A photograph of a prune tree with green leaves and clusters of small, round, purple and pinkish fruits. The background is a clear blue sky. On the right side of the image, there is a white silhouette of a person's head and shoulders, facing left.

Early Detection of Cytospora and Other Canker Diseases of Prune

**Themis J. Michailides,
Yong Luo, Franz Niederholzer,
Richard Rosecrance, Becky Wheeler-Dykes,
Karla Caldera, & Jaime Ott**

Definition

What is a canker?

- A continuous mass of killed tissues in trunks, scaffolds, branches, and shoots of plants.
- The canker pathogen grows and colonizes the entire cankered tissue and beyond.
- A canker can result to a blight, but a blight to occur does not always need a canker.



Cytospora canker caused by *Cytospora leucostoma*



Photo F. Neiderholzer

Bacterial canker caused by *Pseudomonas syringae*

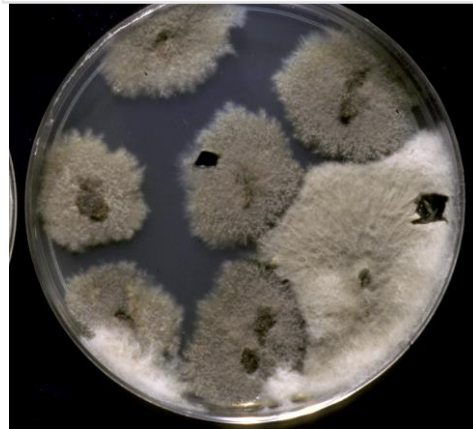


Canker-pathogen fungi isolated from cankers in prune trees

Cytospora leucostoma
Cytospora cincta



Botryosphaeria dothidea



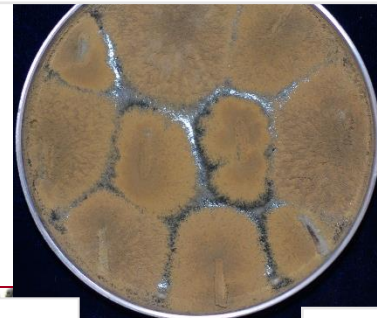
Lasiodiplodia citricola



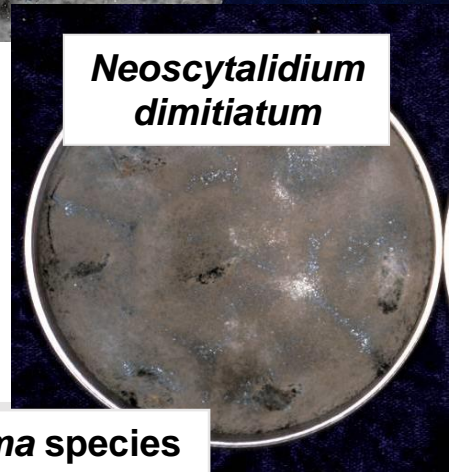
Fusarium sp.



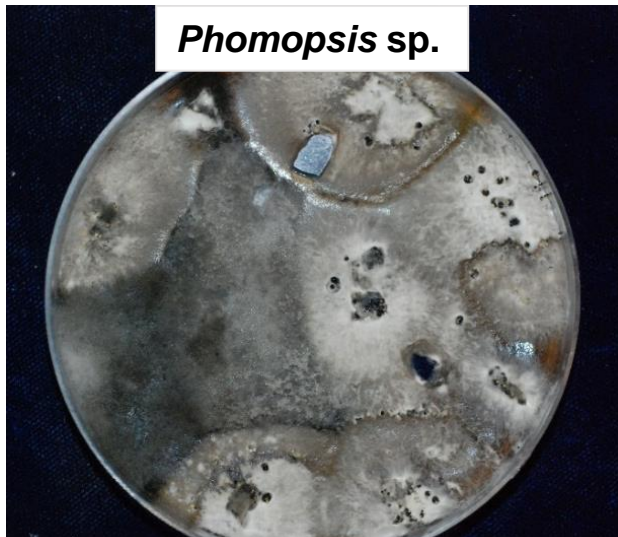
Paecilomyces variotii



Neoscytalidium dimitiatum



Phomopsis sp.



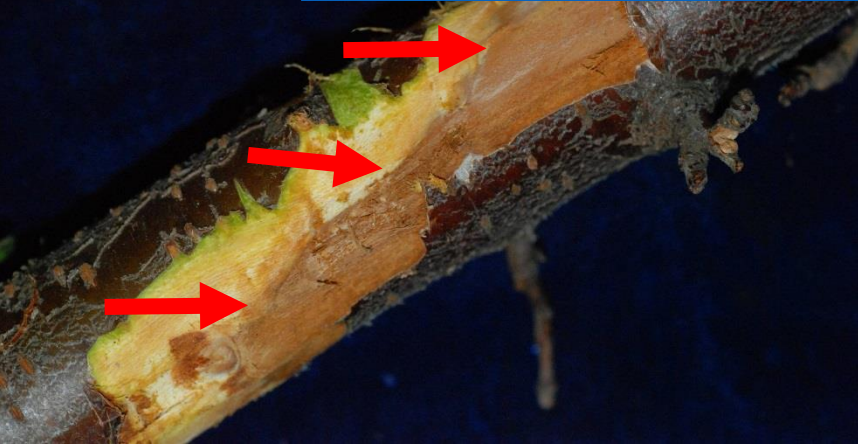
Diplodia seriata



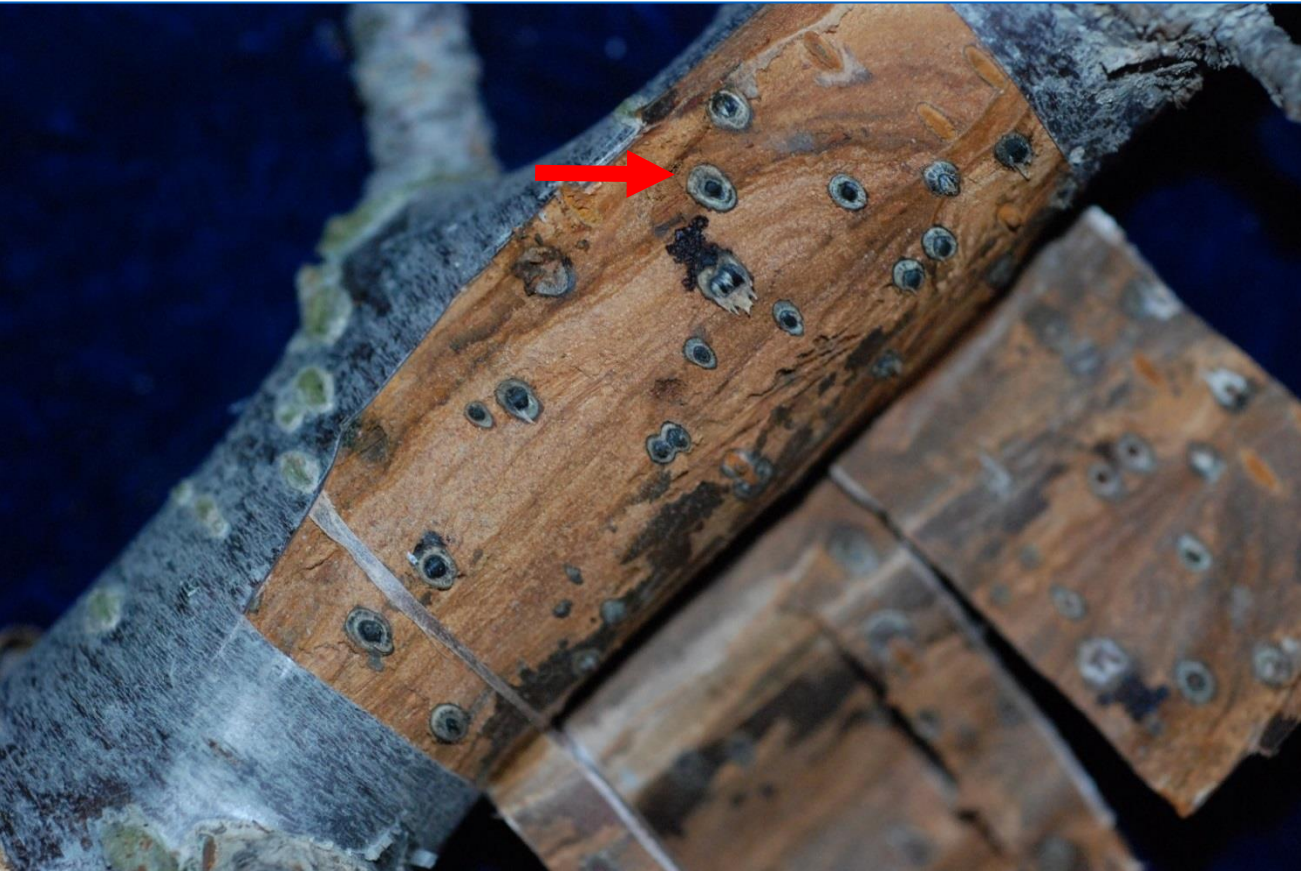
Phoma species



Cytospora and other canker fungi produce pycnidia



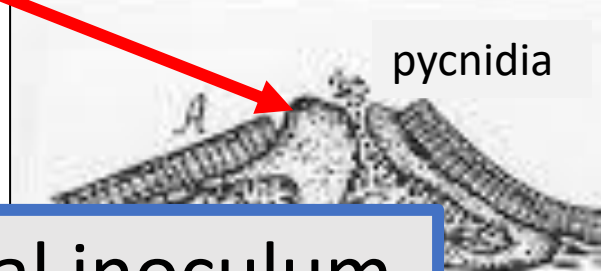
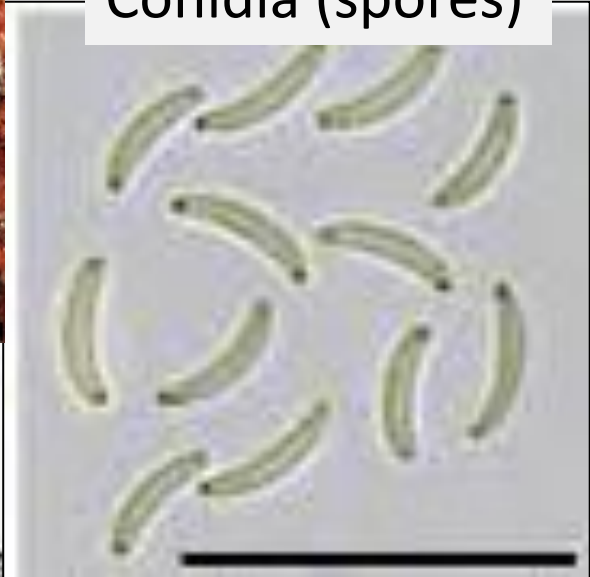
Frequently, *Cytospora* & *Botryosphaeria* canker fungi can occur in the same canker



