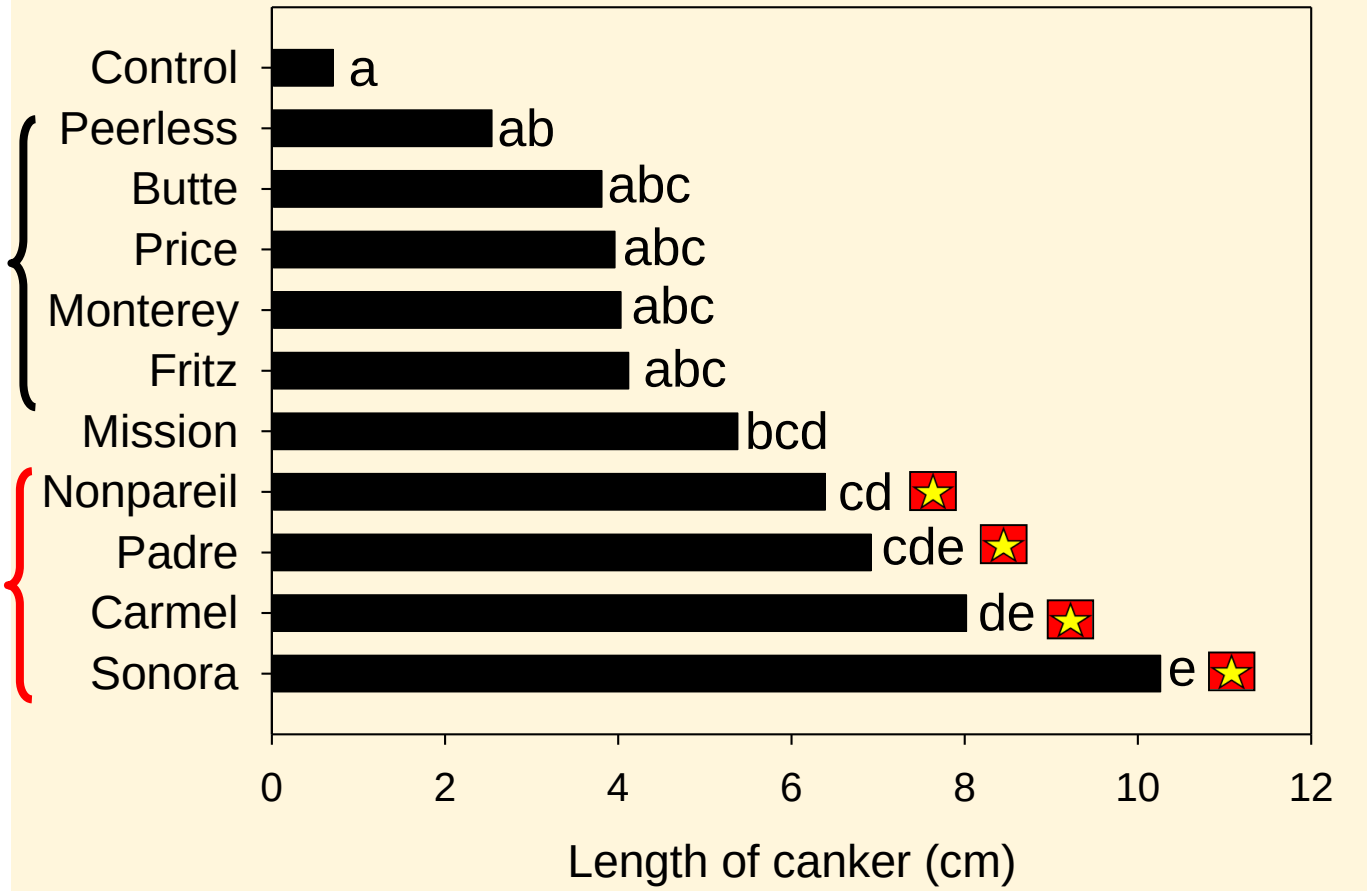
ascospores

airborne

Ascocarps

pseudothecia

Almond cultivars inoculated with *Neofusicoccum* (results of 2 years)

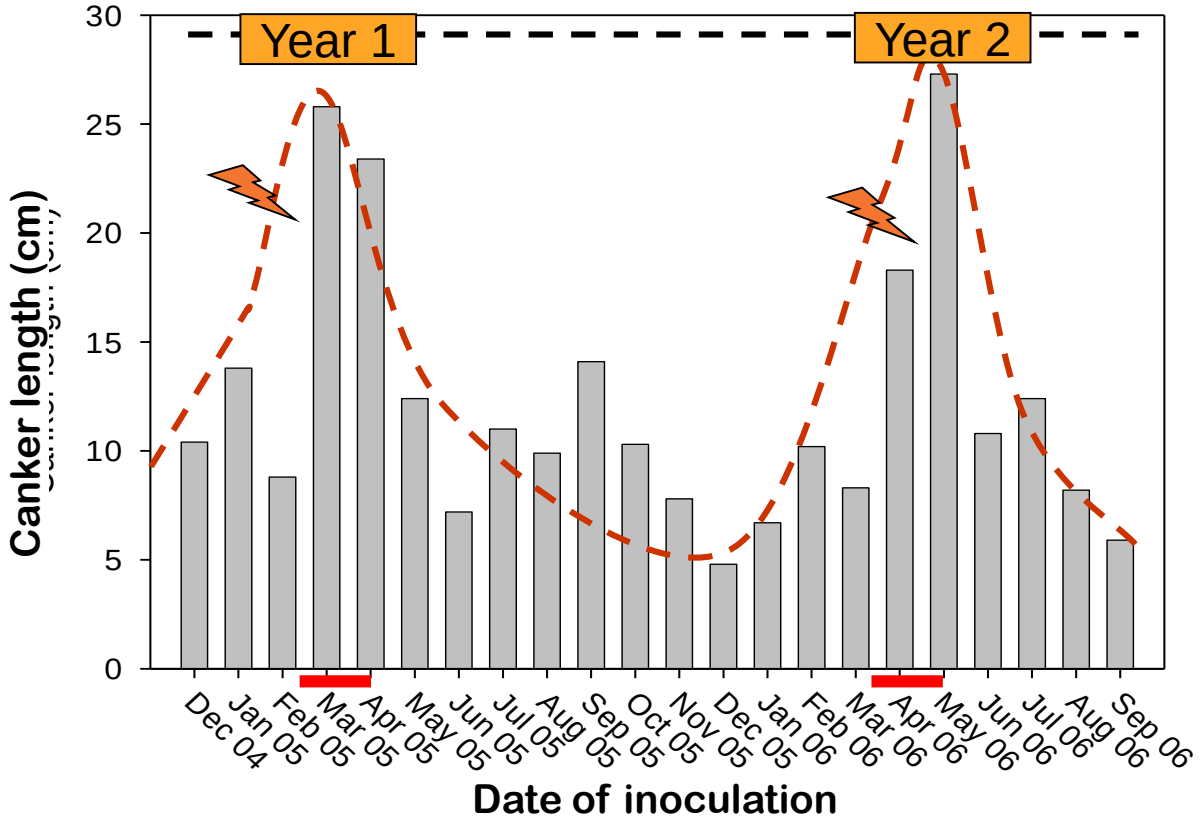


- ✓ Vigorously growing varieties
- ✓ 2 to 6-years-old trees

- ✓ Nonpareil
- ✓ Carmel
- ✓ Padre
- ✓ Aldrich
- ✓ Wood Colony
- ✓ & others

When do infections occur?

