Cytospora Canker of Dried Plum

Latent (endophytic) infection and prospects for management

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Outline of Cytospora diseases:

- ✓ Surveys (importance)
- ✓ Symptoms (diagnosis)
- ✓ Pathogen(s)
- ✓ Disease management (general, common sense practices)

Unknown:

- ✓ **Methods of detection before symptoms.**
- **Pathogen built up, infection, & epidemiology (factors affecting how and where the disease develops)**
- **New approaches for disease management (keep the trees healthy from young age, etc …)**

Cytospora Canker (general info)

- Caused by the fungus *Cytospora leucostoma* (more species ???)
- Usually visible as dark depressed areas in the bark (too late for control, except to prune it).
- Look for small, grey-white pimple-like spore bodies protruding through the outer bark of the canker (too late to do anything, except to prune it).
- Factors that favor Cytospora are water stress, potassium deficiency, heavy clay soils, ring nematode and sunburn (pay close attention to these).
- Most infection occurs in sunburn injuries or other injuries (including tissues killed by bacterial canker) from rain-splashed spores (inoculum could come immediately after the injury? Or, is it present in the tissues as tissues are injured?)

Surveys: Fungi isolated from cankers of dried Plum

2012

- ***Cytospora leucostoma******
- *Lasiodiplodia theobromae***
- *Nattrassia mangiferae****
- *Diplodia seriatta***
- *Phomopsis* species*
- *Paecilomyces variotii** ?*
- *Fusarium* species*

2013

- ***Cytospora leucostoma******
- *Lasiodiplodia theobromae***
- *Diplodia seriatta**
- *Paecilomyces variotii **?*
- *Fusarium* species*
- *Chondrostereum purpurescens**

2014

- ***Cytospora leucostoma******
- *Lasiodiplodia theobromae***
- *Botryosphaeria dothidea*
- *Diplodia seriatta***
- *Nattrassia mangifera*,*
- *Phomopsis* species*
- *Paecilomyces variotii **?*
- *Fusarium* species*

2014

- **Cytospora canker*****
- *Phellinus* species**
- *Schizophyllum commune*

2015

- Bacterial canker***
- **Cytospora canker*****
- Botryosphaeria canker*

2016

- Bacterial canker***
- **Cytospora canker*****
- Botryosphaeria canker*

Canker-pathogen fungi isolated from dried plums

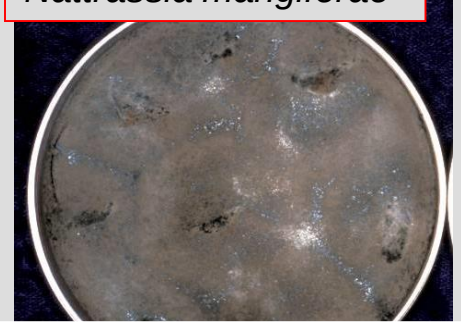
Cytospora leucostoma

Botryosphaeria dothidea
Neofusicoccum mediterraneum

Lasiodiplodia citricola

Fusarium sp.