Oozing pycnidia

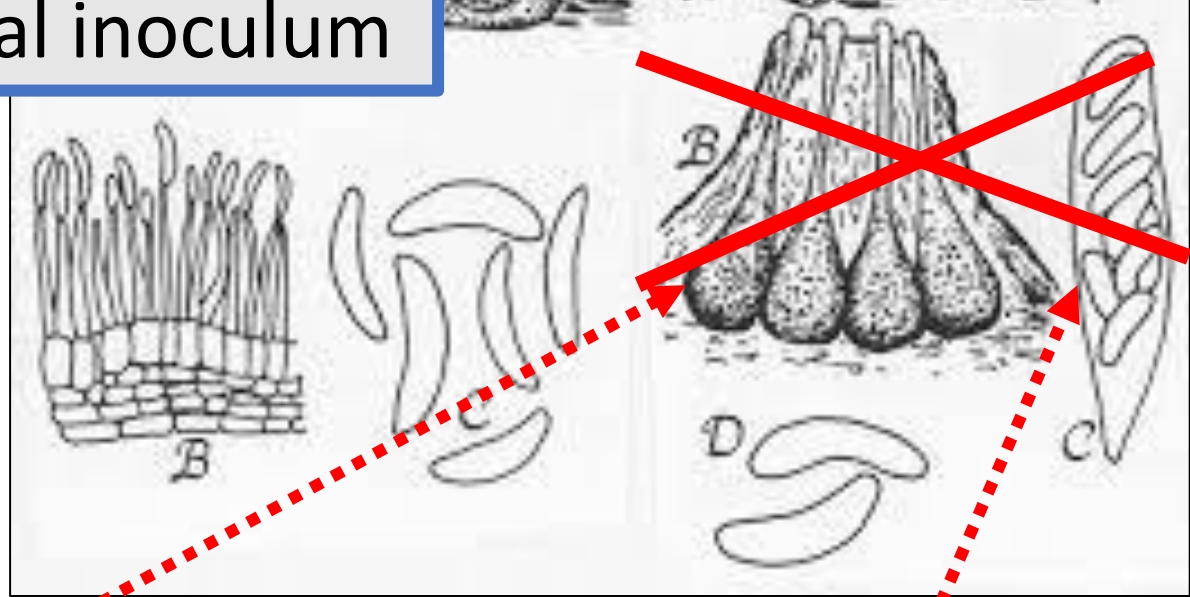
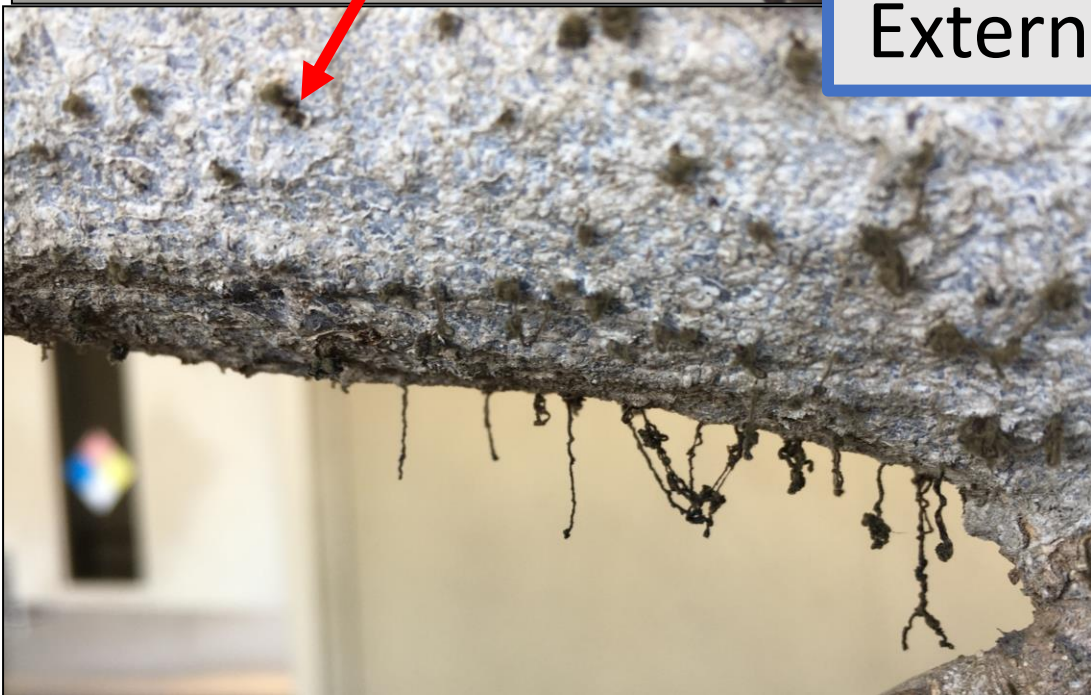


Conidia (spores)



pycnidia

External inoculum



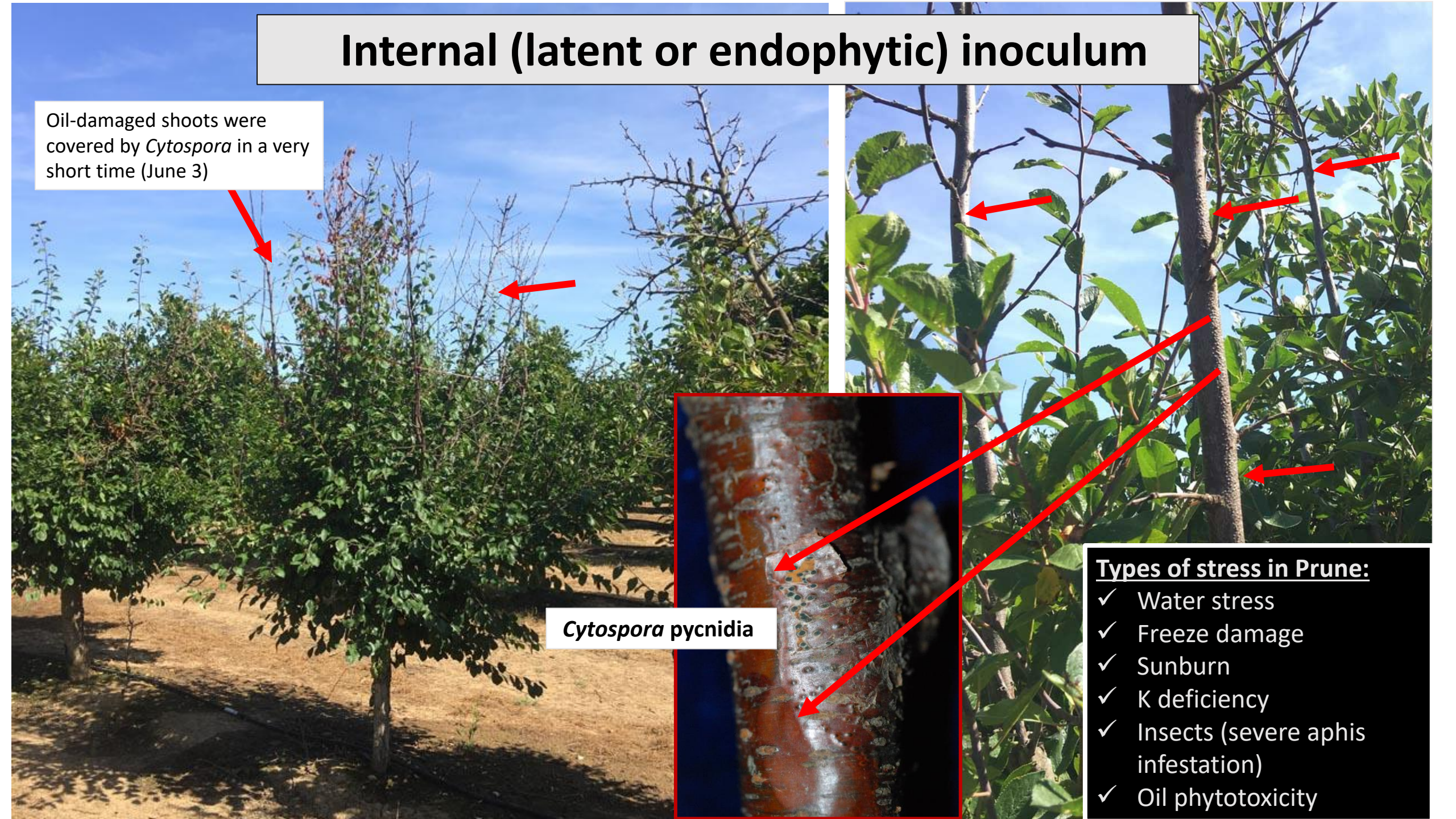
Perithecia (producing airborne ascospores)?



Typical canker symptoms in pruning wounds due to external inoculum

Internal (latent or endophytic) inoculum

Oil-damaged shoots were covered by *Cytospora* in a very short time (June 3)