Potted trees inoculated with *Neofusicoccum*

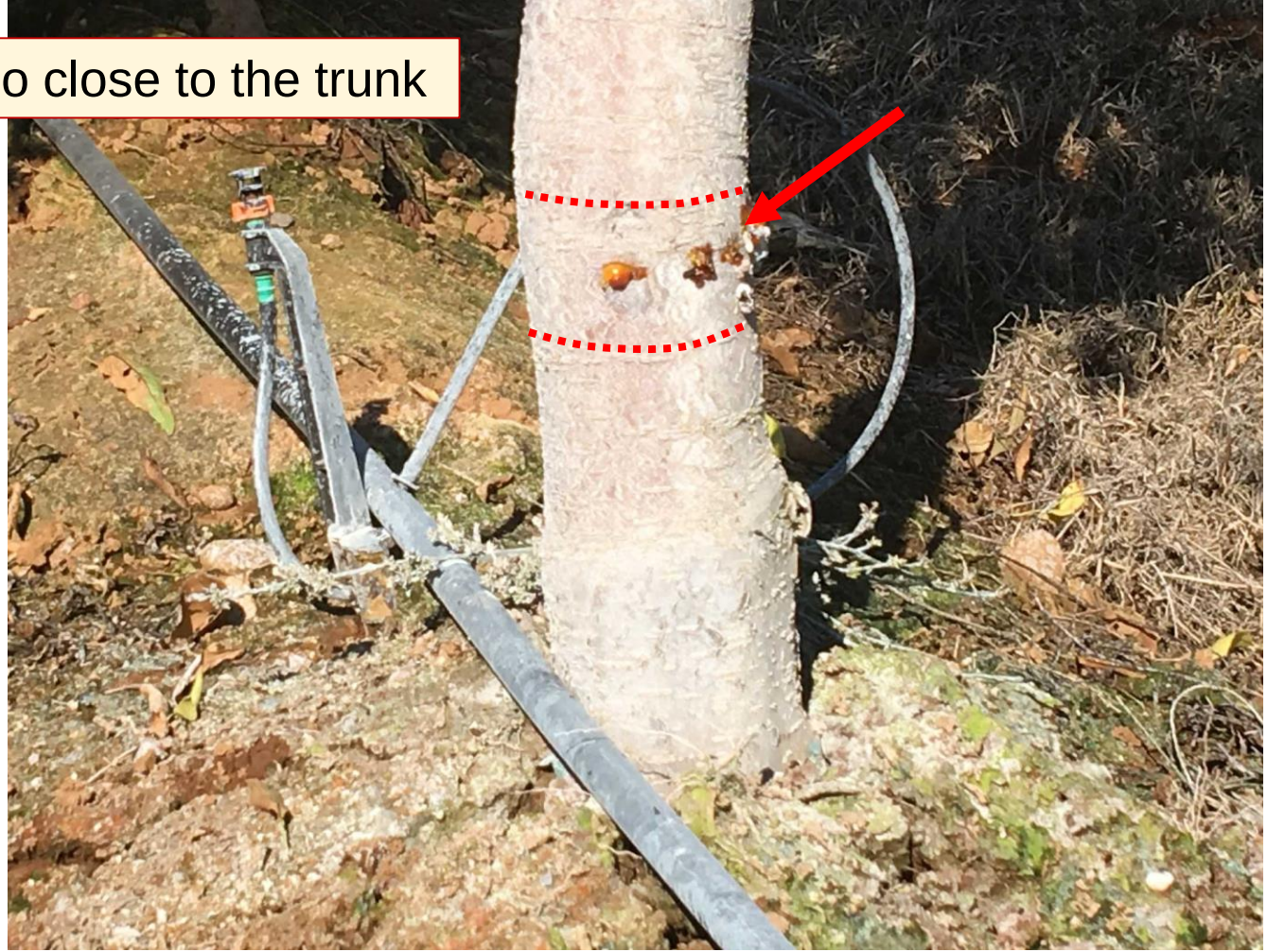


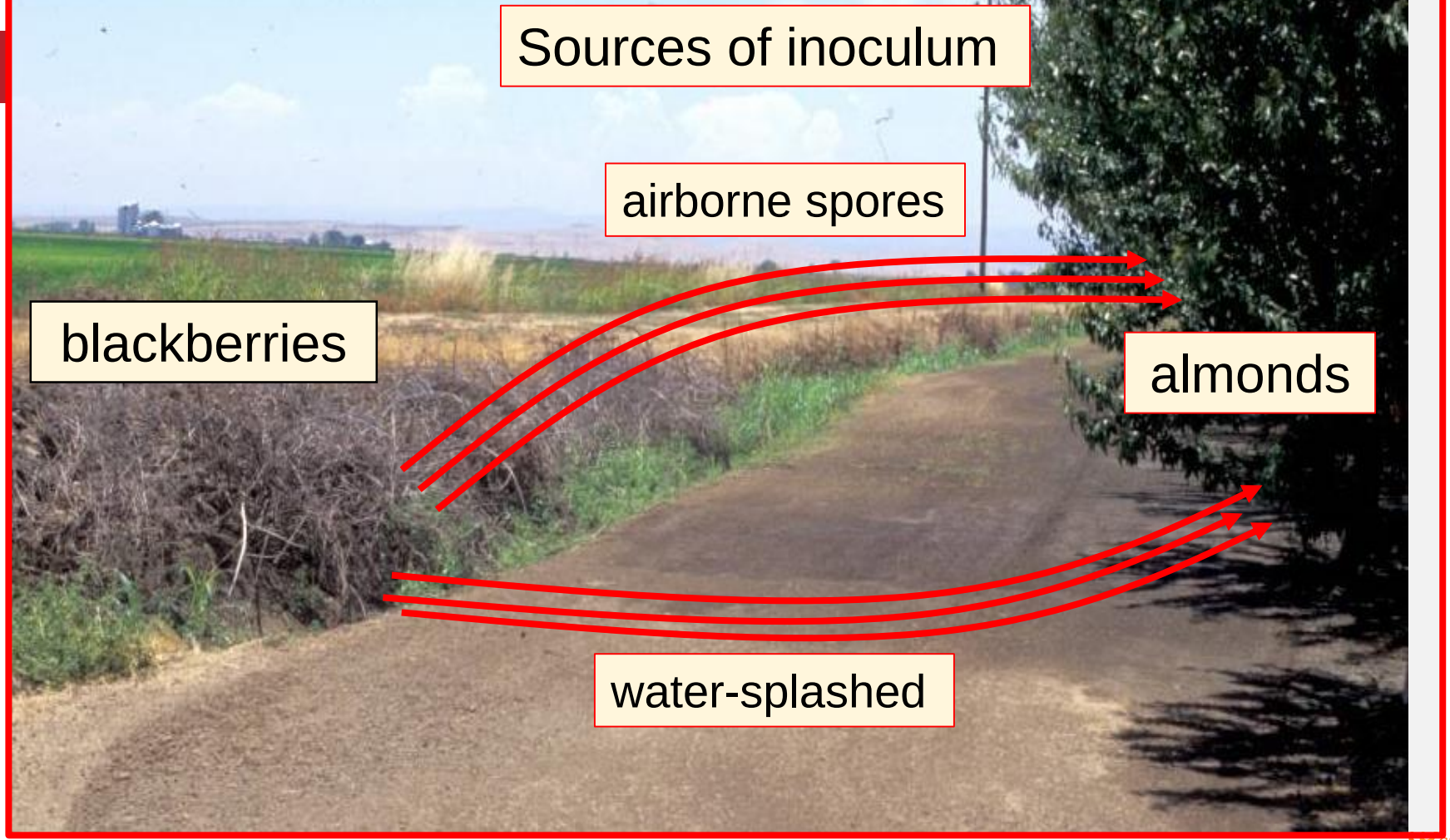
Management during March to May…

Disease Management: Fungicide injection experiments

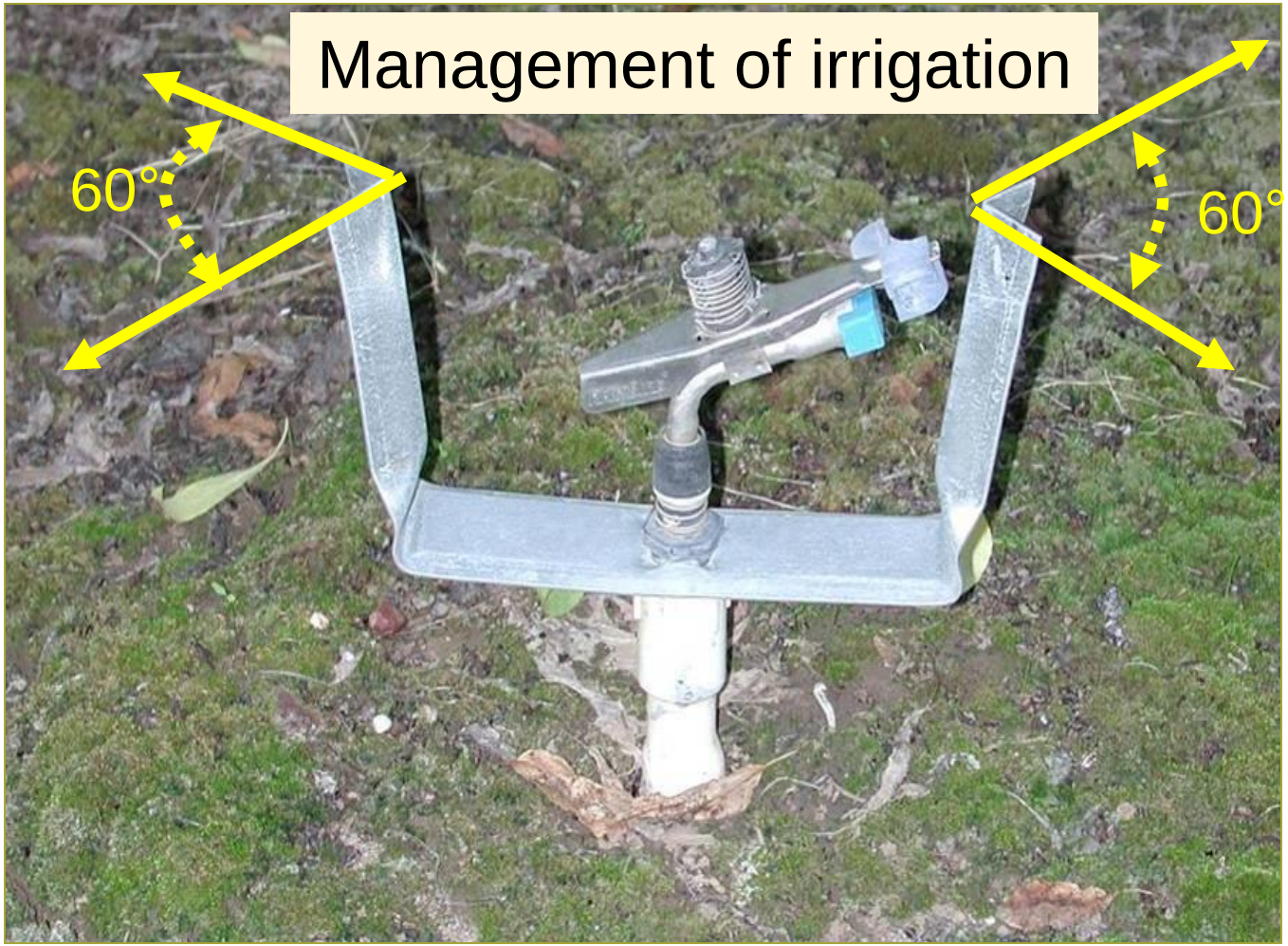
Treatment of trunks with fungicide sprays

Fanjet too close to the trunk

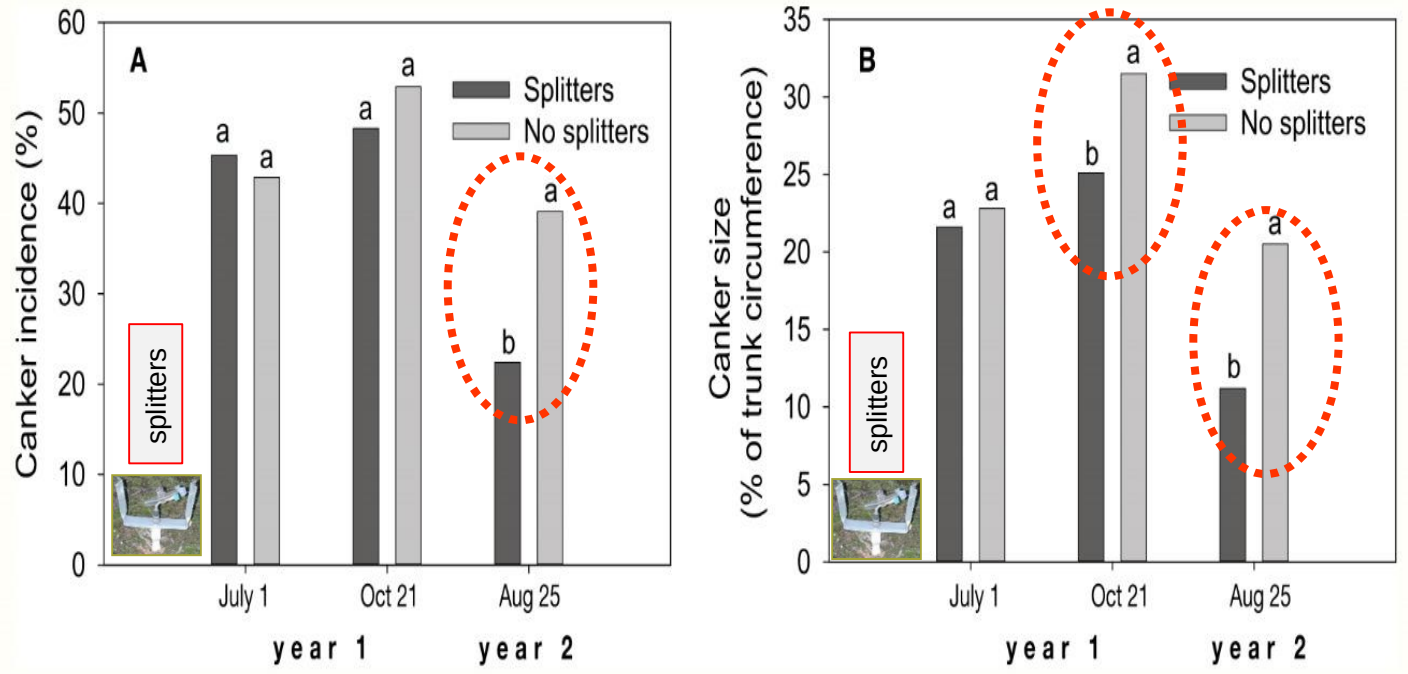




Management of irrigation



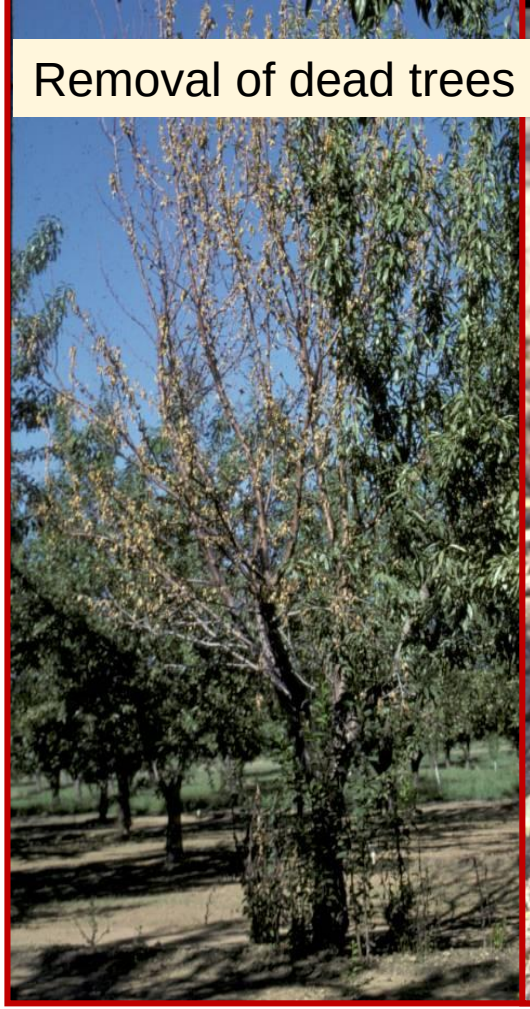
Effect of water splitters installed in sprinklers on band canker of almond



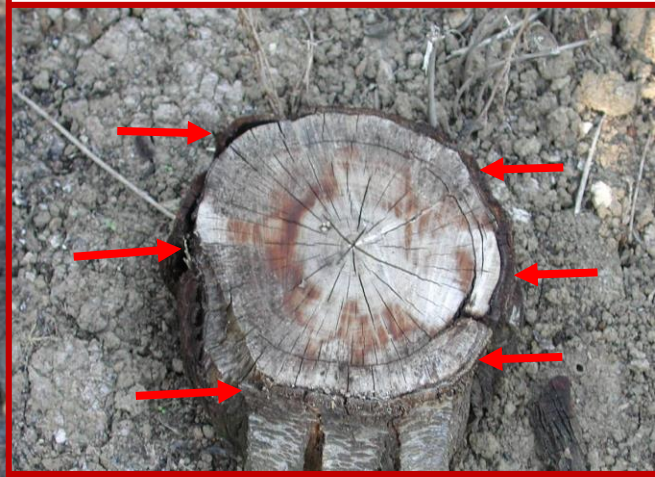
Keeping the trunk dry reduced infection and slowed down the growth of existing cankers