Nattrassia mangiferae

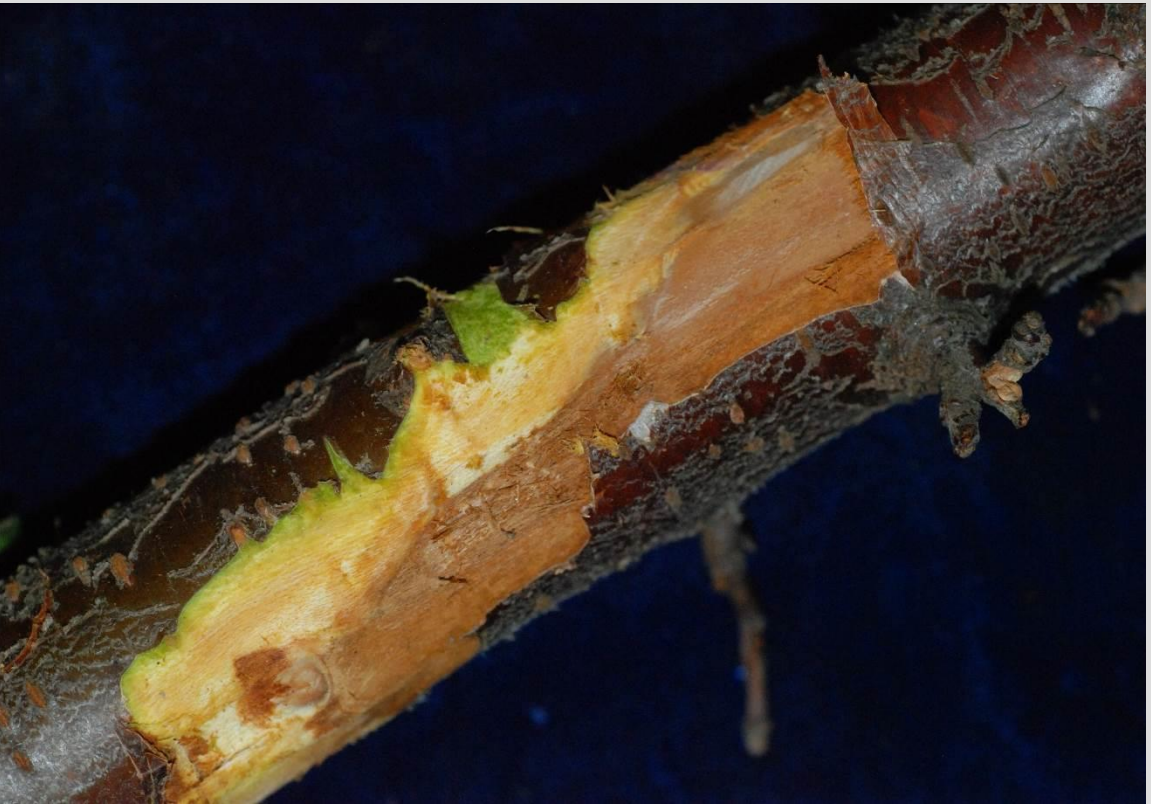


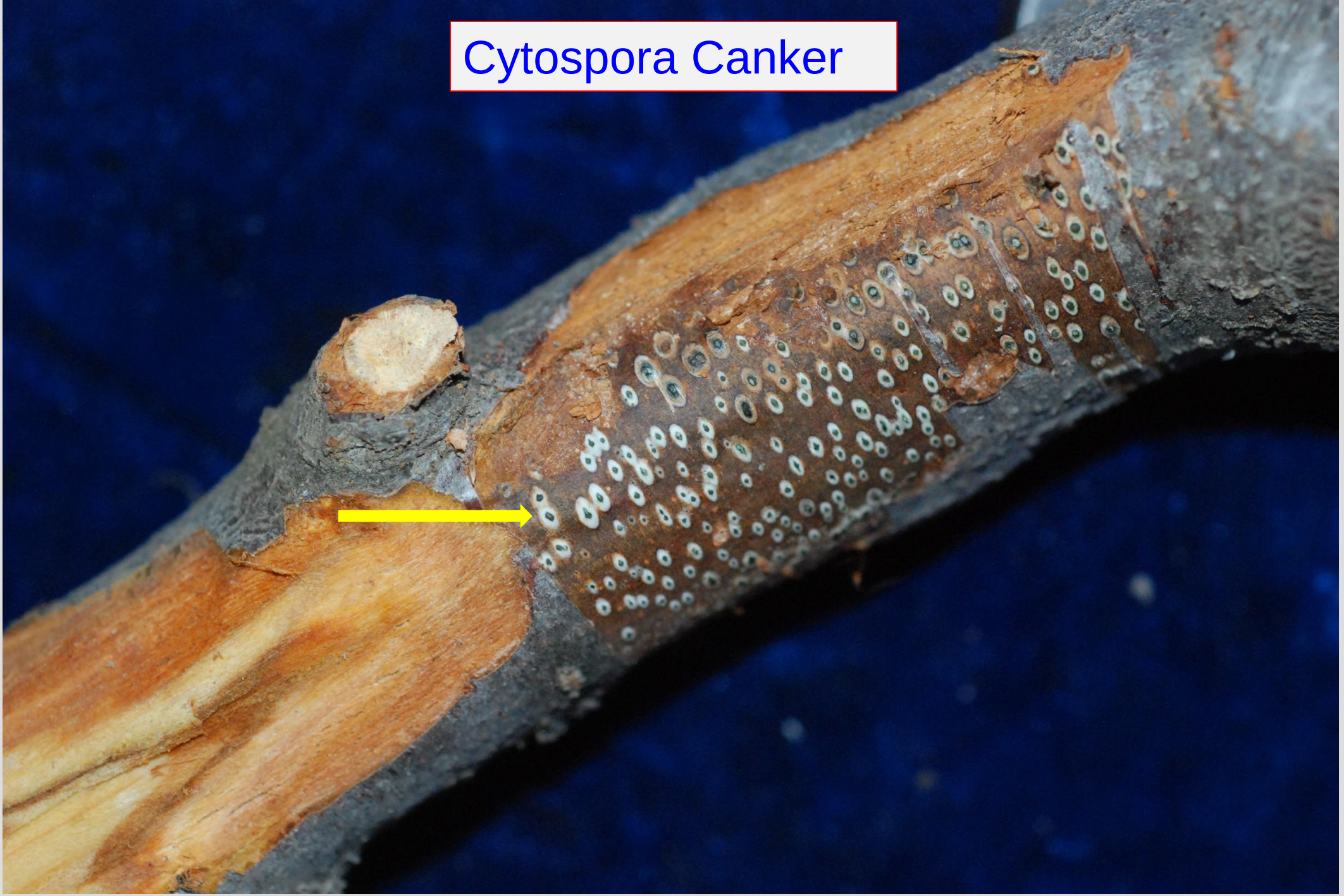
Phomopsis sp.