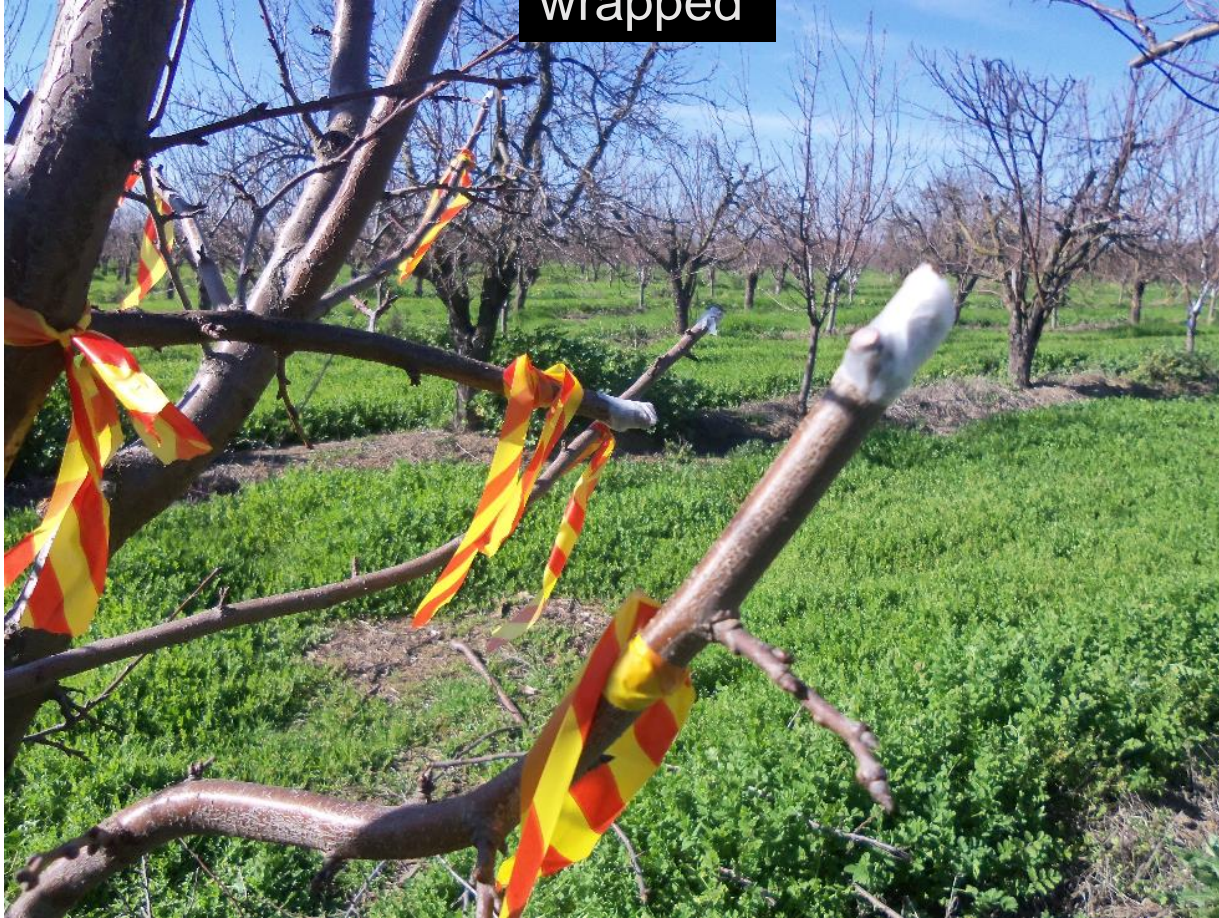


Cytospora pycnidia

Types of stress in Prune:

- ✓ Water stress
- ✓ Freeze damage
- ✓ Sunburn
- ✓ K deficiency
- ✓ Insects (severe aphid infestation)
- ✓ Oil phytotoxicity

wrapped

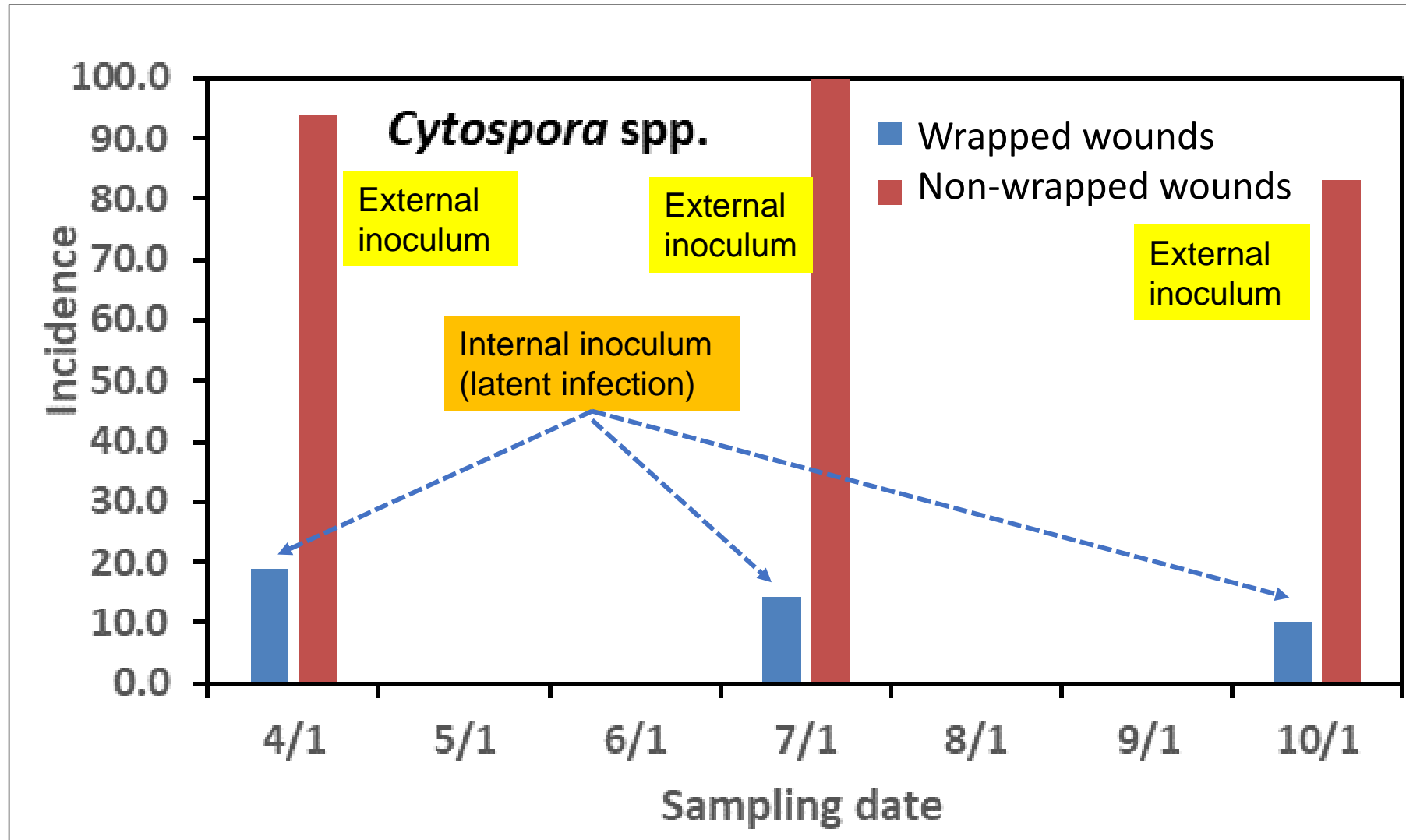


not wrapped



- Experiments in two orchards with severe *Cytospora* disease.
- Two treatments: 1, wrapped wounds; & 2, not-wrapped wounds.
- Periodic collection of shoot samples per each treatment.
- Application of *qPCR* assay to quantify the DNA of latent infection levels.

Comparison of infection of wrapped and non-wrapped pruning wounds
by *Cytospora*



How does *Cytospora* infect a tree?

Two mechanisms of infection:

1. **Internal inoculum**, or latent infection (“endophytic stage”) } **stress, wound** → Canker disease

2. **External inoculum** **wound, stress** → Canker disease

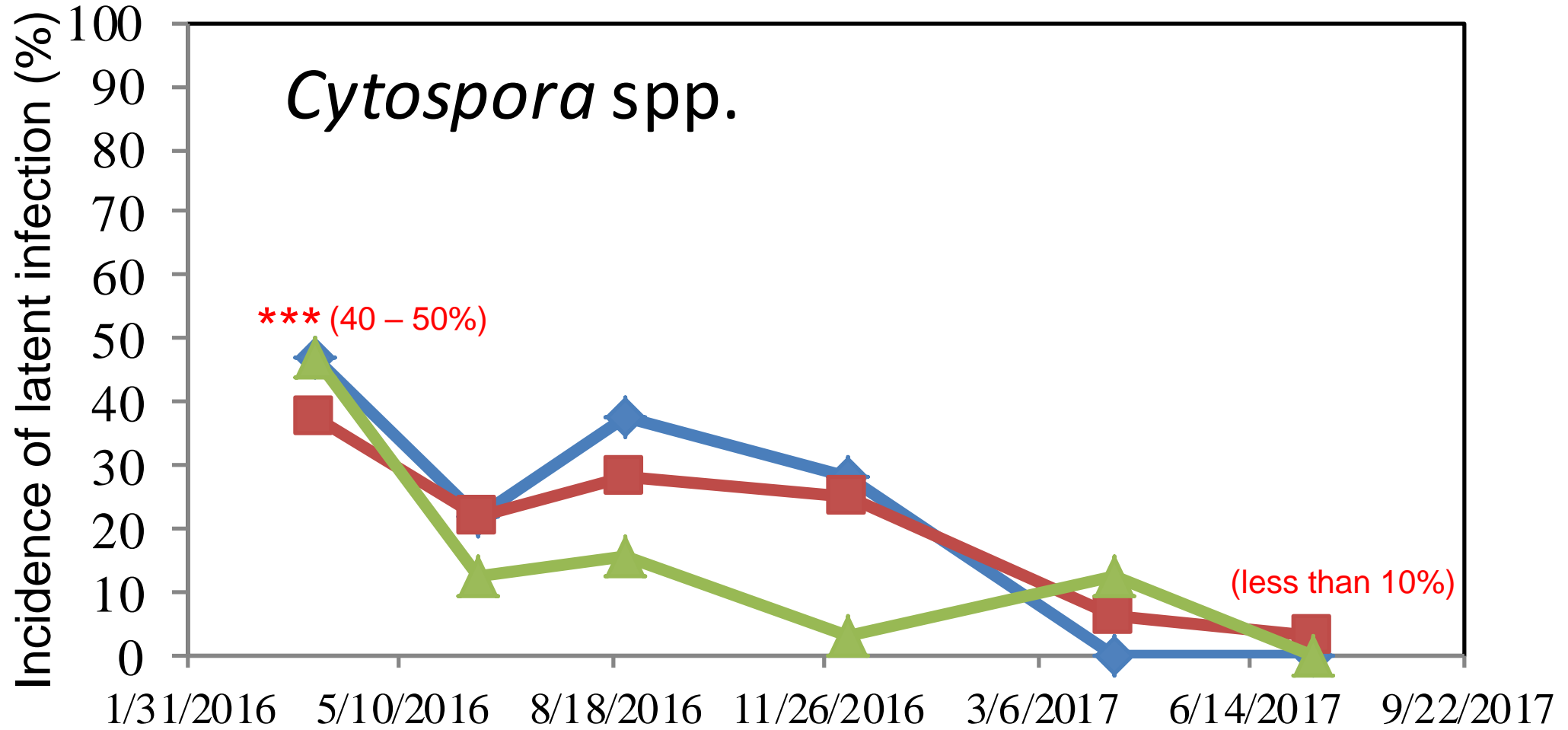
For the early detection we determined seasonal patterns of latent infection in newly-emerged and one-year-old shoots