Commercial water splitters to keep tree trunk dry

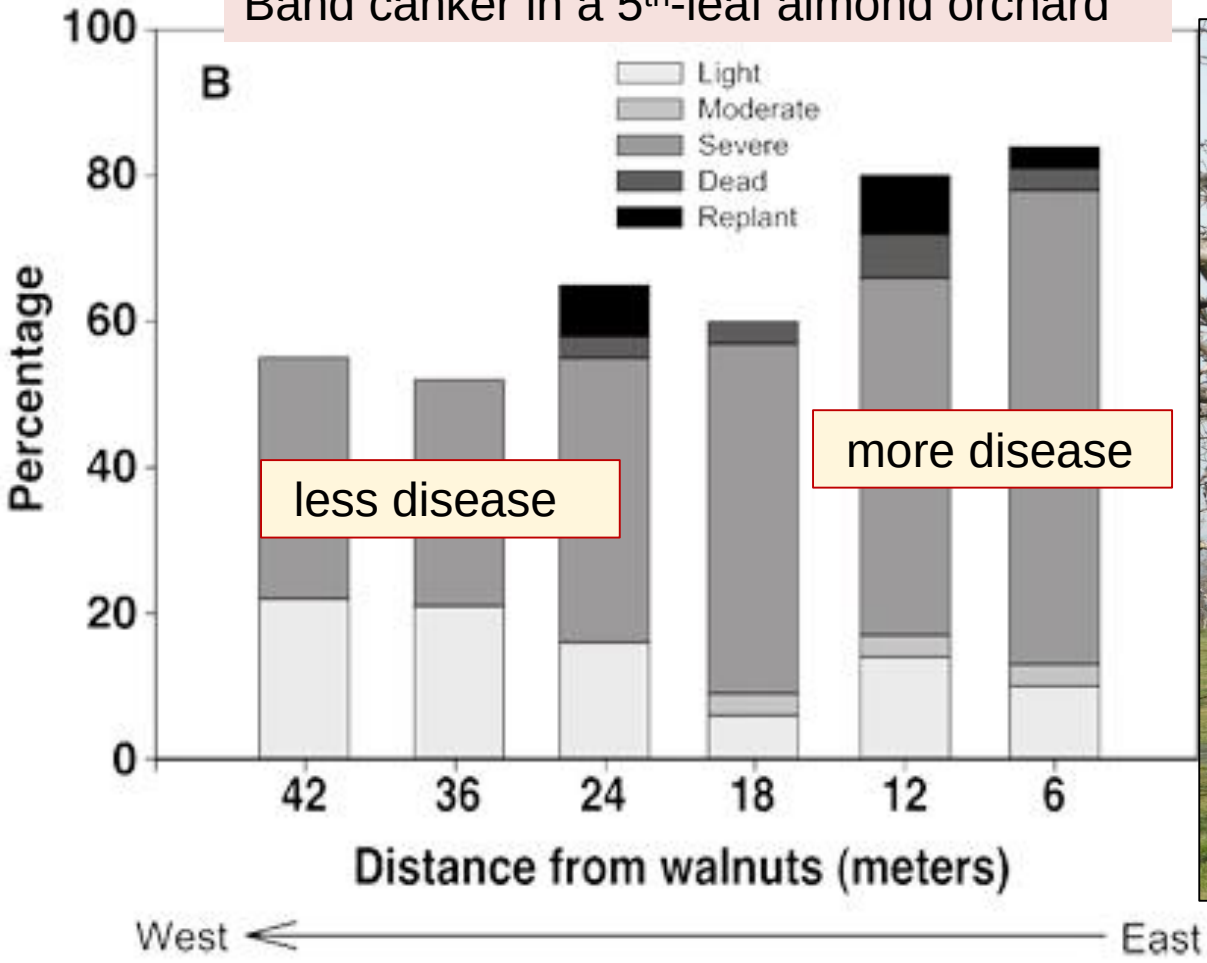
Removal of dead trees and their stumps

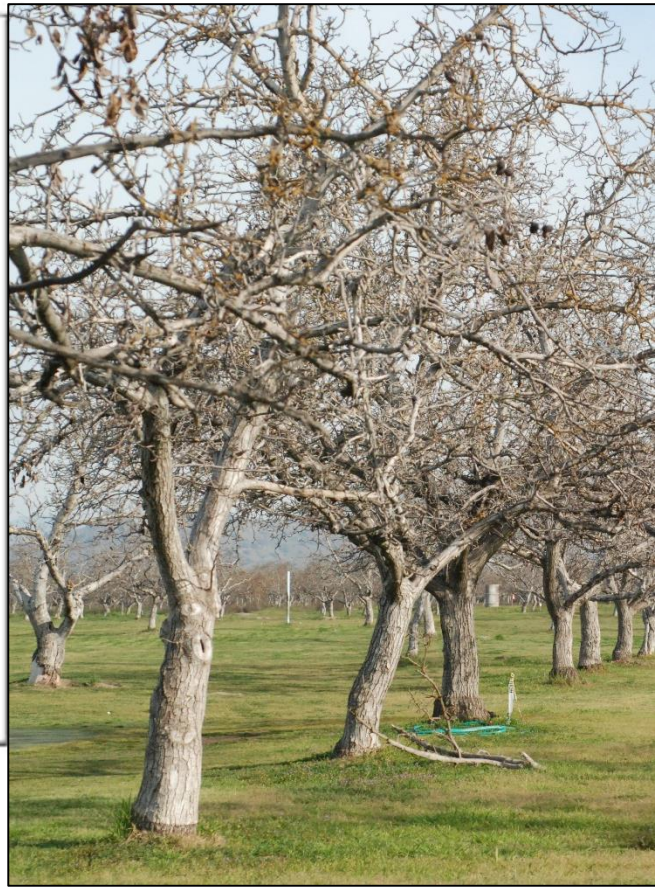


Pycnidia and pseudothecia



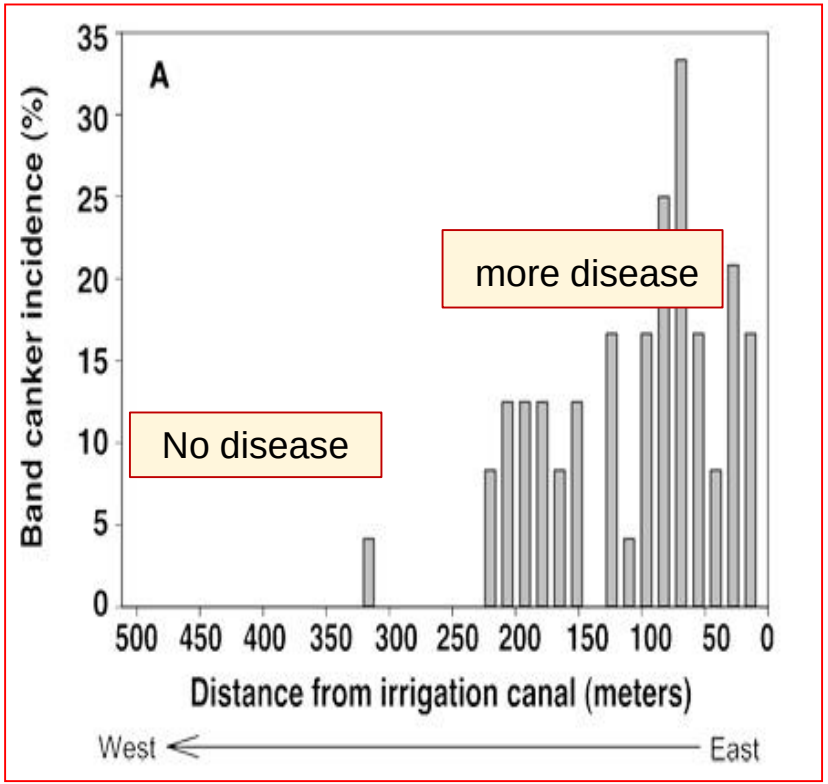
Band canker in a 5th-leaf almond orchard





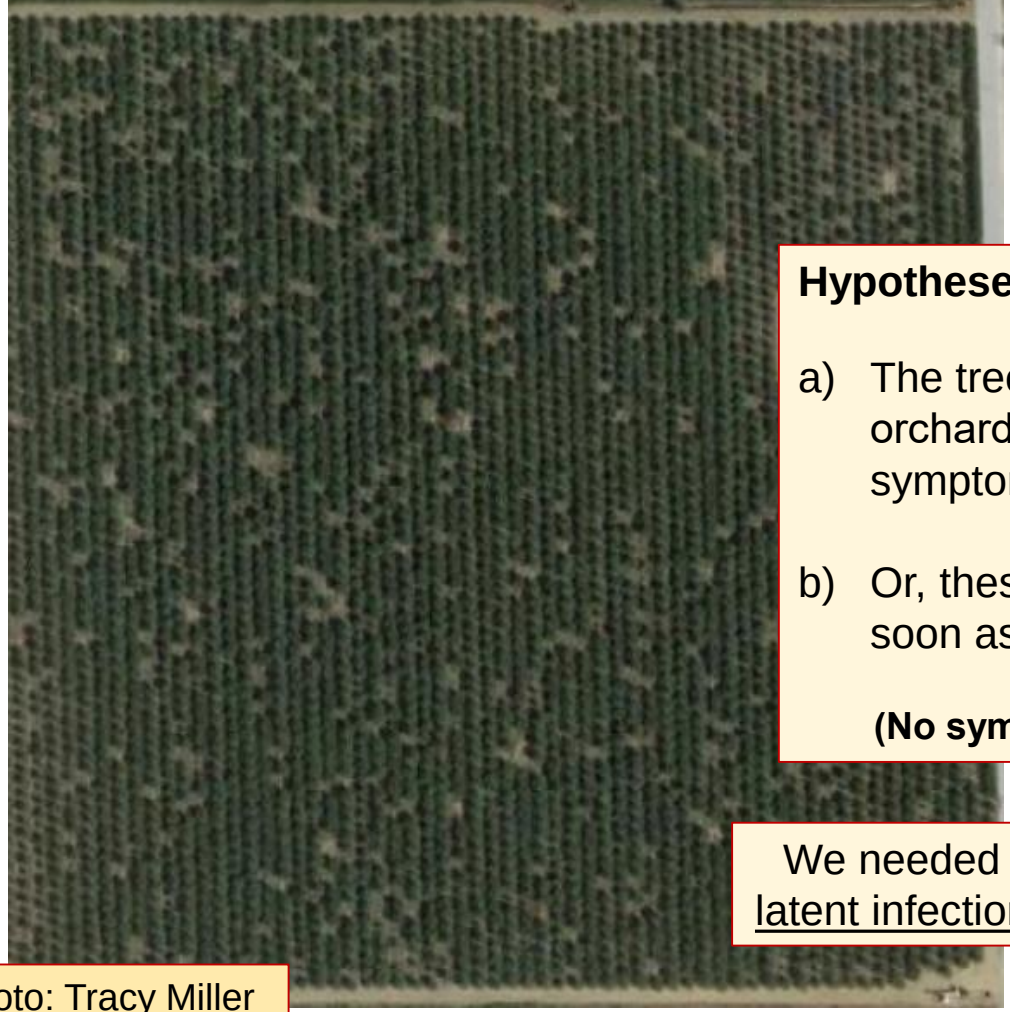
Walnut

Spread of band canker from the inoculum source



3rd leaf Nonpareil/Padre; inoculum source: riparian trees and water canal

2nd-leaf almond orchard with gaps due to Band Canker



3rd-leaf almond orchard with gaps due to Band Canker (Stanislaus Co.)

Hypotheses:

a) The trees were either delivered to the orchard “infected” (but with no disease symptoms).

b) Or, these trees were infected uniformly as soon as they were planted.

(No symptoms at planting… latent infections?)

We needed then to develop a method to <u>detect latent infections early</u> in tissues with no symptoms

Photo: Tracy Miller

For early detection:

1. We wanted to find out if latent infections start in trees at the nursery, or as soon as trees are planted.

and,

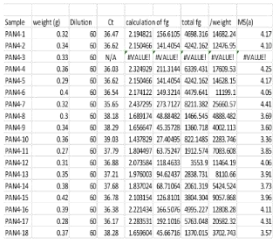
2. If so, to begin protective sprays in very young orchards to prevent infection.

Methodology to quantify latent infections of canker pathogens (qPCR method)

1. Sample collection and processing

2. Grinding and DNA extraction of samples

3. Real-time PCR assay



Sample	weight (g)	Dilution	Ct	calculation of fg	total fg	/weight	MS(a)
PANA-1	0.32	60	36.47	2.194821	1364.035	4098.316	14682.24	4.17
PANA-2	0.34	60	36.62	2.150466	141.4054	4242.162	12476.95	4.10
PANA-3	0.33	60	N/A	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
PANA-4	0.36	60	36.03	2.324029	211.3144	6339.431	17609.53	4.25
PANA-5	0.29	60	36.62	2.150466	141.4054	4242.162	14628.15	4.17
PANA-6	0.4	60	36.54	2.174122	149.3214	4479.641	11199.1	4.05
PANA-7	0.32	60	35.65	2.437295	273.7127	8211.382	25660.57	4.41
PANA-8	0.3	60	38.18	1.689174	48.88482	1466.545	4888.482	3.69
PANA-9	0.34	60	38.29	1.656647	45.35726	1360.718	4002.113	3.60
PANA-10	0.36	60	39.03	1.437829	27.40495	822.1485	2283.746	3.36
PANA-11	0.27	60	37.79	1.804497	63.75247	1912.574	7083.608	3.85
PANA-12	0.31	60	36.88	2.073584	118.4633	3553.9	11464.19	4.06
PANA-13	0.35	60	37.21	1.978003	94.42457	2828.731	8110.66	3.91
PANA-14	0.38	60	37.68	1.837024	68.71054	2061.159	5424.524	3.73
PANA-15	0.42	60	36.78	2.103154	126.8103	3804.304	9057.868	3.96
PANA-16	0.39	60	36.38	2.221434	166.5074	4995.227	12808.28	4.11
PANA-17	0.28	60	36.17	2.283531	192.1016	5763.048	20582.32	4.31
PANA-18	0.37	60	38.28	1.659604	45.66716	1370.015	3702.743	3.57