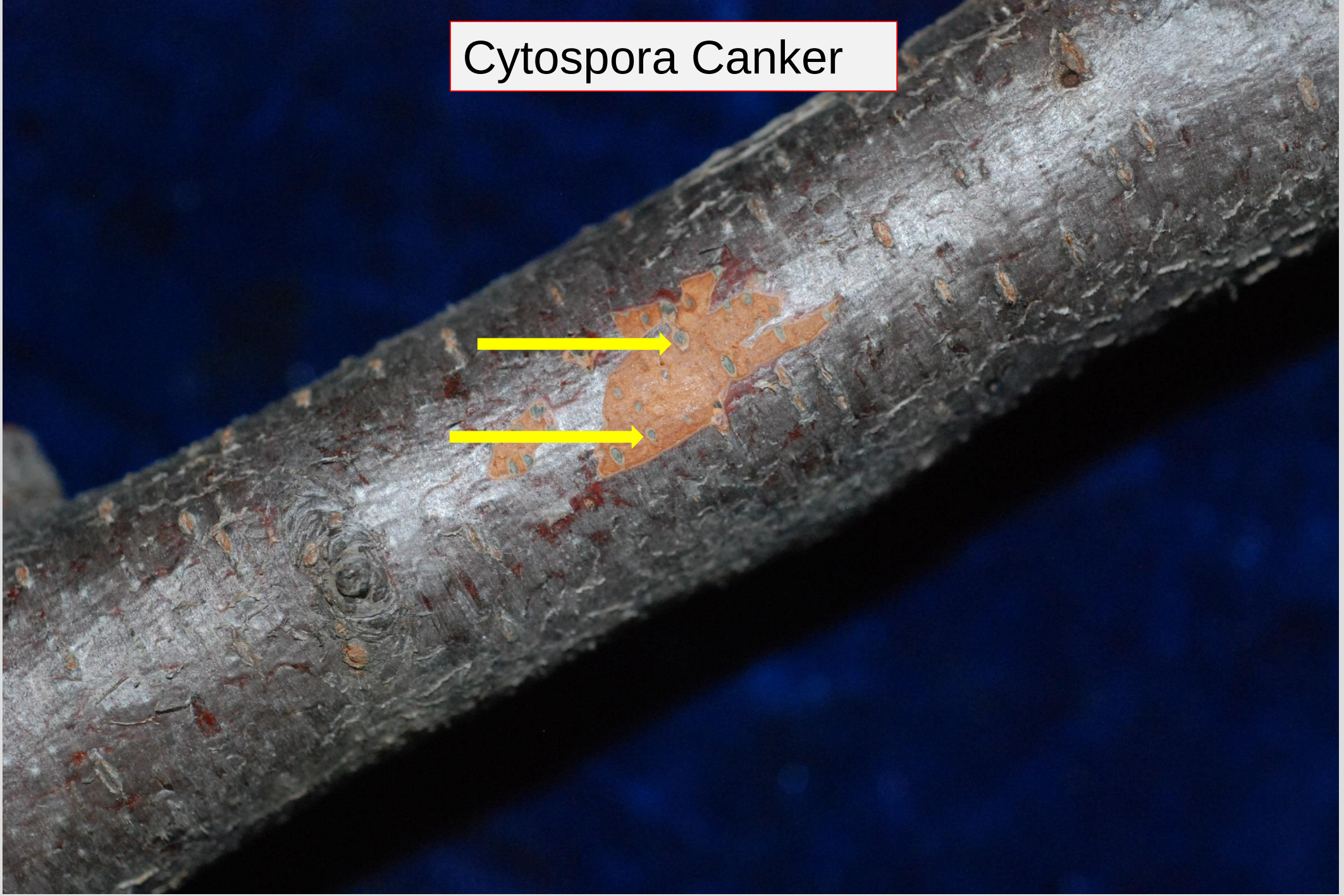
Diplodia seriata

Phoma species

Cytospora in dried plum

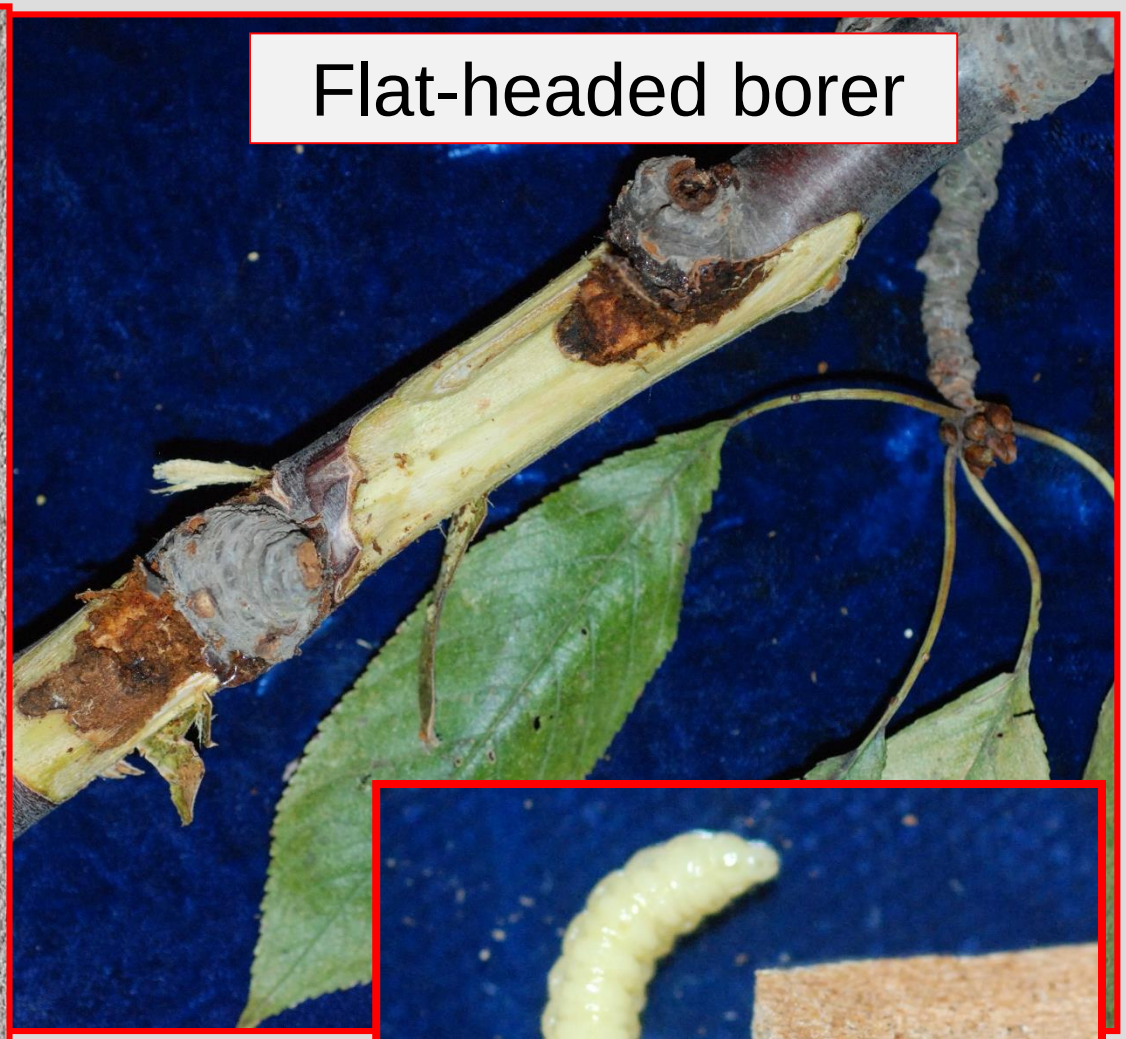



Cytospora Canker


Cytospora Canker

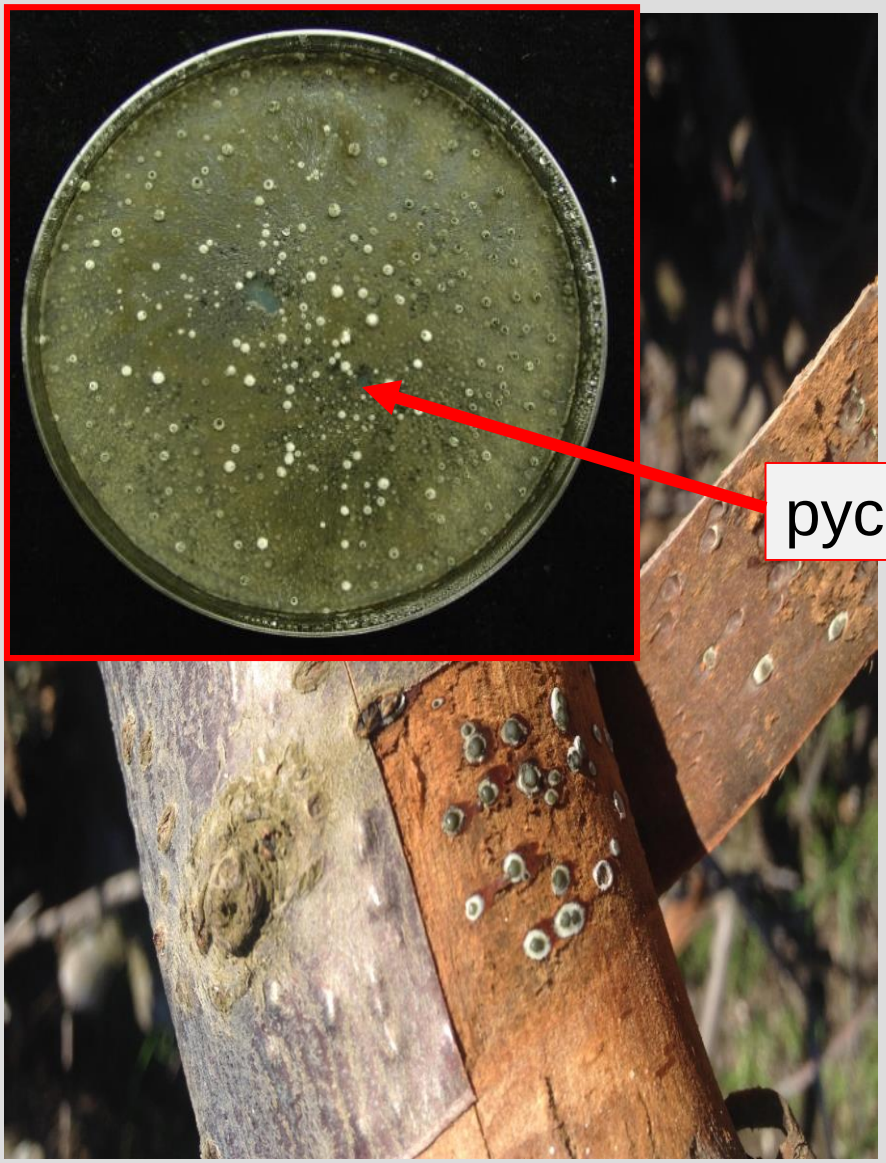