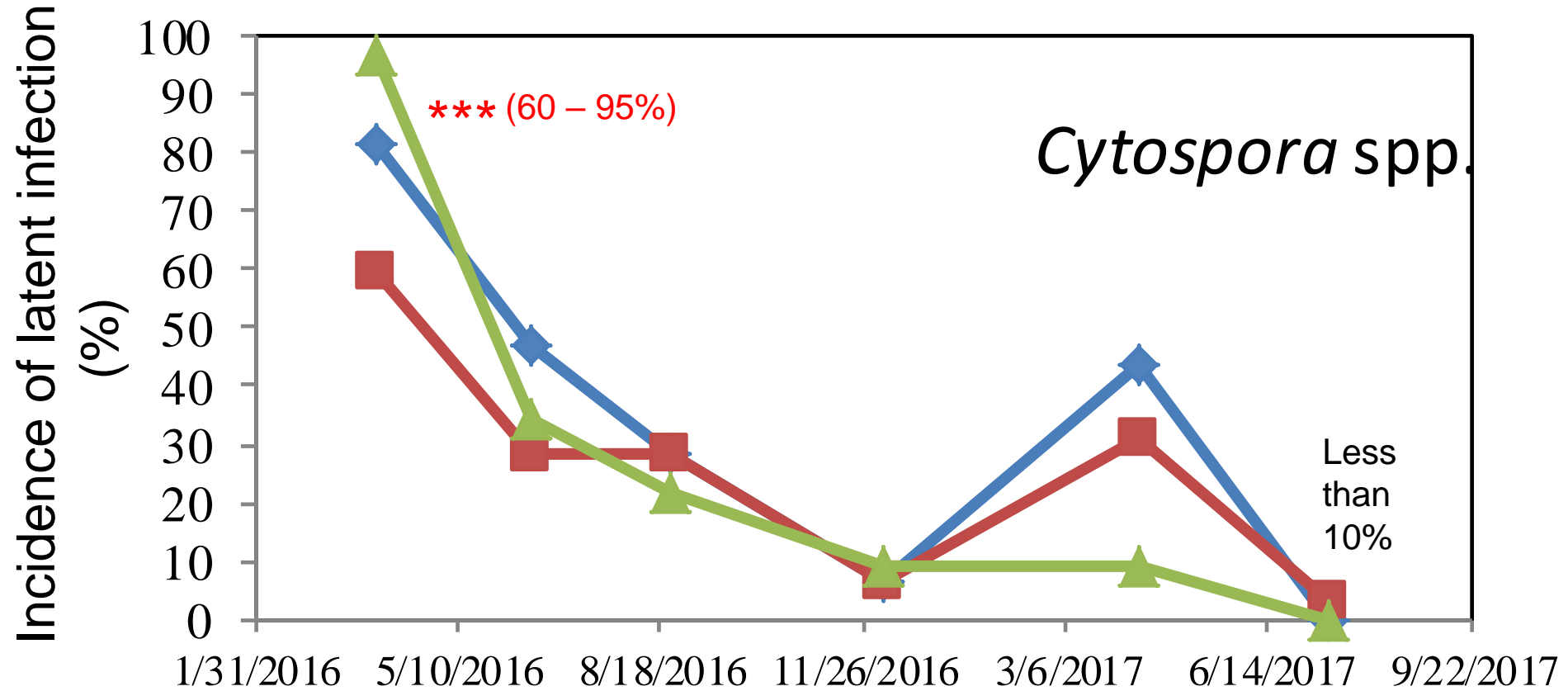
➤ Newly-emerged (current growth) and 1-year-old shoot samples were collected from 3 prune orchards every three months.

- Shoot samples were processed to extract DNA.
- Six primer pairs were used to target 6 canker-causing pathogen groups.

Newly-emerged shoots from three prune orchards

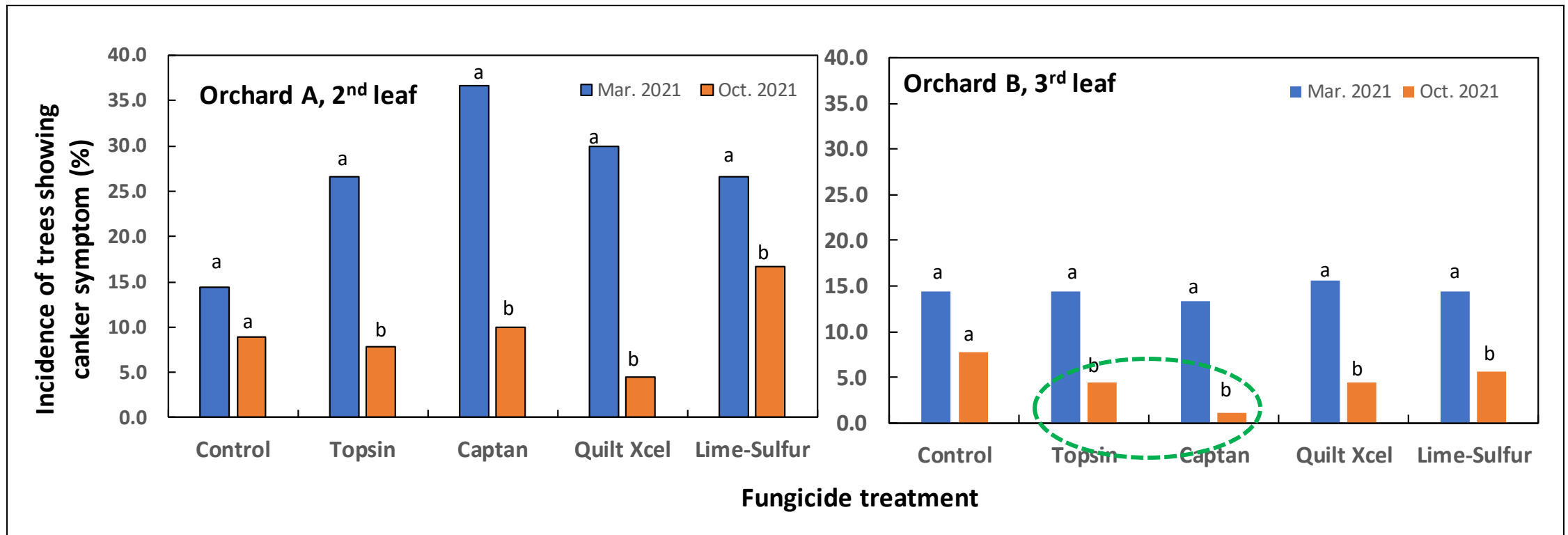


1-year-old shoots from three prune orchards



Protection from external & internal inoculum: Efficacy of selected fungicides to control prune Cytospora and other cankers in young orchards