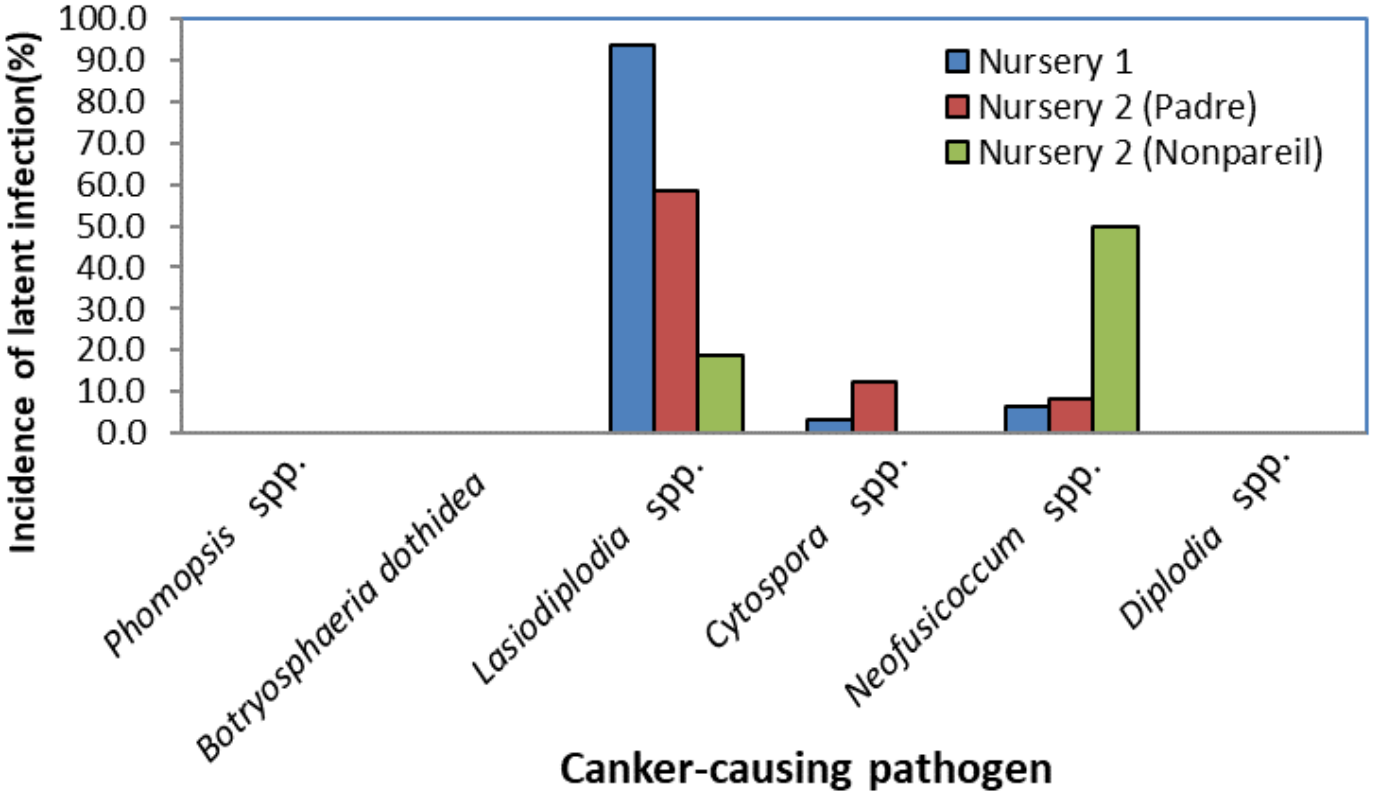
4. Data analysis

<u>Six Pathogen Groups</u>: ***Phomopsis* spp.; *Botryosphaeria dothidea; Lasiodiplodia* spp.; *Cytospora* spp.; *Neofusicoccum* spp.; *Diplodia* spp.**

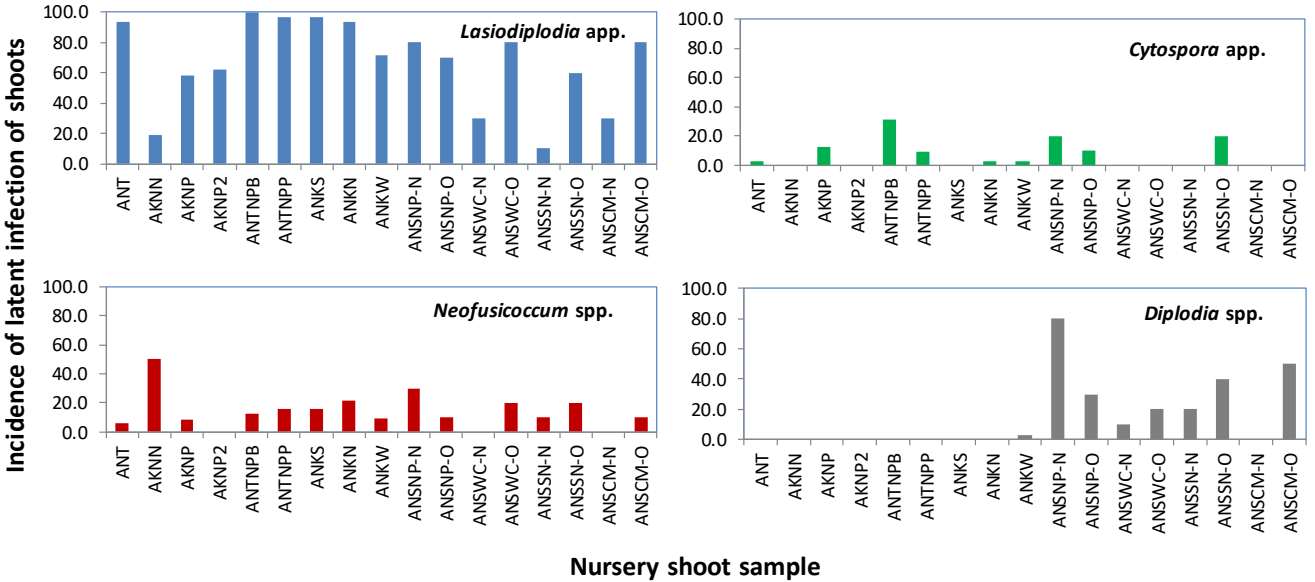
Quantification:

<u>Incidence of latent infection:</u> Positive samples / total number of samples × 100.

Incidence of latent infections of three canker pathogens in trees from two nurseries

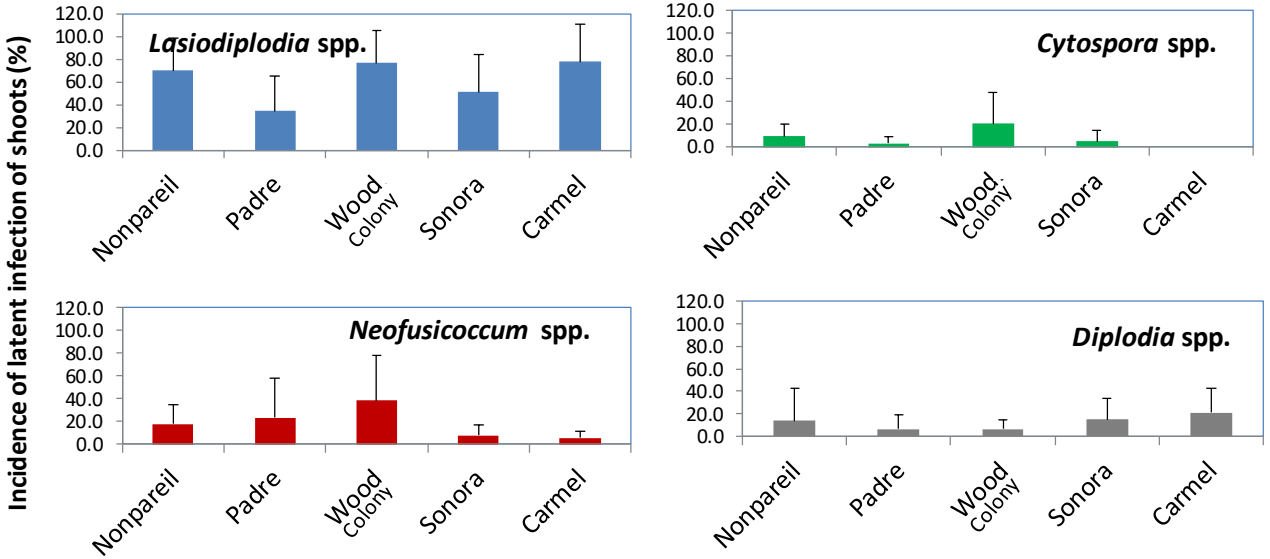


Incidence of latent infections of four canker pathogens from trees in various nurseries



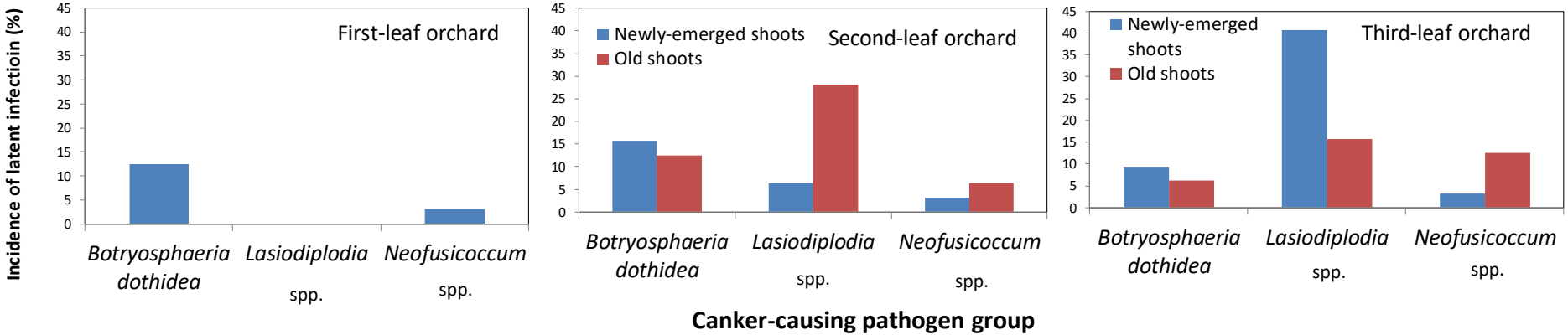
- High incidence *of Lasiodiplodia* spp. (up to 100%); *Cytospora* spp. and *Neofusicoccum* spp. (up to 50%); *Diplodia* spp. (up to 80%).

Incidence of latent infections by four canker pathogens on various almond cultivars obtained from a nursery

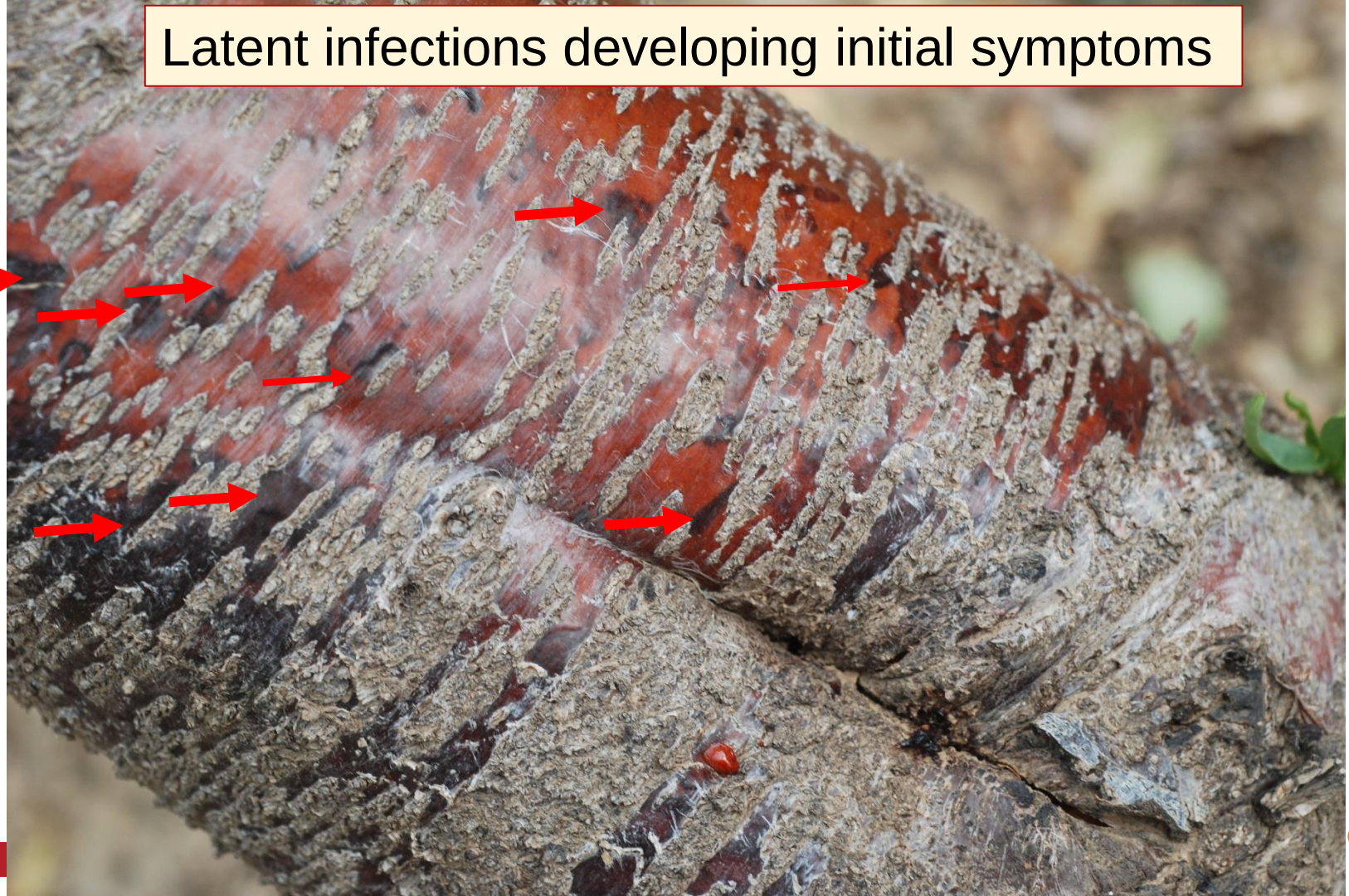


- Wood Colony showed the highest incidence of *Lasiodiplodia*, *Neofusicoccum,* and *Cytospora* spp.
- All cultivars had infection; high incidence *of Lasiodiplodia* spp. (up to 80%); *Cytospora* spp. (up to 20%); *Neofusicoccom* spp. (up to 40%); *Diplodia* spp. (up to 20%).