Cytospora canker

Flat-headed borer

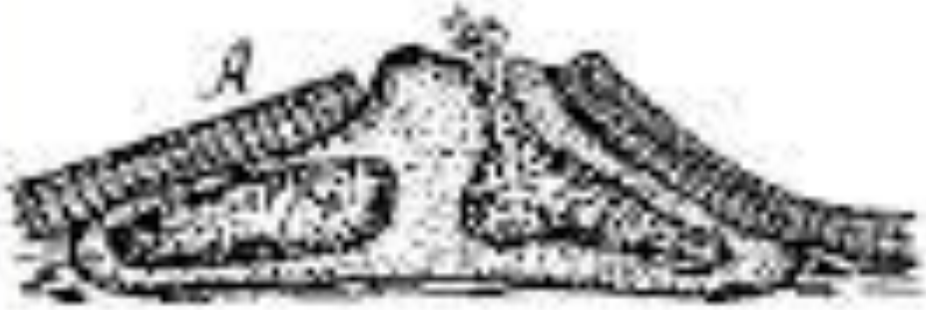


Cytospora Canker

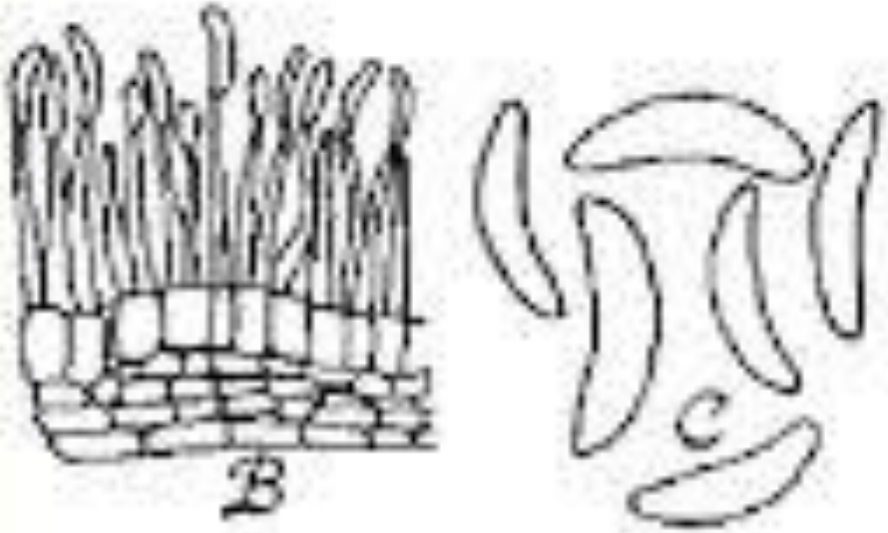
Inoculum sources for Cytospora canker:



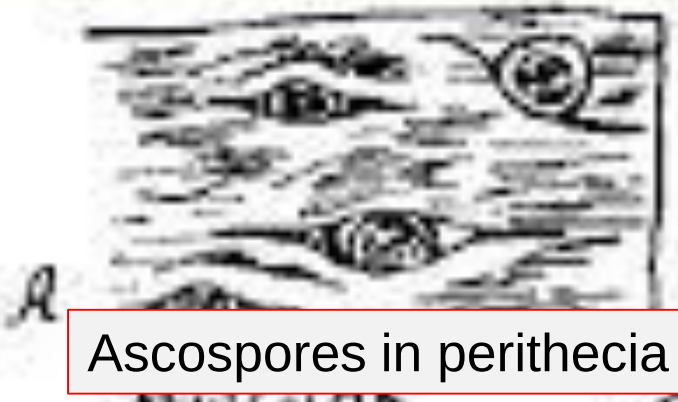
pycnidia



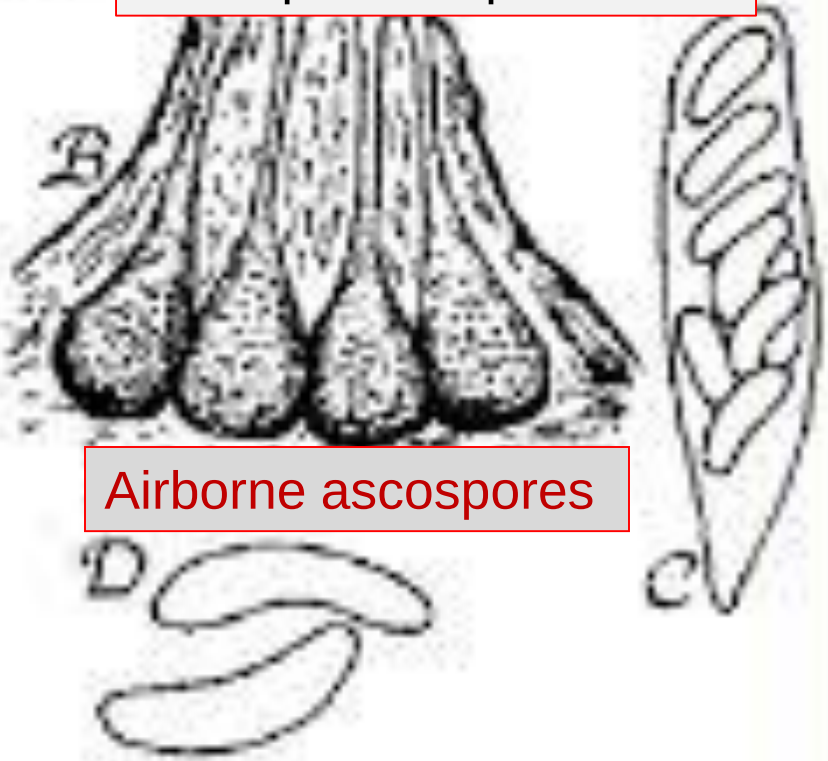
Spores in pycnidia



Water-splashed spores



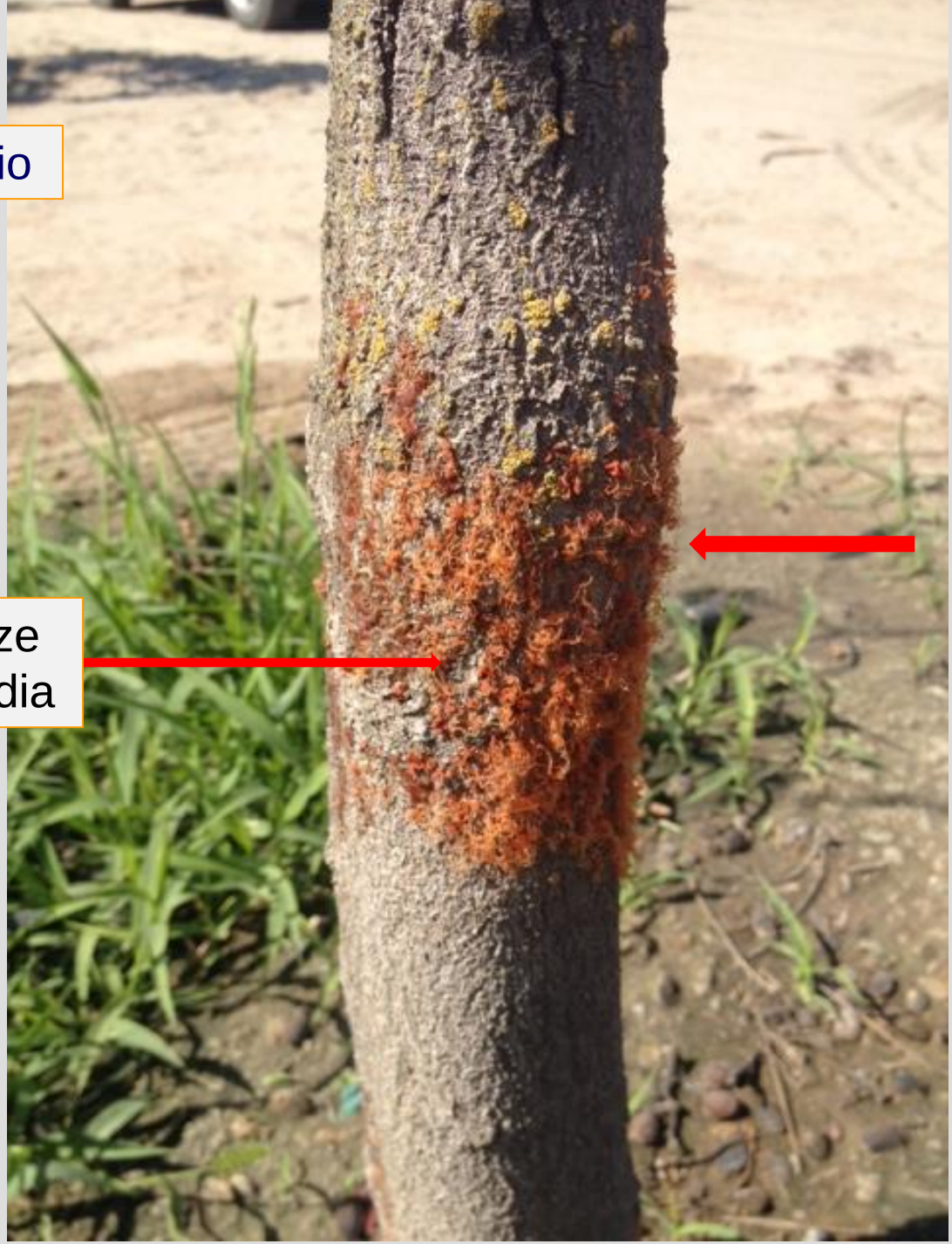
Ascospores in perithecia



Airborne ascospores

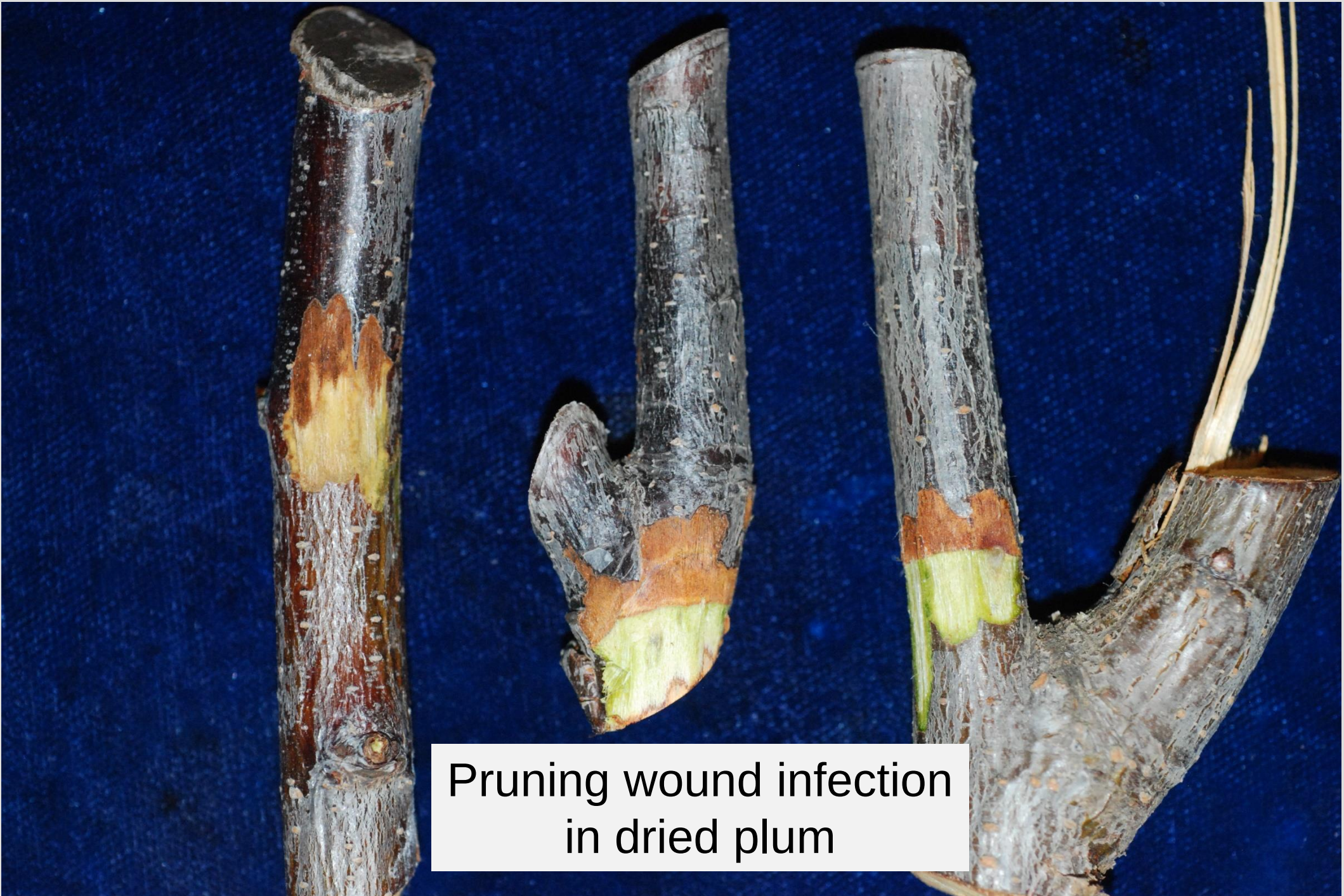

Spores ooze
from pycnidia

Cytospora chrysosperma on pistachio

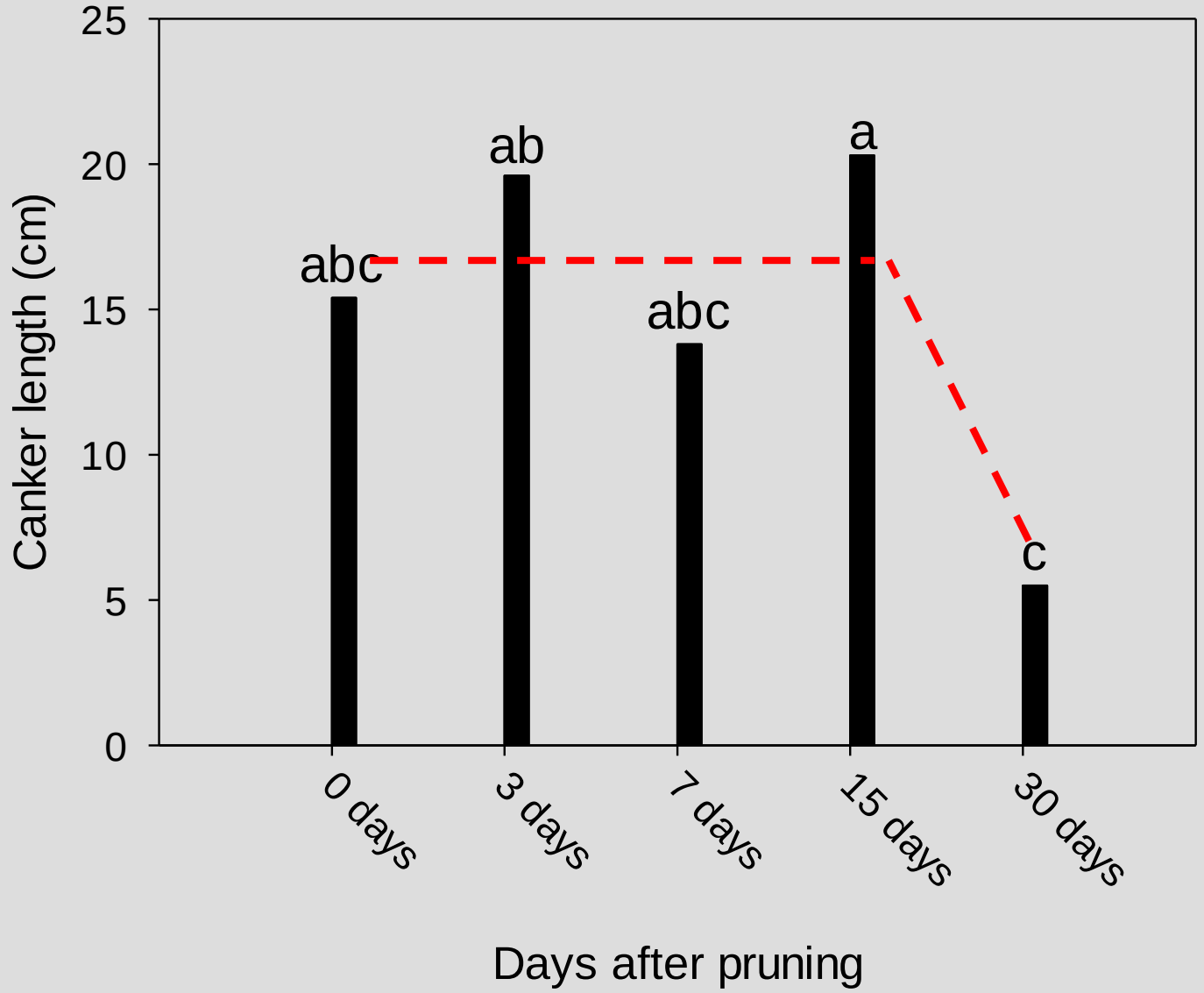


Killed peach trees because of *Cytospora leucostoma* in Colorado (Grand Junction area)

Cytospora canker symptoms on peach


Pruning wound infection
in dried plum

Susceptibility of pruning wounds to *Cytospora leucostoma* (2014/2015)



Cytospora Canker Management

- The pathogen is considered a weak “parasite”: it requires a wound as a mode of entry (*pruning cuts, sunburn, bark cracks, insect wounds*)
- Trees decrease production in each growing season from time of infection to eventual death of shoots and scaffolds.