Trunk and crotch were sprayed 25 March 2021; canker recording on 29 Oct 2021

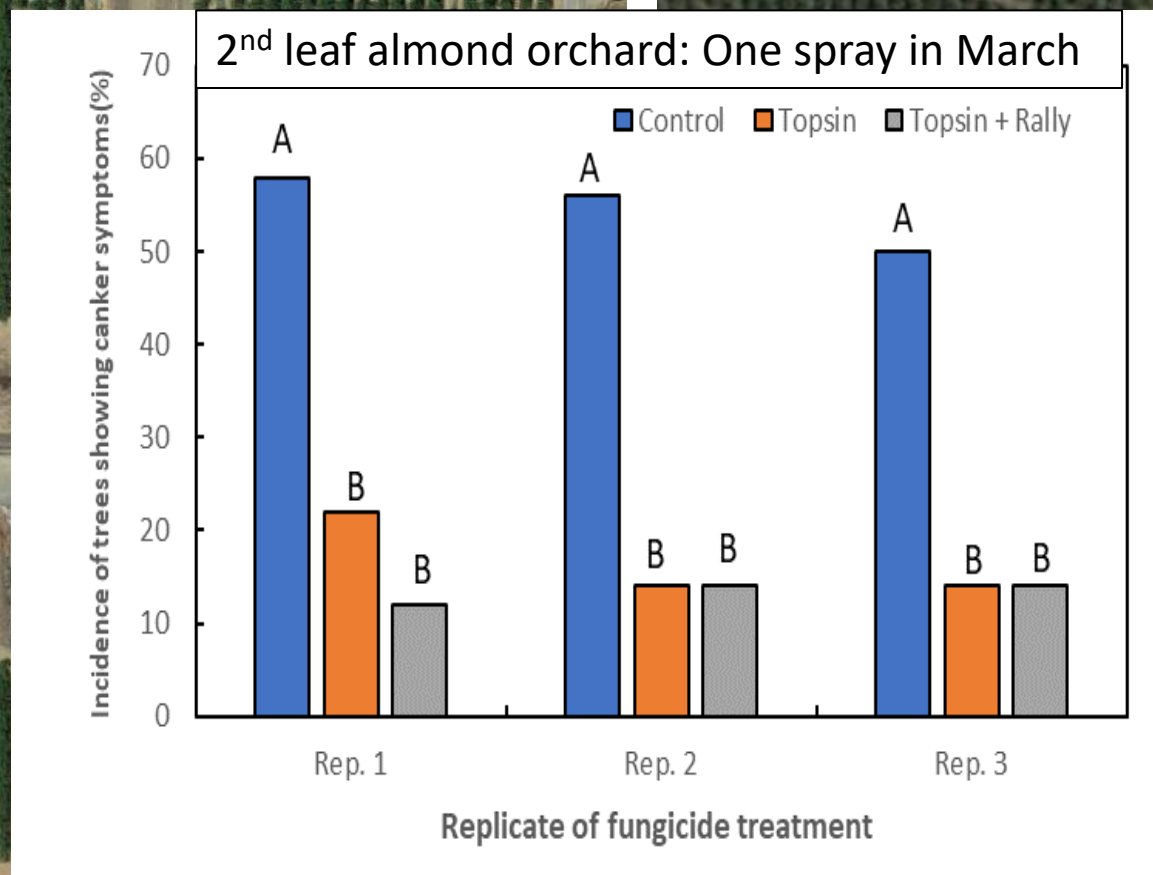
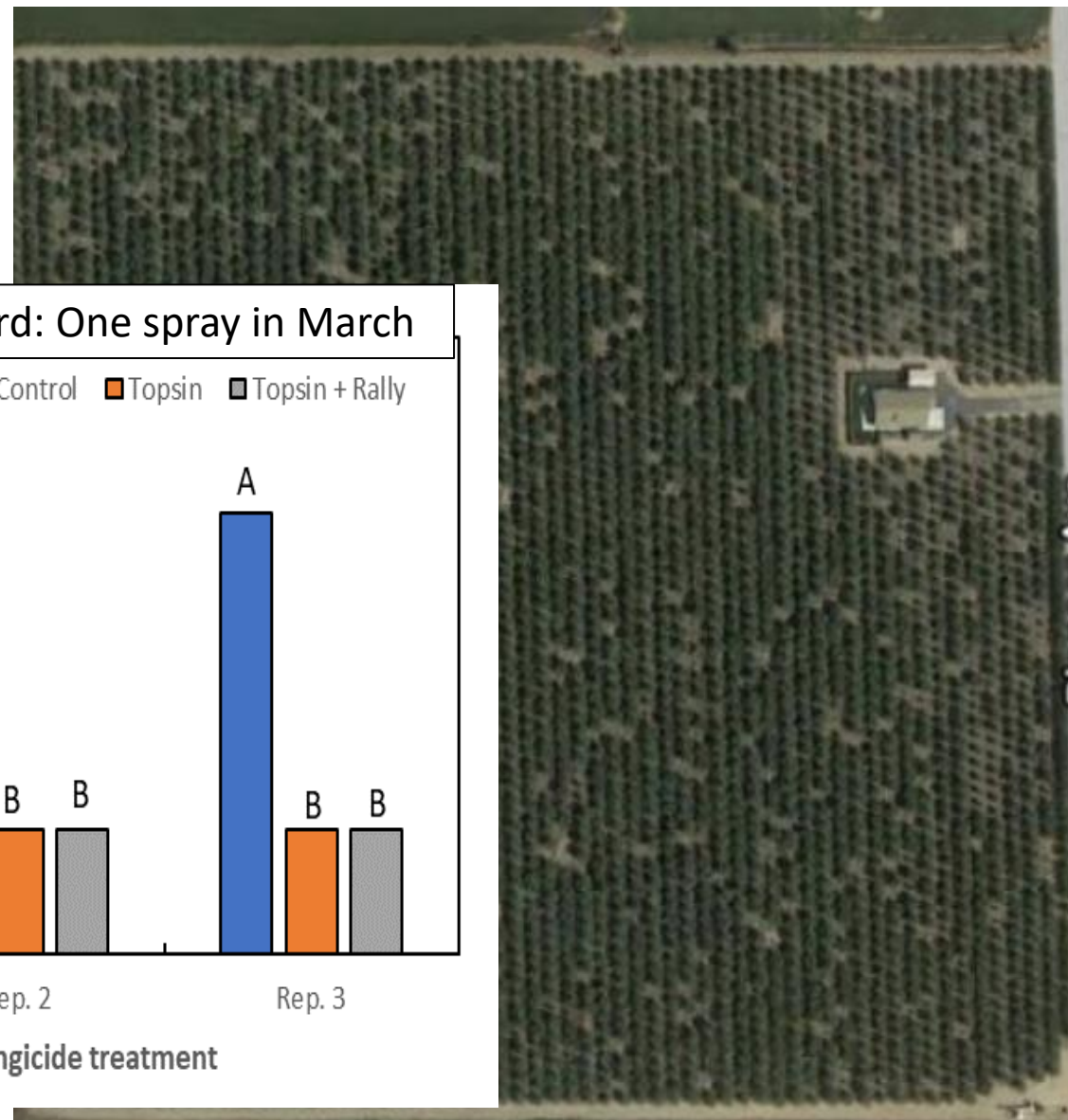
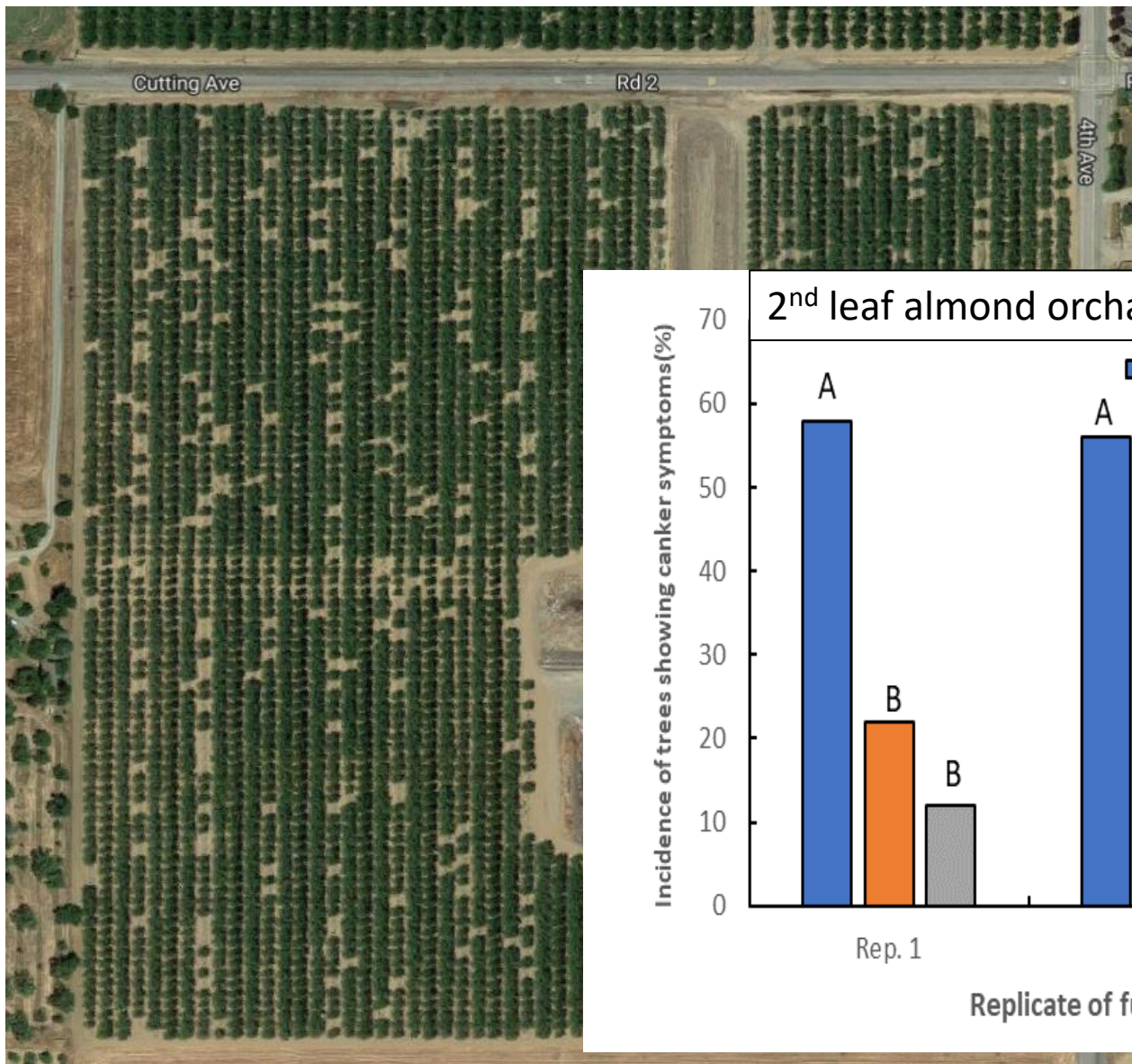


CONCLUSION:

Spraying the trunk and the crotch of young trees seems to make a big difference!

2nd-leaf almond orchard severely damaged by band canker (Butte County)

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



Objectives - 2023

1. To compare the efficacy of Topsin-M, Captan, Vintec (a.i., *Trichoderma atroviride*), and Bio-Mend+ (a cocktail of 10 different biologicals) against *Cytospora* and other canker-causing pathogens after pruning (Richard Rosecrance's Pruning Trial).
2. To determine the various species causing cankers in the pruning/hedging trial in Red Bluff (Richard Rosecrance's Pruning Trial).
3. To study the survival of *Cytospora* in wood chips (whole orchard recycling).

a.i. *Trichoderma atroviride* SC1:
 1×10^{10} CFU per gram

Mechanism: Colonization of the
pruning wound!



Untreated



Treated

Bio-Mend+

Soluble Concentrate

CONTAINS NON-PLANT FOOD INGREDIENTS

Contains the following species of beneficial microorganisms

<i>Bacillus subtilis</i>	187,300,000 CFU/g
<i>Bacillus licheniformis</i>	187,300,000 CFU/g
<i>Paenibacillus polymyxa</i>	187,300,000 CFU/g
<i>Bacillus pumulis</i>	187,300,000 CFU/g
<i>Paenibacillus durum</i>	162,300,000 CFU/g
<i>Bacillus amyloliquefaciens</i>	162,300,000 CFU/g
<i>Streptomyces lydicus</i>	8,300,000 CFU/g
<i>Streptomyces greiseus</i>	8,300,000 CFU/g
<i>Trichoderma harzianum</i>	7,900,000 CFU/g
<i>Trichoderma viride</i>	1,700,000CFU/g

Directions for use:

Use as Microbial Inoculant for Soils

Seed/Sod Installation: Apply 1-2 oz per 2500 square feet

Prep for Gardens and Flower Beds: Apply 0.5-1 oz per

very poor soil, double the rate Potting Mix: Mix into pot

pound per cubic yard. Fertilizer Mix: Add 5 pounds per

Top Dressing: Apply one 1-2oz per 2500 square feet.

Reservoir: 1-2 grams per 10 gallons of reservoir.

Field Application: Apply 2lbs per acre.

Mix concentrated product with water, mixing thorough

Product is suitable for most irrigation systems.

Caution: Store product in cool dry conditions. Do

not expose to direct sunlight for extended periods of time. Always

use proper care when handling.

Statement of Claims: CoastBio certifies that the pr

micro-organism listed on the label at the CFU cou

organism(s) are sensitive to environmental condit

unable to make any guarantees beyond the above

Objective #1: Fungicides & biologicals on pruning wounds



- Prune orchard: Lindauer River Ranch, Red Bluff.
- Activity: Hand pruning (morning) and fungicide application (afternoon) on June 2, 2023.
- A total of 51 trees were used (from north to south).
- Pruning on the shoots of two sides of each tree. 10 pruning wounds per tree were generated.
- Flagging: For each tree, 5 colors of tapes were used, representing 5 fungicide treatments.



A sponge brush was used to paint the fungicide/bio-fungicide on the pruning wound.