Incidence of latent infection of young symptomless almond shoots using the molecular (finger-printing) qPCR technique



Latent infections developing initial symptoms



Compare Botryosphaeriaceae fungi from latent infections and band-cankered trees

DNA of Species and Genera from asymptomatic tissues (qPCR assay)	Species causing band canker isolated with the conventional method from asymptomatic tissues
Lasiodiplodia spp.	*Lasiodiplodia theobromae*
Neofusicoccum spp.	*Neofuscicoccum nonquaesitum*
Botryosphaeria dothidea	*Neofusicoccum parvum*
Diplodia spp.	*Neofusicoccum mediterraneum*
Cytospora spp.	*Botryosphaeria dothidea*
	Microphomina phaseolina
Phomopsis spp.	*Diplodia seriata*
	Dothiorella sarmentorum

N. mediterraneum, L. theobromae, and *B. dothidea* were identified from latent infections

Fungicide sprays to protect young trees.

Orchard 1 treated on 2/16/2018:

1) Topsin M WP 70, 1.51 lb/acre

2) Pristine, 14.5 oz/acre

4 rows- reps with 67 trees/ row

3rd-leaf trees

Orchard 2 treated on 3/30/2018:

1) Topsin M WP 70, 1.51 lb/acre

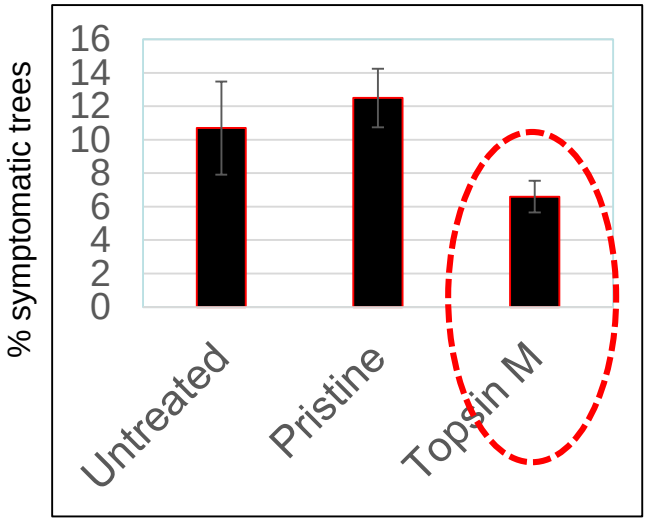
2) Pristine, 14.5 oz/acre

3) Quilt-Xcel, 17.5 fl oz/acre

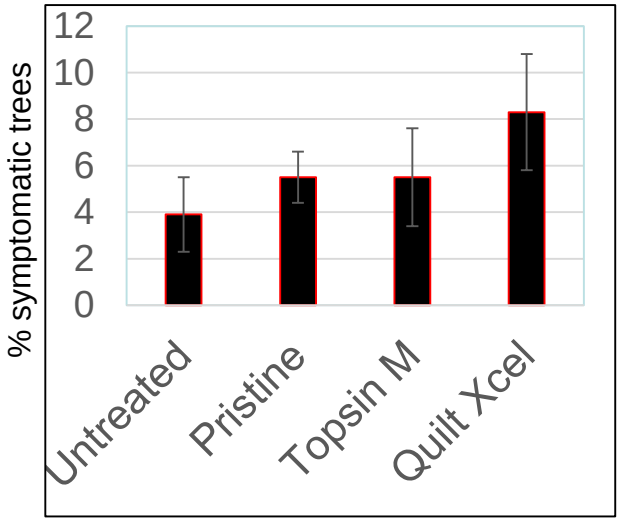
4 rows-reps with 45 trees/ row

3rd-leaf trees

Sprays to protect young trees



Orchard 1 – 272 trees / treatment



Orchard 2 – 180 trees / treatment

Conclusion: Topsin M reduced infection but only in Orchard 1; perhaps the treatment of Orchard 2 was too late. ???

Conclusions:

1. Keep trunks of trees dry.
2. Remove dead trees and their stumps.
3. Band-canker pathogens can be found in symptomless tissues of young trees obtained from nurseries and trees planted in the field (1st to 3rd leaf).
4. Trees may need protection at a very young age! (research in progress...)

Thank you for your attention

We also thank the Almond Board of California for funding this research and participating nurseries and almond growers.

california almonds
Almond Board of California

Hull rot and causes

Severe Hull Rot Disease (Last Several Years)

Rhizopus stolonifer

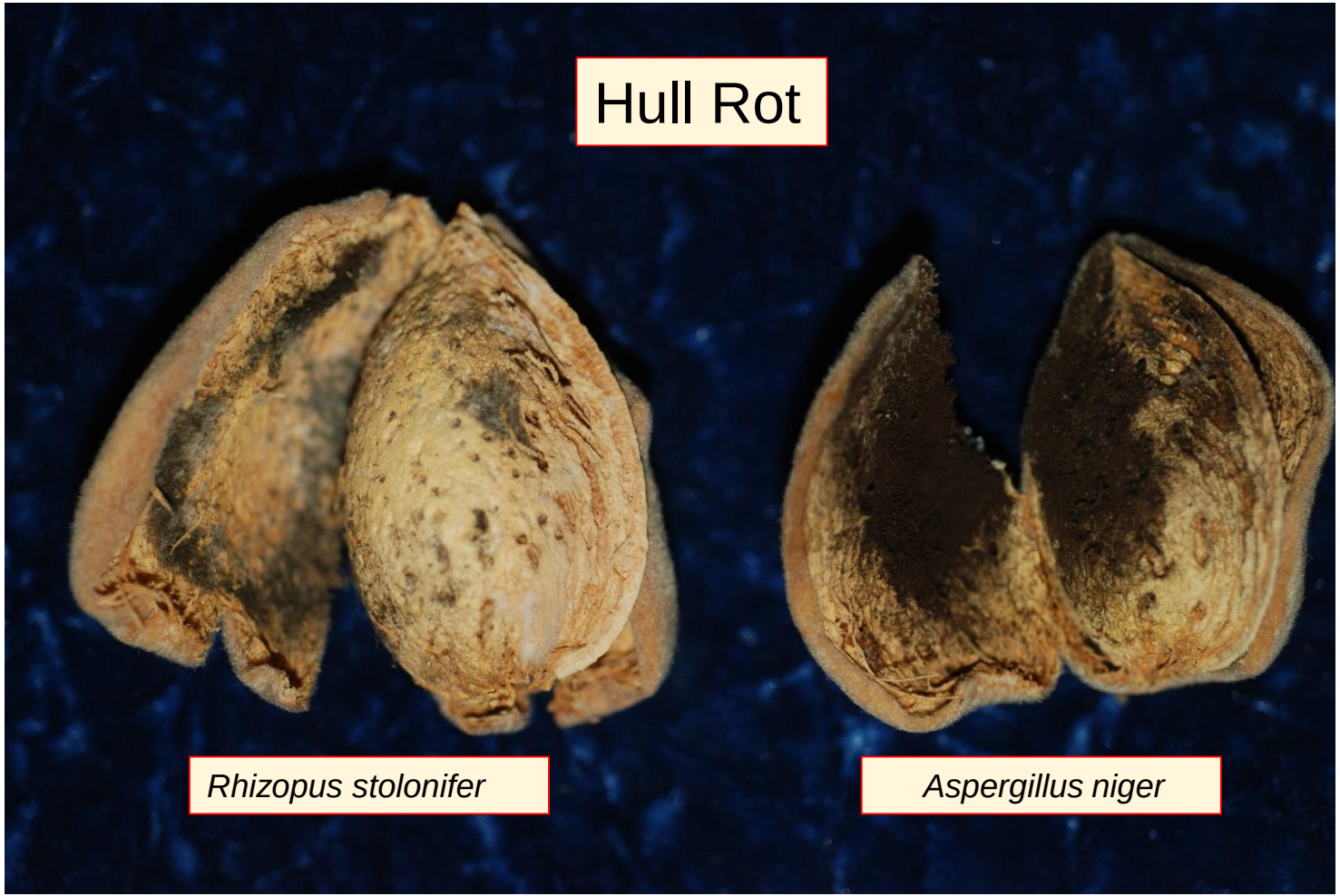
Aspergillus niger

Incidence of ***Aspergillus niger*** and ***Rhizopus stolonifer*** in samples of almond with **hull rot** (2018)

Fruit Sample #	County	A. niger (%)	R. stolonifer (%)
18081a	Fresno	89	11
18081b	Fresno	100	0
18082[a]	Stanislaus	55	--
18084	Fresno	100	0
18102[b]	Madera	54	0
18124	Fresno	42	68
18125[c]	Glenn	20	0
18126c	Glenn	20	0
18127	Glenn	100	50
18130	Stanislaus	90	100
18130 (spurs)[d]	Stanislaus	70	0

[a] 7% *A. niger* + *A. flavus*
[b] 13% *Fusarium*
[c] 80% *Phomopsis*
[d] Spurs bearing fruit with hull rot