- Genetic resistance: None; or, unknown.

Control Measures for Cytospora canker

- ✓ Maintain healthy tree vigor.
- ✓ Prune out and destroy dead or diseased twigs and branches.
- ✓ Prevent sunscald and freeze damage - paint (latex).
- ✓ Control borers and other wood-attacking insects.
- ✓ Avoid water stress → some defoliation → sunburn.
- ✓ Avoid potassium deficiency → defoliation → sunburn.
- ✓ Avoid mechanical injury to tree – especially main scaffolds and/or the trunks of trees.
- ✓ Woodpiles are an important source of inoculum – burn or remove them.
- ✓ The use of copper hydroxide as a dormant application will help prevent infection of pruning cuts and/or wounds.

Why is it any damaged tissue is easily infected?

Oil-damaged shoots were covered by Cytospora in a short time
(June 3, 2016)


Water stressed? Potassium deficiency?

Establishment of *q*PCR system to quantify latent infection level and determine “endophytic” phases

Six canker-causing pathogen groups were considered:

- ✓ *Cytospora* spp.
- ✓ *Botryosphaeria dothidea*
- ✓ *Lasiodiplodia* spp.
- ✓ *Neofusicoccum* spp.
- ✓ *Phomopsis* spp.
- ✓ *Diplodia* spp.

Definition of latent infection: a close parasitic relationship of the pathogen and the plant, which initially shows no symptoms: eventually induces macroscopic symptoms.

PhBT-R1	ACGAGATTTGAAGACAGGGAATAG			
BdF	CAGCGTGGGAGAACATCAA /	**Botryospaeria dothidea**	103	81.5
BdR	GTGAGAGAGTACCTCGTTGAAATAG			
LcBT-F2	CTGCTTTCTGGTTTGTTGCC /	**Lasiodiplodia spp.**	128	86
LcBT-R2	GAGAAGGCGCACACTTACA			
CtBTFF1	GAGCGCATGAACGTCTACTT /	**Cytospora spp.**	106	82.6
CtBtFR1	GGAAGAAAGCGCGTCAGTAA			
NpBT-F2	ACCACAGGCAGACCATTTC/	**Neofusicoccum spp.**	118	86.4
NpBT-R2	GTCGGAGGTGCCATTGTAG			
DpF	GTGTAAGTTTGCGCTGTCTTTG /	**Diplodia spp.**	118	84.8
DpR	GTAGAGAGTACCTCGTTGAAGTAGA			

How our system works

a) Sample collection and processing

b) Grinding and DNA extraction of samples

c) Real-time PCR assay

Sample	weight (g)	Dilution	Ct	calculation of fg		total fg	/weight	MS(a)	
PAN4-1	0.32	60	36.47	2.194821	156.6105	4698.316	14682.24		4.17
PAN4-2	0.34	60	36.62	2.150466	141.4054	4242.162	12476.95		4.10
PAN4-3	0.33	60	N/A	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	
PAN4-4	0.36	60	36.03	2.324929	211.3144	6339.431	17609.53		4.25
PAN4-5	0.29	60	36.62	2.150466	141.4054	4242.162	14628.15		4.17
PAN4-6	0.4	60	36.54	2.174122	149.3214	4479.641	11199.1		4.05
PAN4-7	0.32	60	35.65	2.437295	273.7127	8211.382	25660.57		4.41
PAN4-8	0.3	60	38.18	1.689174	48.88482	1466.545	4888.482		3.69
PAN4-9	0.34	60	38.29	1.656647	45.35728	1360.718	4002.113		3.60
PAN4-10	0.36	60	39.03	1.437829	27.40495	822.1485	2283.746		3.36
PAN4-11	0.27	60	37.79	1.804497	63.75247	1912.574	7083.608		3.85
PAN4-12	0.31	60	36.88	2.073584	118.4633	3553.9	11464.19		4.06
PAN4-13	0.35	60	37.21	1.976003	94.62437	2838.731	8110.66		3.91
PAN4-14	0.38	60	37.68	1.837024	68.71064	2061.319	5424.524		3.73
PAN4-15	0.42	60	36.78	2.103154	126.8101	3804.304	9057.868		3.96
PAN4-16	0.39	60	36.38	2.221434	166.5076	4995.227	12808.28		4.11
PAN4-17	0.28	60	36.17	2.283531	192.1016	5763.048	20582.32		4.31
PAN4-18	0.37	60	38.28	1.659604	45.66716	1370.015	3702.743		3.57

d) Data analysis

Quantification of latent infection/endophytic situation --- definitions

1. **Incidence of latent infection (I): Number of samples positive in pathogen DNA / total number of samples × 100.**

2. **Molecular Severity (MS): MS = $\log_{10}(P/H)$,**
   ***P* = the weight of the pathogen's DNA in femtograms (fg) from the standard curve.**
   ***H* = the shoot weight in grams (g); the range of MS value is 0 – 15.**