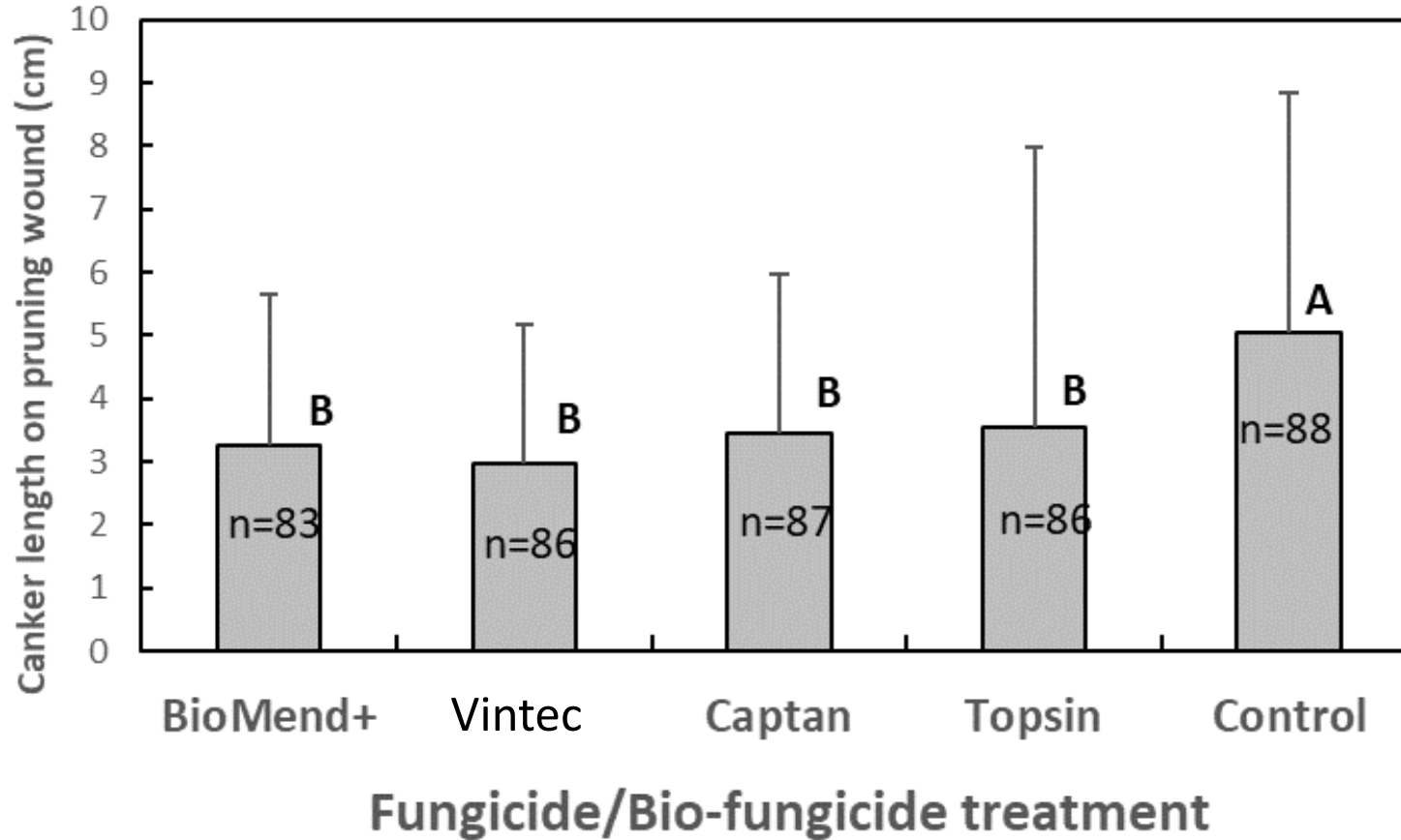
Fungicide/bio-fungicide Treatments

- 1) Topsin-M: $0.36 \text{ g} / 200 \text{ ml} = 1.51 \text{ lbs} / \text{acre}$
- 2) Captan: $0.88 \text{ g} / 200 \text{ ml} = 3.75 \text{ lbs} / \text{acre}$
- 3) Vintec (Trichoderma spp.):
 $0.2 \text{ g} / 200 \text{ ml} = 0.83 \text{ lbs} / \text{acre}$
- 4) Bio-Mend+ (multiple biocontrol agents): $0.24 \text{ g} / 200 \text{ ml} = 1 \text{ lb} / \text{acre}$.
- 5) Untreated control (UTC).



Measurement of canker length

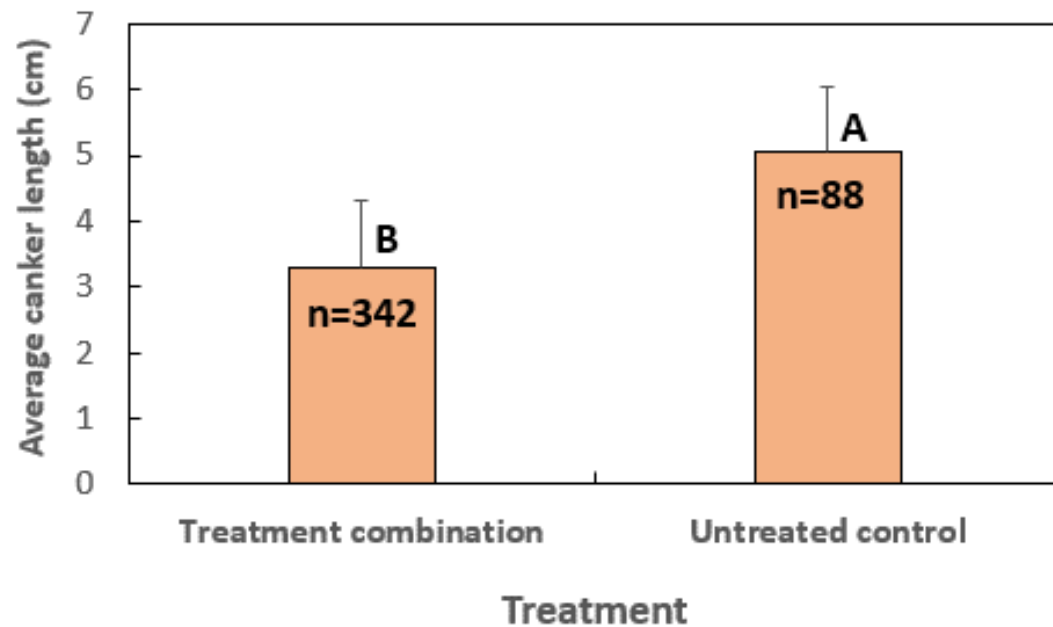
Effect of treating pruning wounds with fungicides/biofungicides on the canker development in pruning wounds in a commercial orchard



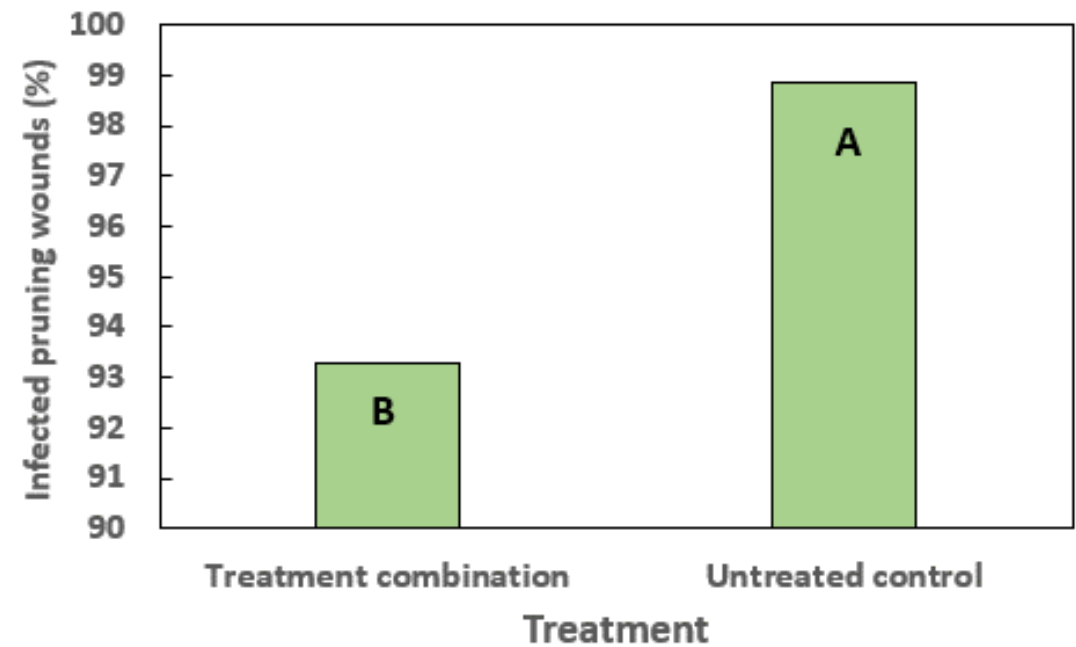
Treatment	N	Mean	S.D.
BioMend+	83	3.25	2.42
Vintec	86	2.97	2.21
Captan	87	3.46	2.50
Topsin	86	3.53	4.44
Control	88	5.04	3.78

- The fungicide/biofungicide treatments significantly reduced the canker length.
- No significant difference among the four fungicide/bio-fungicide treatments.