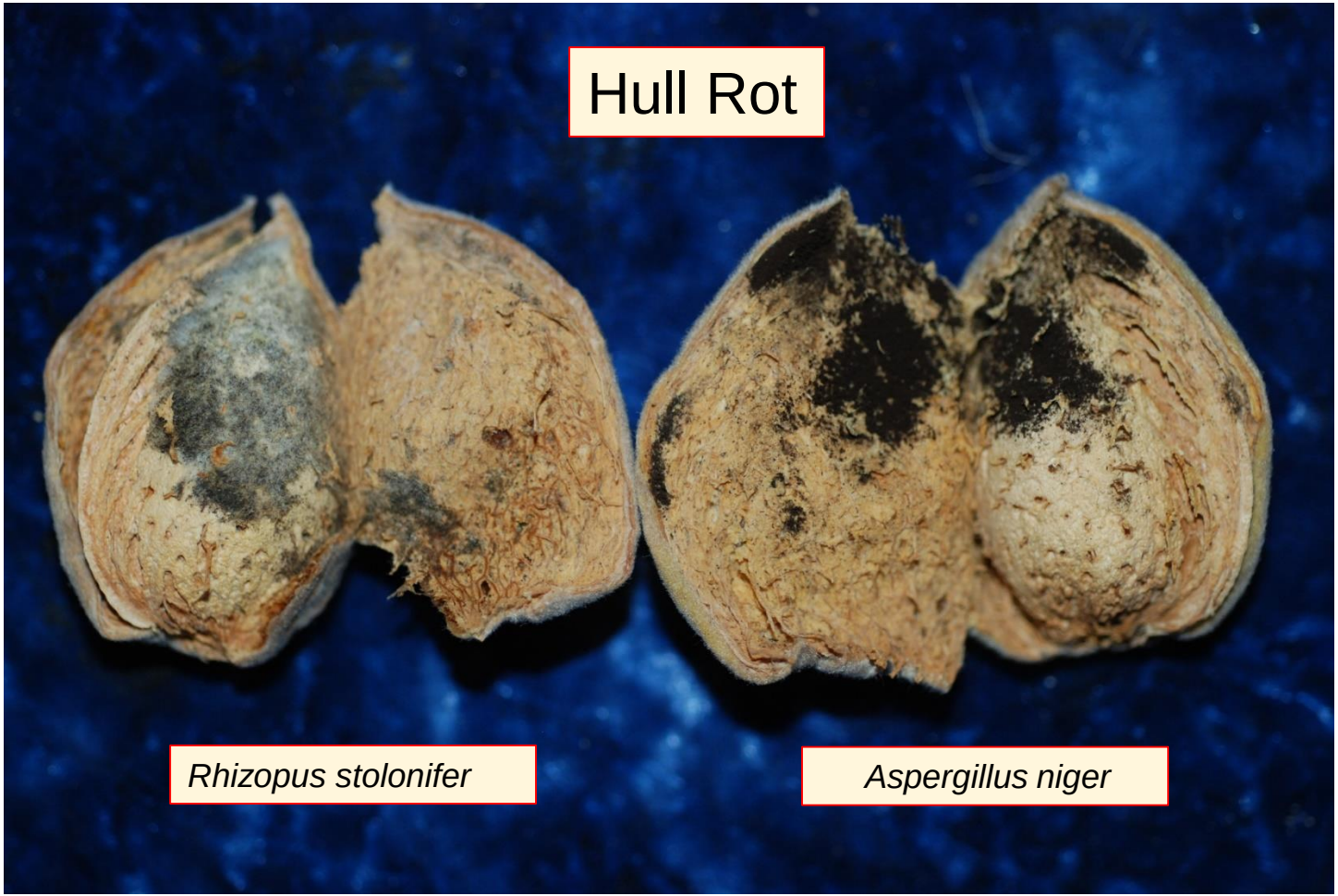
Hull Rot



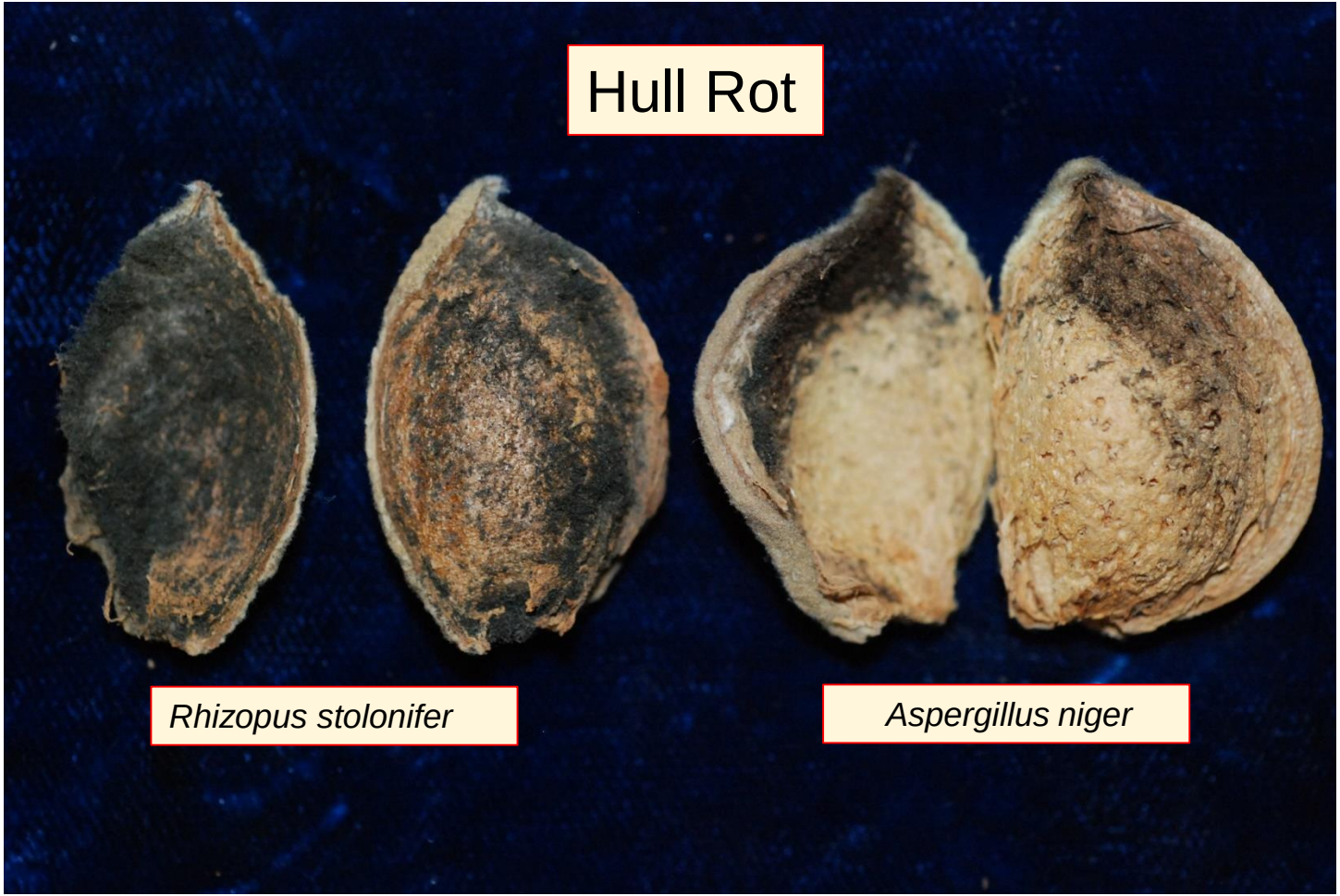
Rhizopus stolonifer

Aspergillus niger

Hull Rot

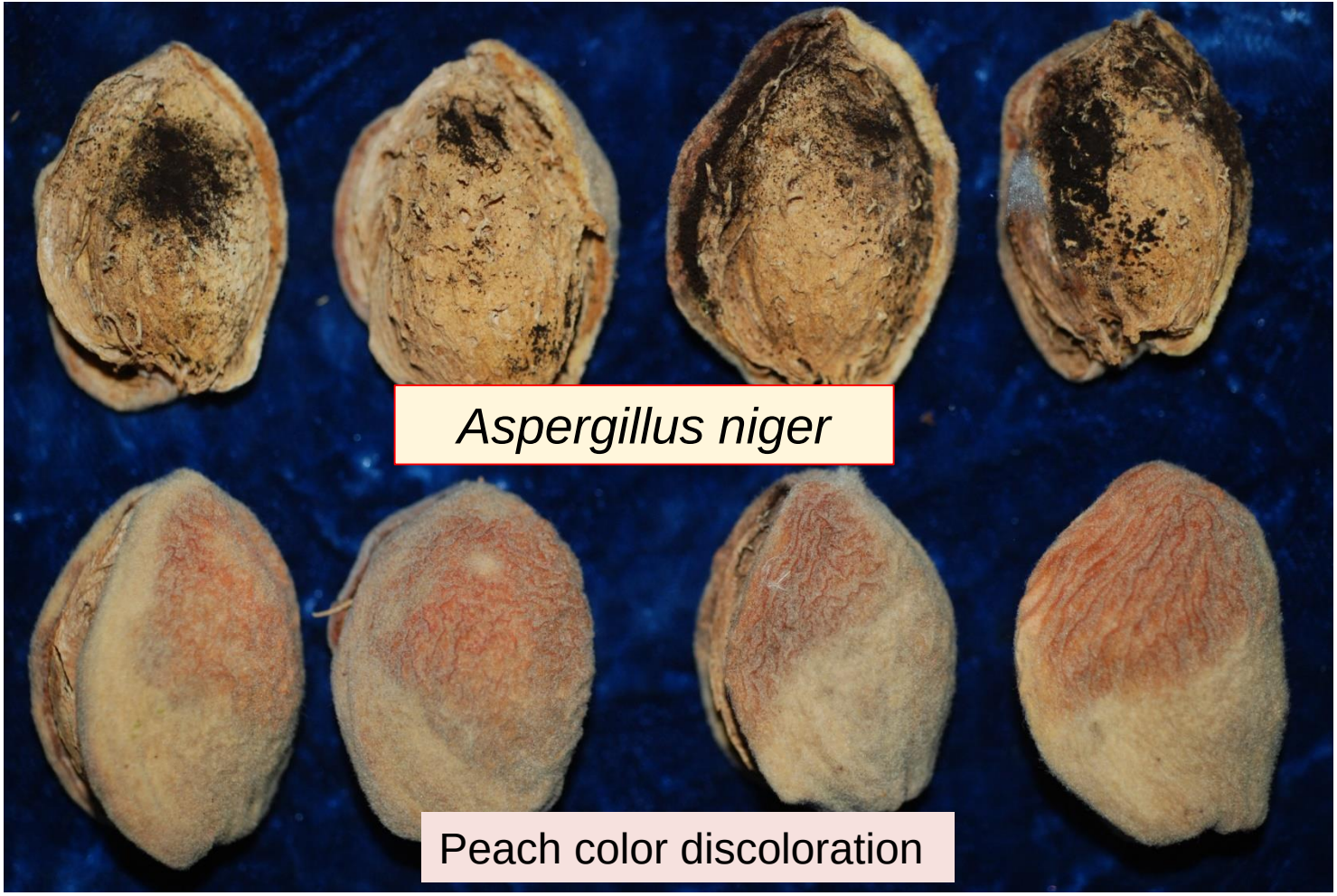


Hull Rot



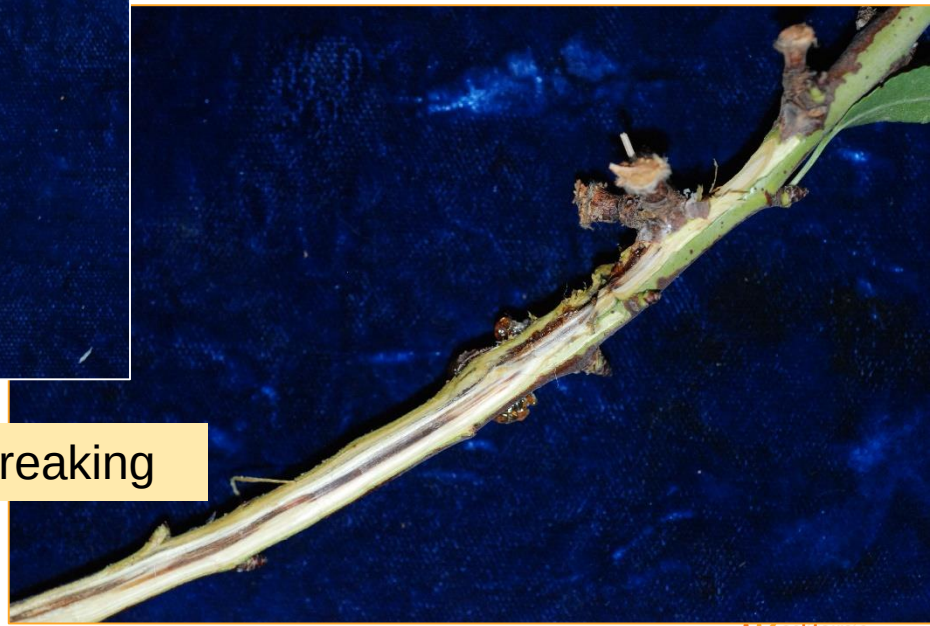
Rhizopus stolonifer

Aspergillus niger



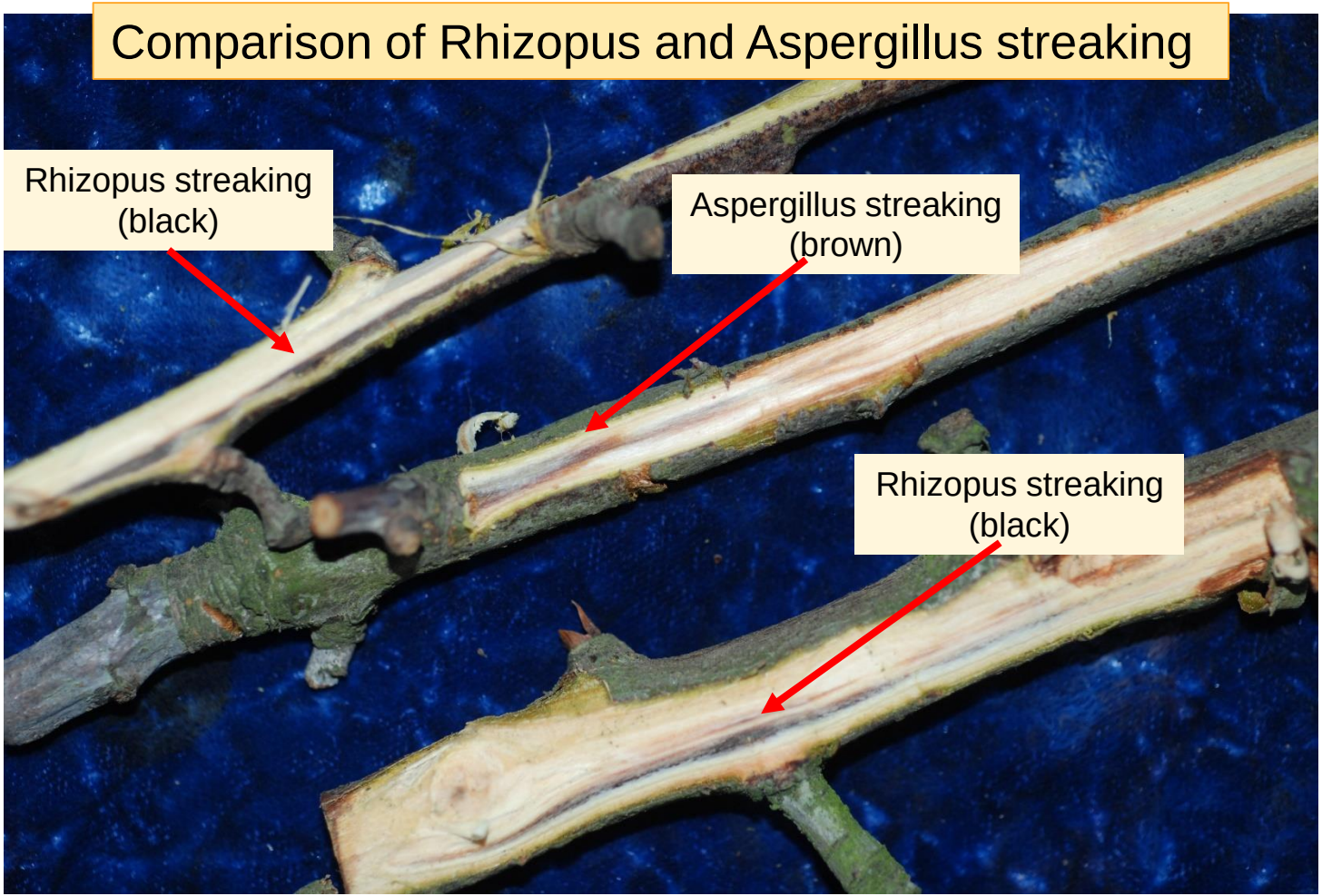
Symptoms and Signs of Hull Rot

Symptoms of Rhizopus hull rot



Black streaking

Comparison of Rhizopus and Aspergillus streaking



Fruit susceptibility to Hull Rot Pathogen *Rhizopus stolonifer*

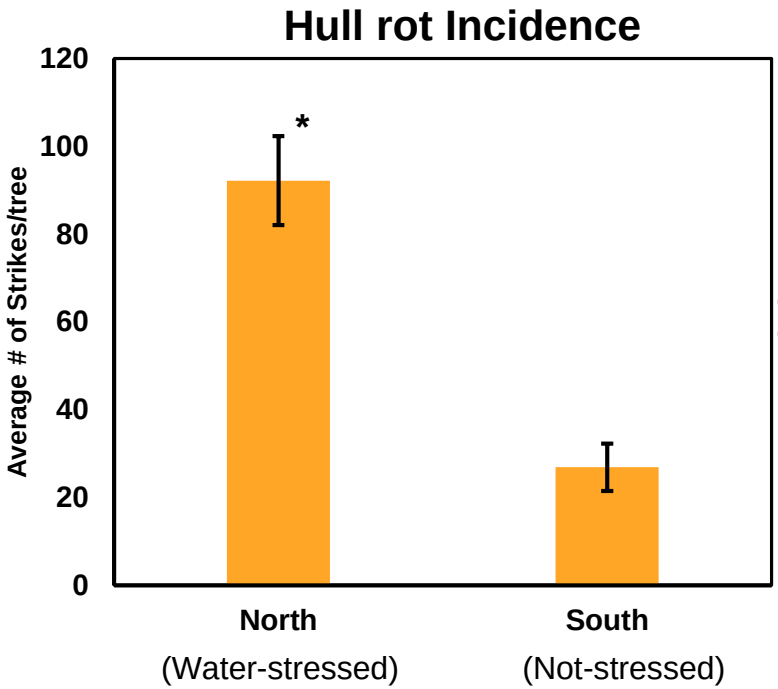
(b1) Initial separation-50% or more of a thin separation line visible

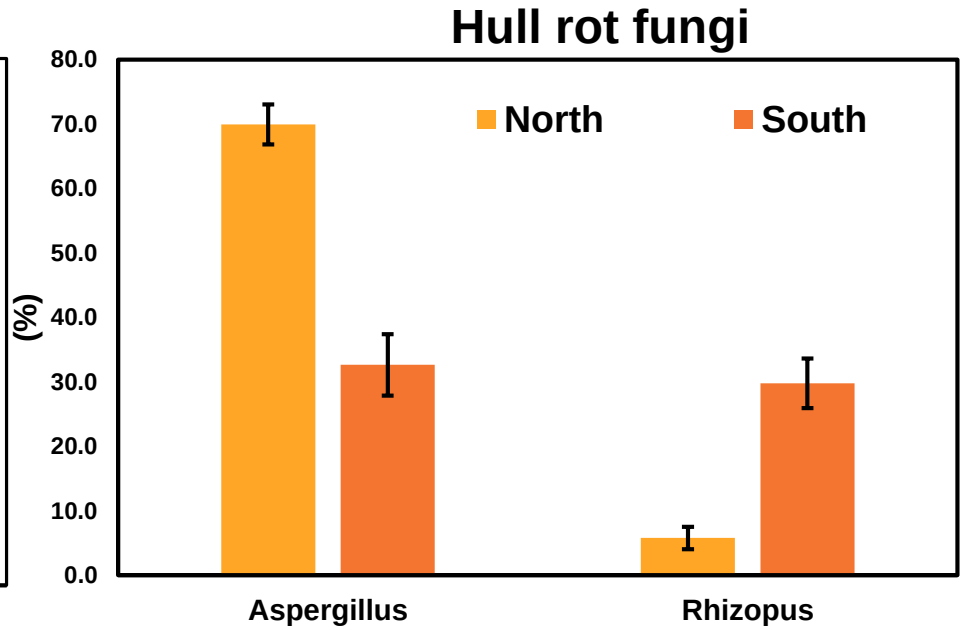
(b2) Deep V, is the most susceptible stage (source: Adaskaveg. 2010. Almond Board of California Research Proceedings # 09-PATH4-Adaskaveg)

(b3) Deep V, split-a deep "V" in the suture, which is not yet visibly separated, but which can be squeezed open by pressing both ends of the hull

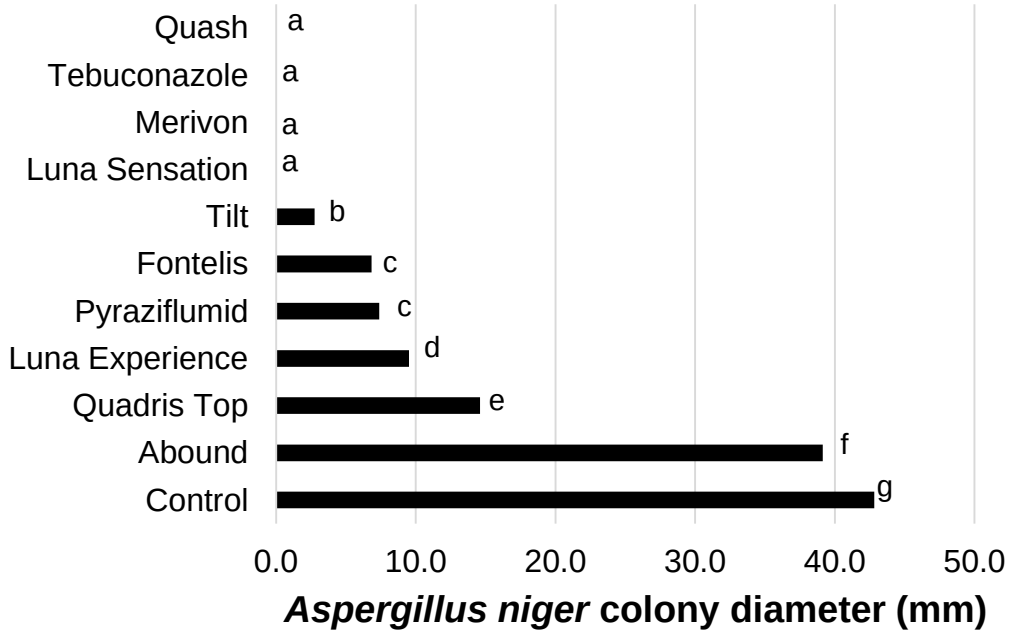
(c) Split, less than 3/8 inch

Percentage of fruit associated with hull rot that has *Aspergillus niger* or *Rhizopus stolonifera* in a water-stressed orchard (North) than not stressed orchard (South)

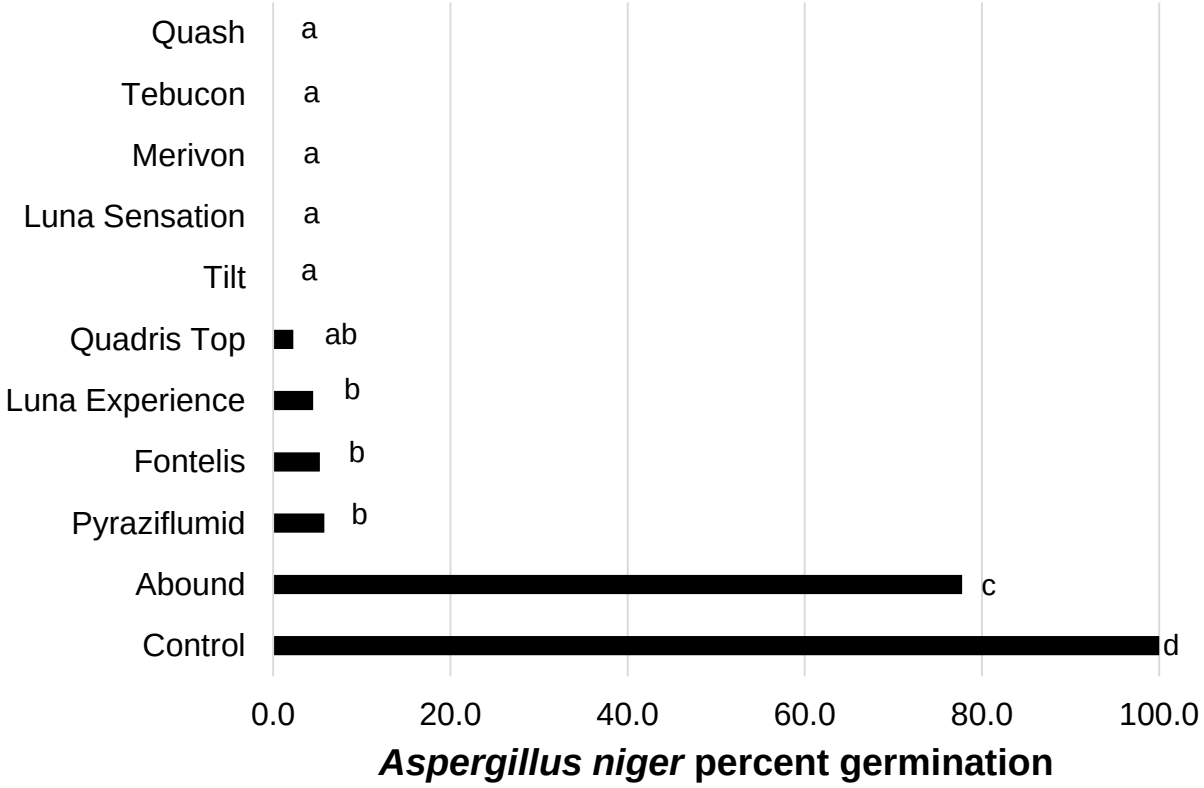




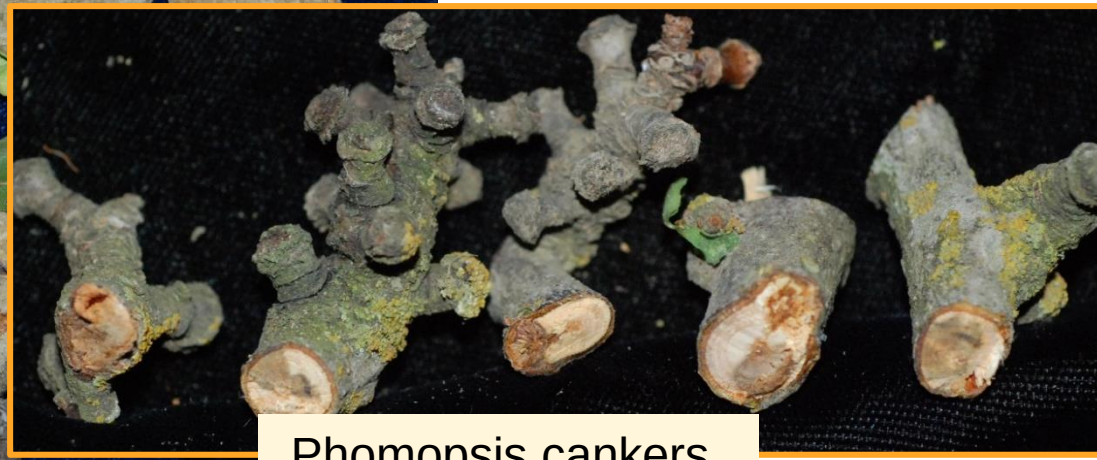
Colony diameter of *Aspergillus niger* on fungicide amended PDA plates