Effect of fungicide/biofungicides on canker length



Effect of fungicide/biofungicides on incidence of infection (%)

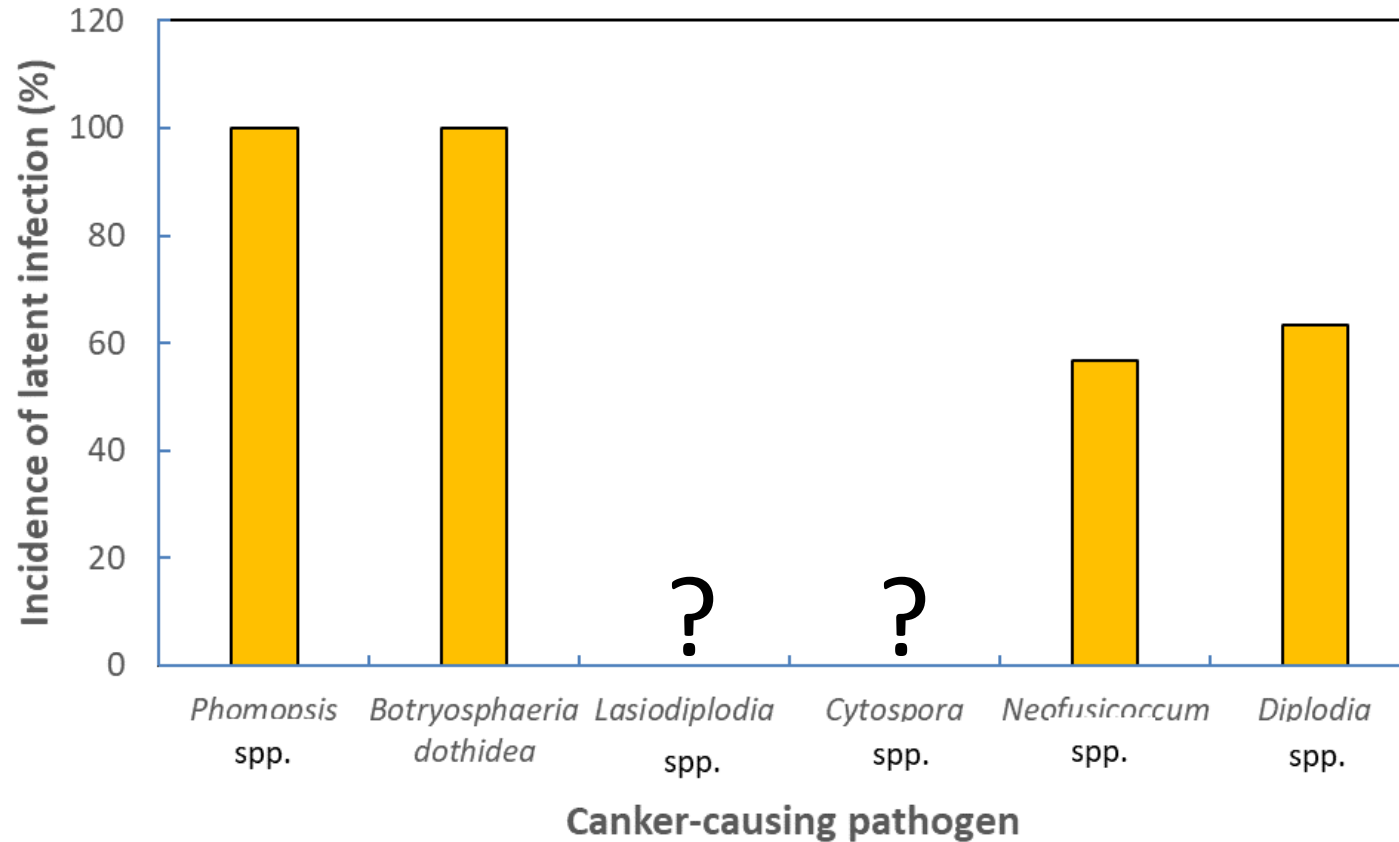


Objective #2: Recovery of canker pathogens



- Samples of pruning wounds from Objective #1 were used.
- For each wound, pieces of tissues from symptom margins were cut.
- Both culturing and real-time PCR (qPCR) methods were applied to identify the canker-causing pathogen species.

The incidence of latent infection caused by different canker-causing pathogen taxa determined by using real-time PCR (qPCR)



- Latent infections by *Phomopsis* spp. and *Botryosphaeria dothidea* were 100%.
- Latent infections by *Neofusicoccum* spp. and *Diplodia* spp. were 57 and 63%, respectively.
- No latent infections by *Lasiodiplodia* spp. and *Cytospora* spp.
- However, no canker-causing pathogens were isolated with culturing isolation method.***

Canker Pathogens isolated from hedging wounds in pruning/hedging trial
(sample #21025 - c/o Luke Milliron)- 11 June 2021

Species isolated	Incidence (%)***
<i>Neoscytalidium dimitiatum</i>	50%
<i>Cytospora leucostoma</i>	10%
<i>Botryosphaeria</i> spp.	40%
<i>Paecilomyces variotii</i>	60%

*** >100% because some of the fungi coexist in the same canker

Canker pathogens isolated from samples with branch cankers
from other orchards (c/o Jaime Ott) - 2023

Sample	Canker fungus	Incidence (%)
#23039 Branch 1	<i>Neoscytalidium dimitiatum</i>	30%
Branch 2	<i>Schizophyllum commune</i>	60%
Branch 3	<i>Paecilomyces variotii</i>	10%
#23070 Branch 1	<i>Cytospora leucostoma</i>	100%
Branch 2	<i>Phellinus</i> sp.	100%
#23125 Branch 1	Wood decay (<i>Schizophyllum</i> sp.)	30%
Branch 2	<i>Neoscytalidium dimitiatum</i>	60%
Branch 3	<i>Paecilomyces variotii</i>	25%

Sample dates: #23039 (April 2023); #23070 (June 2023); & #23125 (November 2023)

Almond pruning wound protection trial (c/o Dr. Florent Troullias)

Products	<i>Cytospora sp.</i>	<i>Eutypa lata</i>	<i>C. fimbriata</i>	<i>B. dothidea</i>	<i>N. parvum</i>	<i>N. mediterraneum</i>	<i>Neosc. dimidiatum</i> ***	Avg. recovery
Control	25	75	50	50	100	50	50	57.1
Luna Experience	75	25	25	25	0	25	25	28.6
Merivon	50	25	25	0	25	50	50	32.1
Topsin M	0	0	0	0	0	0	0	0
Quash	25	50	0	0	25	50	50	28.6
Inspire Super	25	75	0	0	0	25	25	21.4
Quadris Top	100	0	0	0	0	0	100	28.6
Rally	50	25	0	0	25	0	50	21.4
thyme oil #1	100	100	0	75	50	75	50	64.2
thyme oil #2	75	25	0	50	100	75	100	60.7
neem oil	100	100	0	100	100	100	100	85.7
Avg. recovery	56.8	45.4	9.1	27.3	38.6	40.9	54.5	

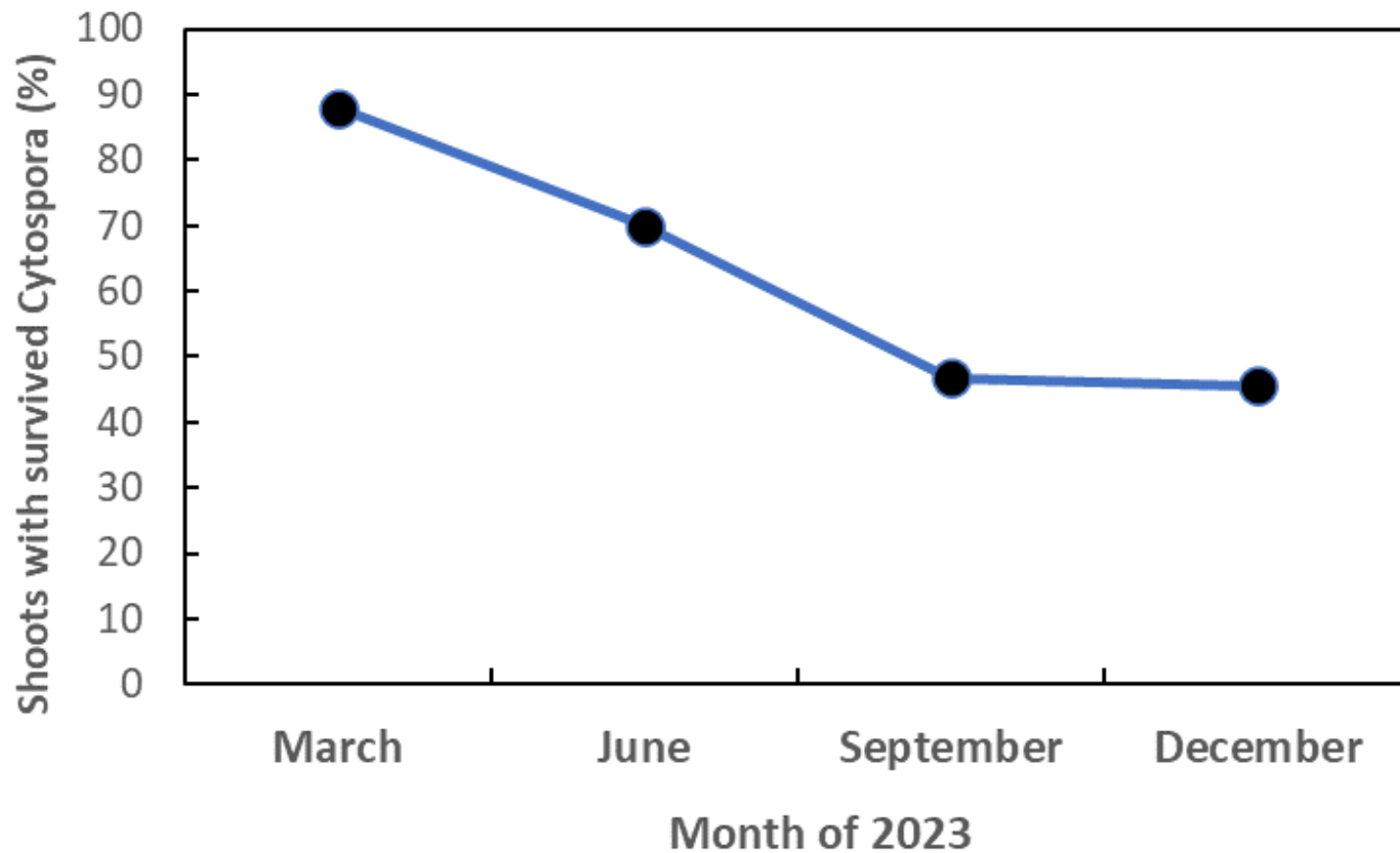
Objective #3: Survival of *Cytospora* in shredded shoots



Shoots were half-buried under prune trees in the Kearney experimental orchard (March 2023).



About 20 – 30 shoots were randomly collected in March, June, September, and December.



- **The percentage of shoots with survived Cytospora decreased from 88% in March to 70% in June, and continuously decreased to about 45% in December (a 9-month period).**
- **An investigation of the overwintering survival is needed.**

2024 proposal

Two objectives:

1. Expand the study on the effect of Topsin treatment on hedging wounds to reduce canker development on a commercial scale. Also study effect of Topsin against *Neoscytalidium dimitiatum* and *Botryosphaeria* fungi in prunes.
2. Continue to study the *Cytospora* survival (overwintering) on shredded shoots over the two seasons (winter 2023/2024, during 2024, and winter 2024/2025).

Objective #1

- A larger plot (200 – 300 trees) in the prune orchard in Red Bluff will be used in 2024.
 - Topsin-M spray will be sprayed commercially after hedging.
 - 50 trees with no spray will be used as untreated control.
 - A large number of shoots with hedging cuts (wounds) from treated and non treated trees will be randomly sampled in October-December 2024 (5 shoots/tree).
-
- Experiments of Topsin effects on controlling *Neoscytalidium* and *Botryopshaeria fungi* will be done after treatment and inoculation in our experimental trees at Kearney.
 - The effect of the Topsin treatment will be compared in sprayed vs. no sprayed pruning wounds.