Percent germination of *Aspergillus niger* on fungicide amended PDA plates

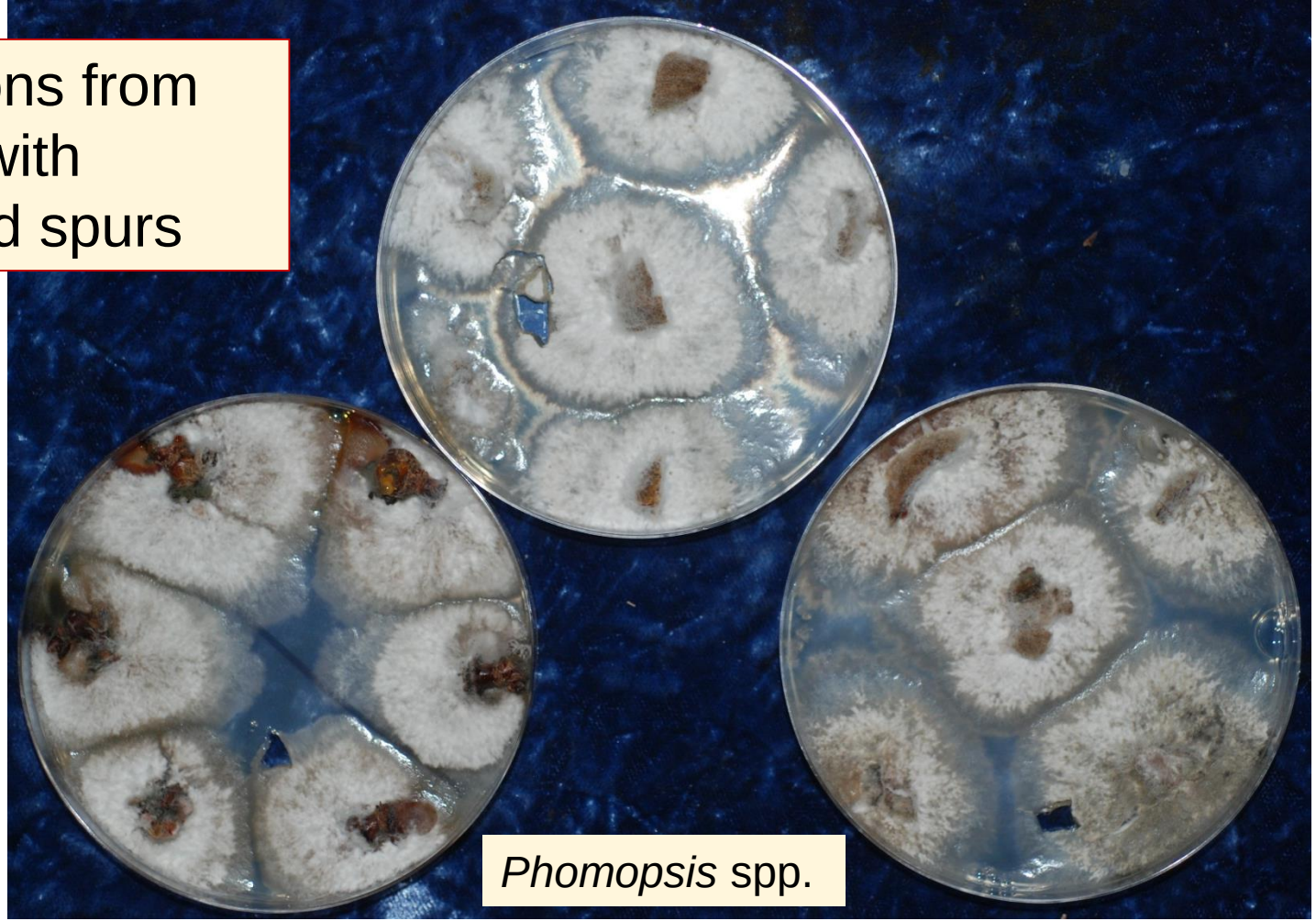


Phomopsis causing stick-tights & killing spurs



Phomopsis cankers

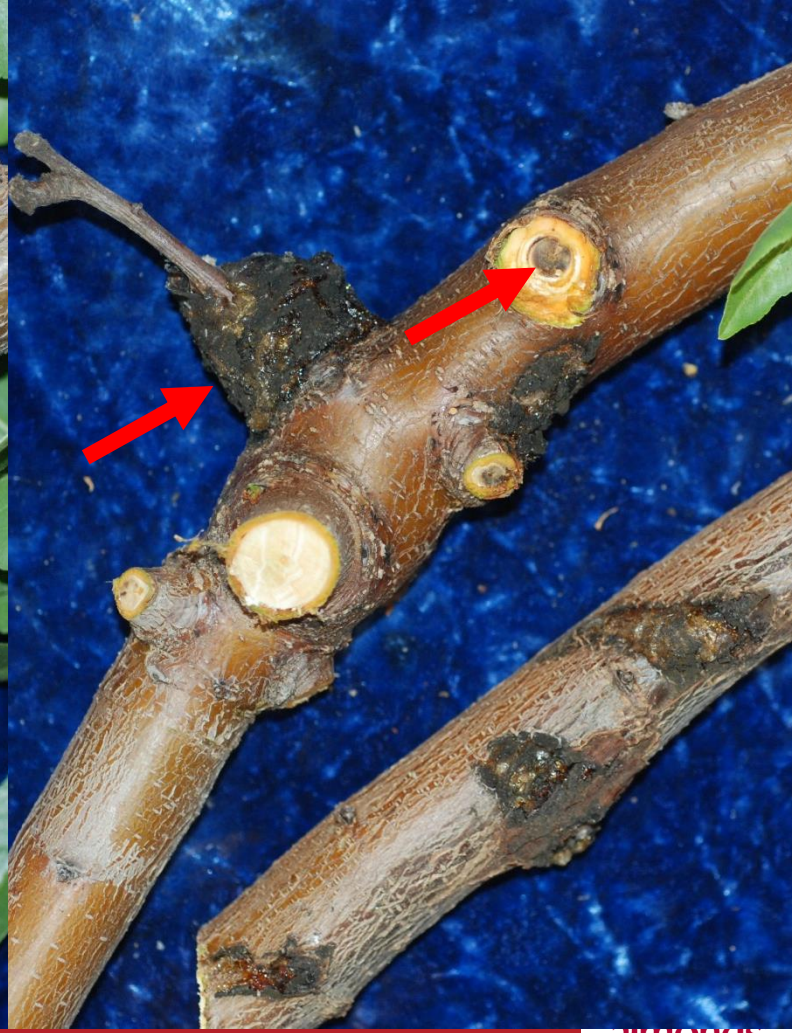
Isolations from spurs with blighted spurs

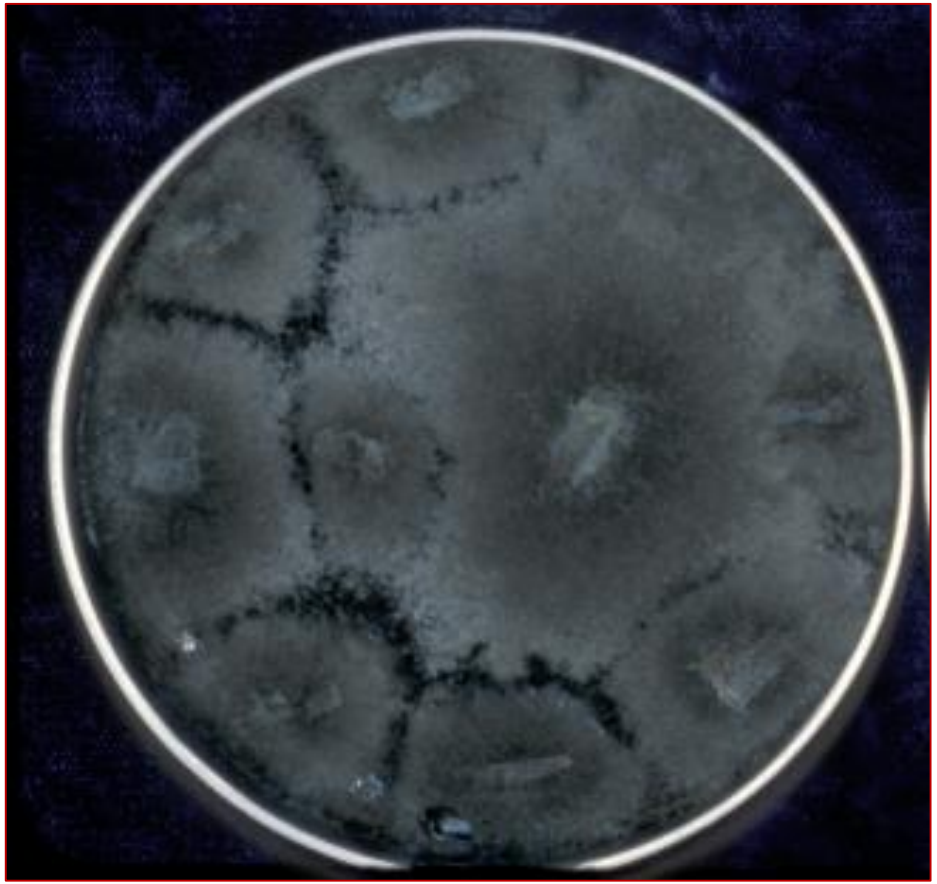


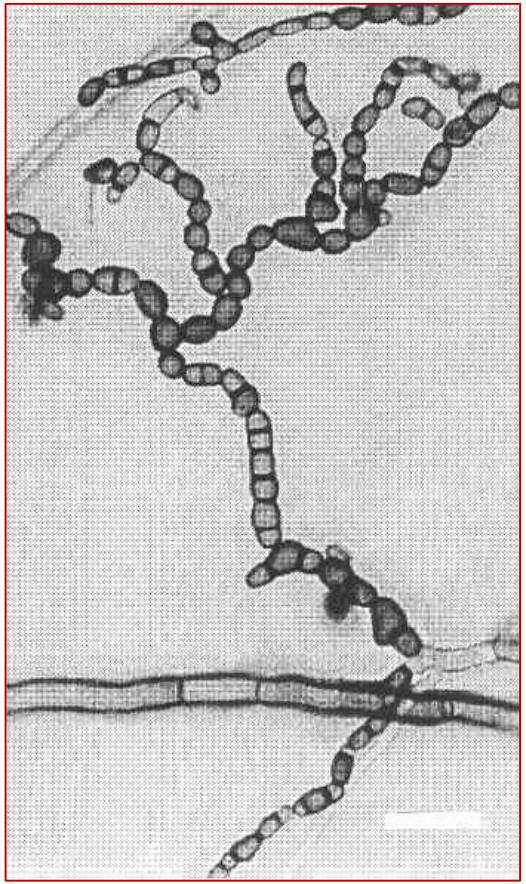
Phomopsis spp.

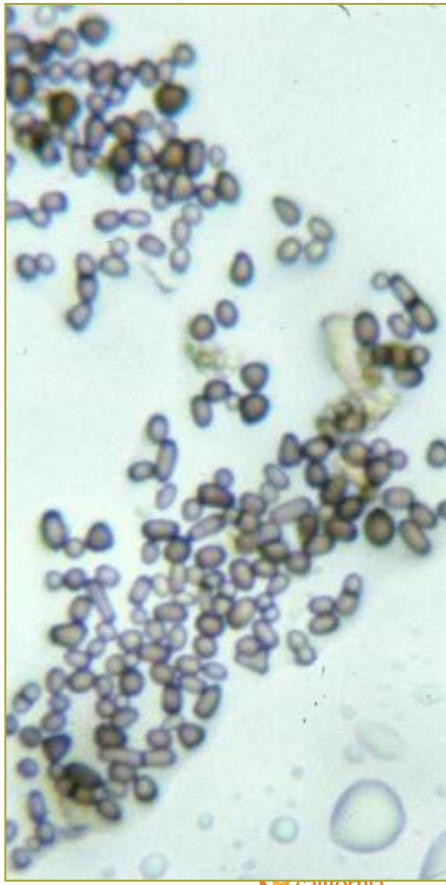
Neoscytalidium canker and fruit rot

Cause: *Neoscytalidium dimitiatum*

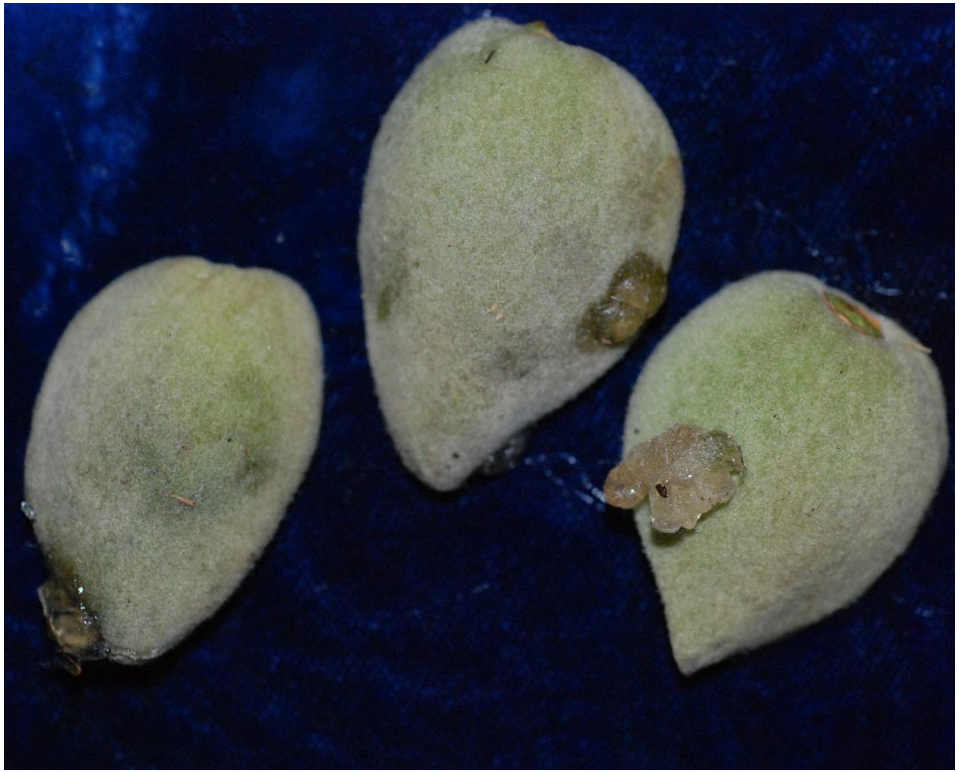


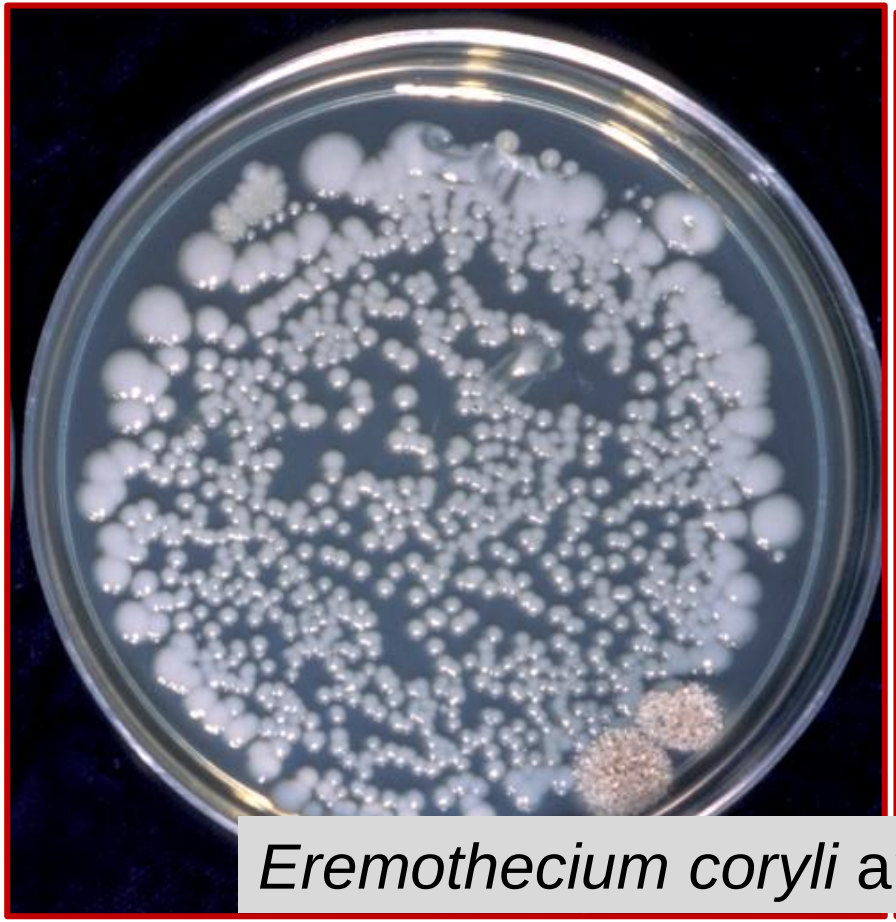






Stigmatomycosis: Damage by Leffooted Bug *Leptoglossus zonatus*





Eremothecium coryli and ascospores

Thank you