Objective #2

- 20 shredded shoots (half-buried at KARE) used in 2023 experiment will be sampled periodically in March, June, September and December 2024.
- 8-10 pieces/shoot will be randomly cut from each shoot sample and cultured on LA plates (pieces of each shoot/plate).
- Plates with at least one *Cytospora* colony will be considered as “shoots with survived *Cytospora*” (= % of shoots with survived *Cytospora*).
- A survival curve of *Cytospora* over two seasons will be obtained.



Thank you

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California Prune Board

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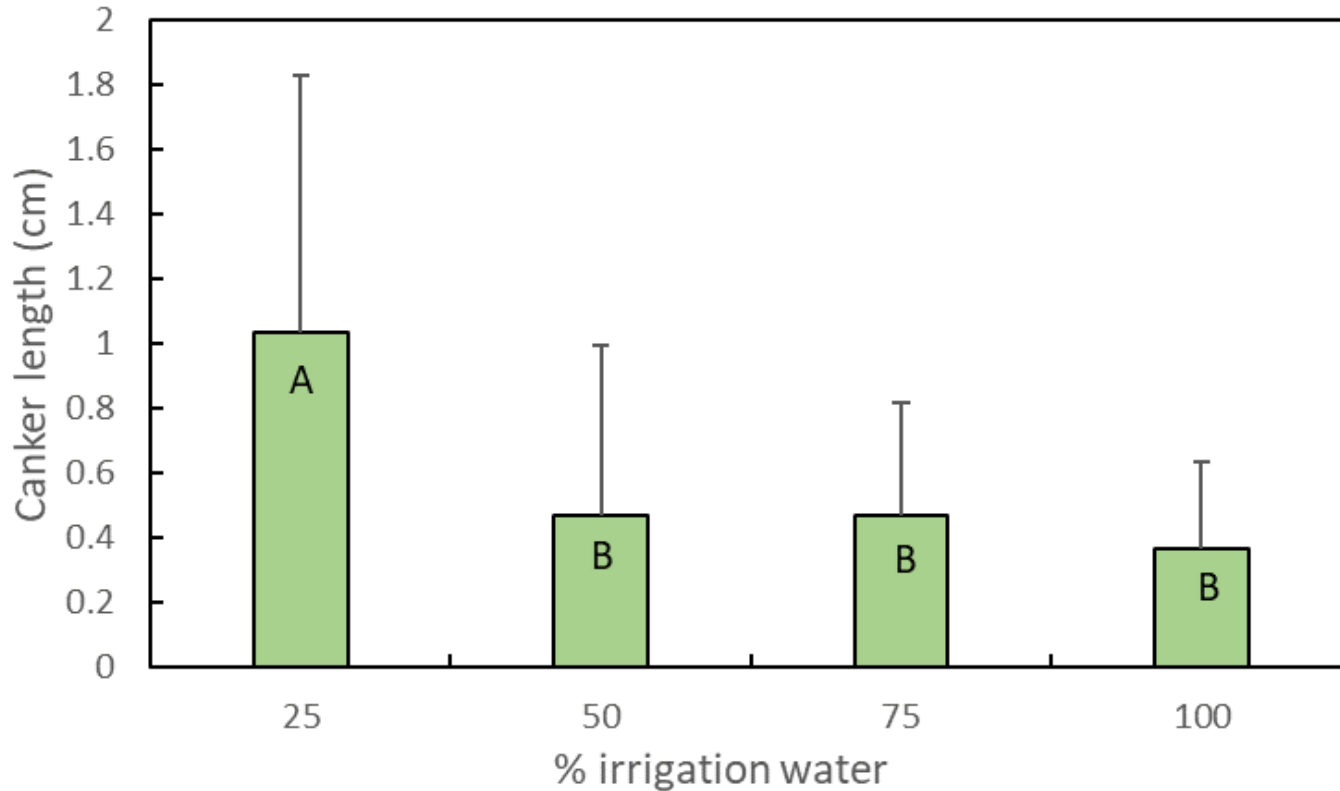
Twitter:

<https://twitter.com/PistachioDoctor>

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- When irrigation was reduced to 25% of the needed water supply, the canker length was significantly longer than those of 50%, 75%, and 100% irrigation treatments.
- There were no significant differences in canker length among the 50%, 75%, and 100% of irrigation treatments.

- Therefore, severe water stress predisposes the trees to develop more severe canker disease.

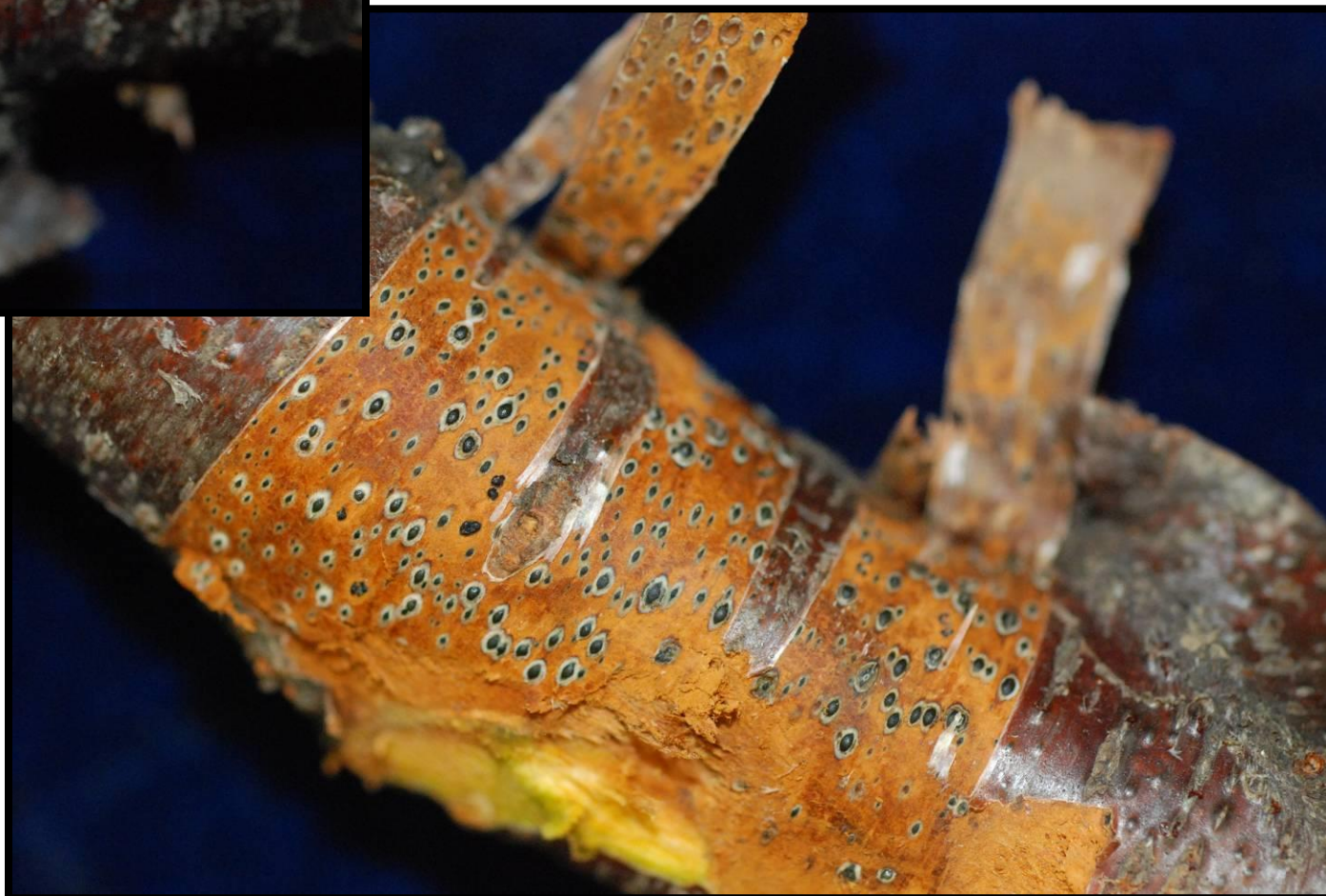
Oil damage → **Cytospora cankers**



Cytospora Canker

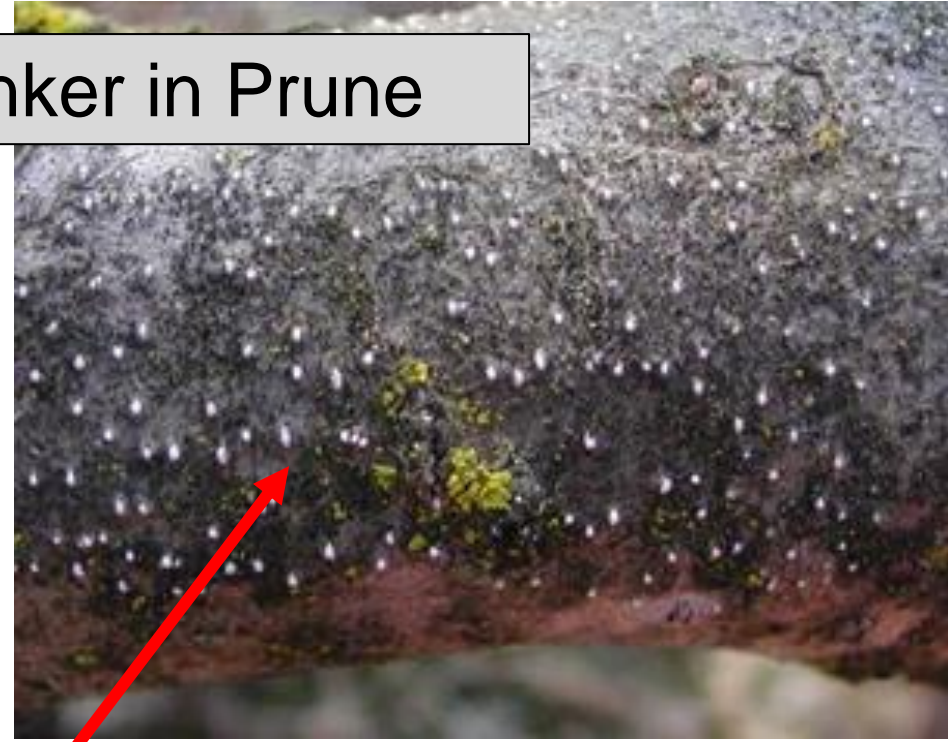


Cytospora leucostoma





Cytospora Canker in Prune



Pycnidia of *Cytospora*