3. **Index of latent infection (ILI): Incidence (I) × MS / 100**

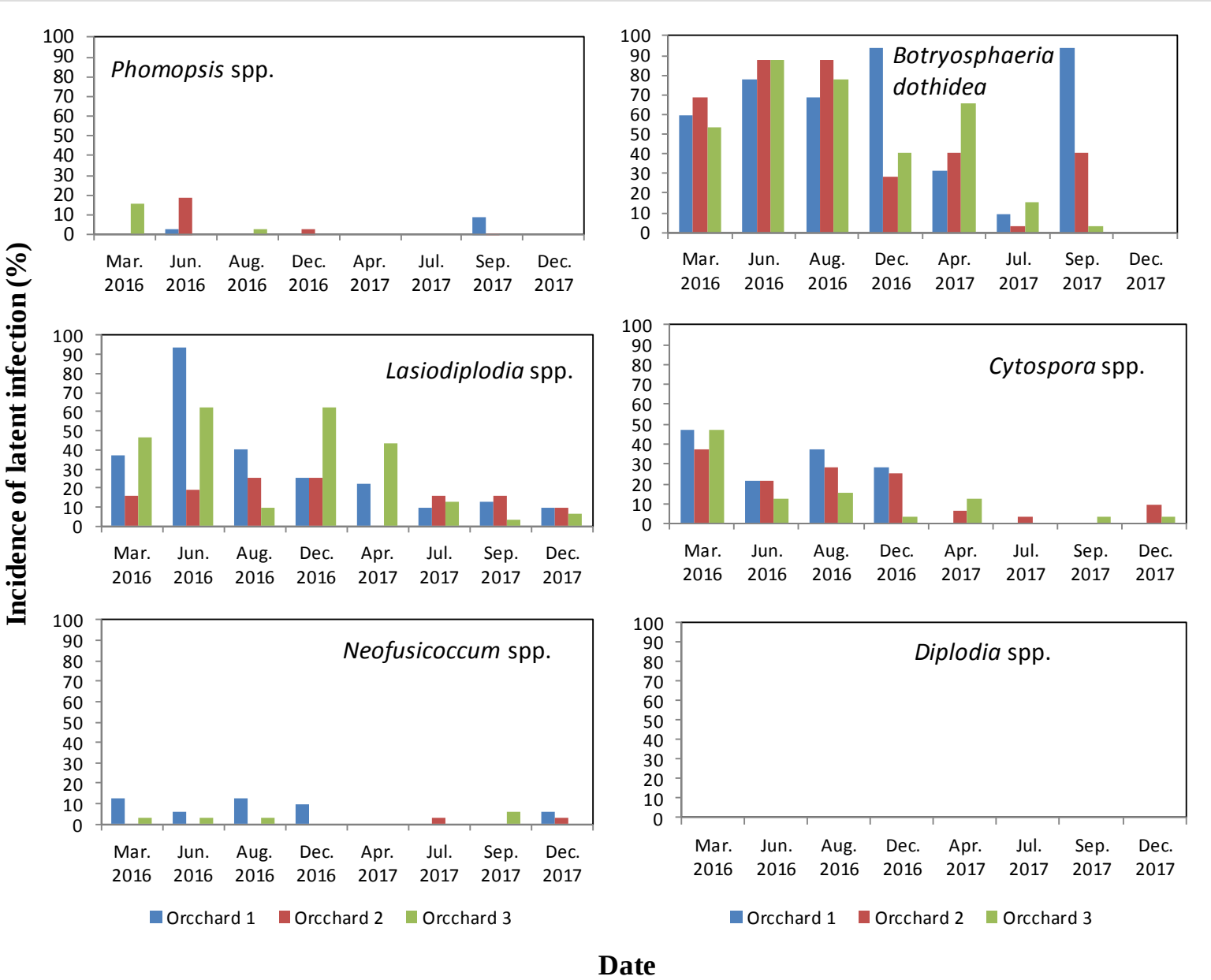
1 femtogram = 10^{-15} grams

Patterns of latent infection in newly-emerged and one-year-old shoots

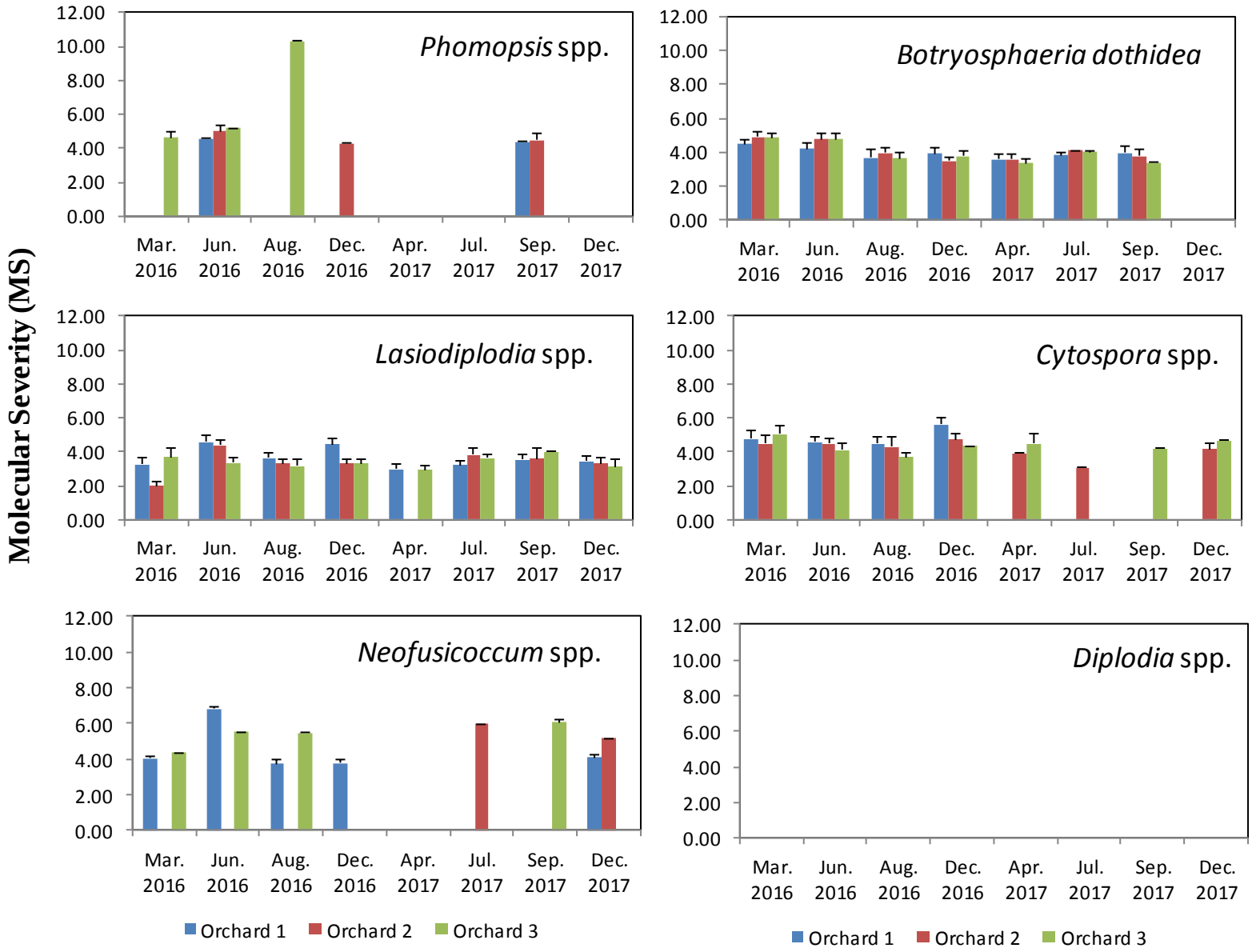
- Newly-emerged (current growth) and 1-year-old shoot samples were collected from 3 prune orchards every three months.

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

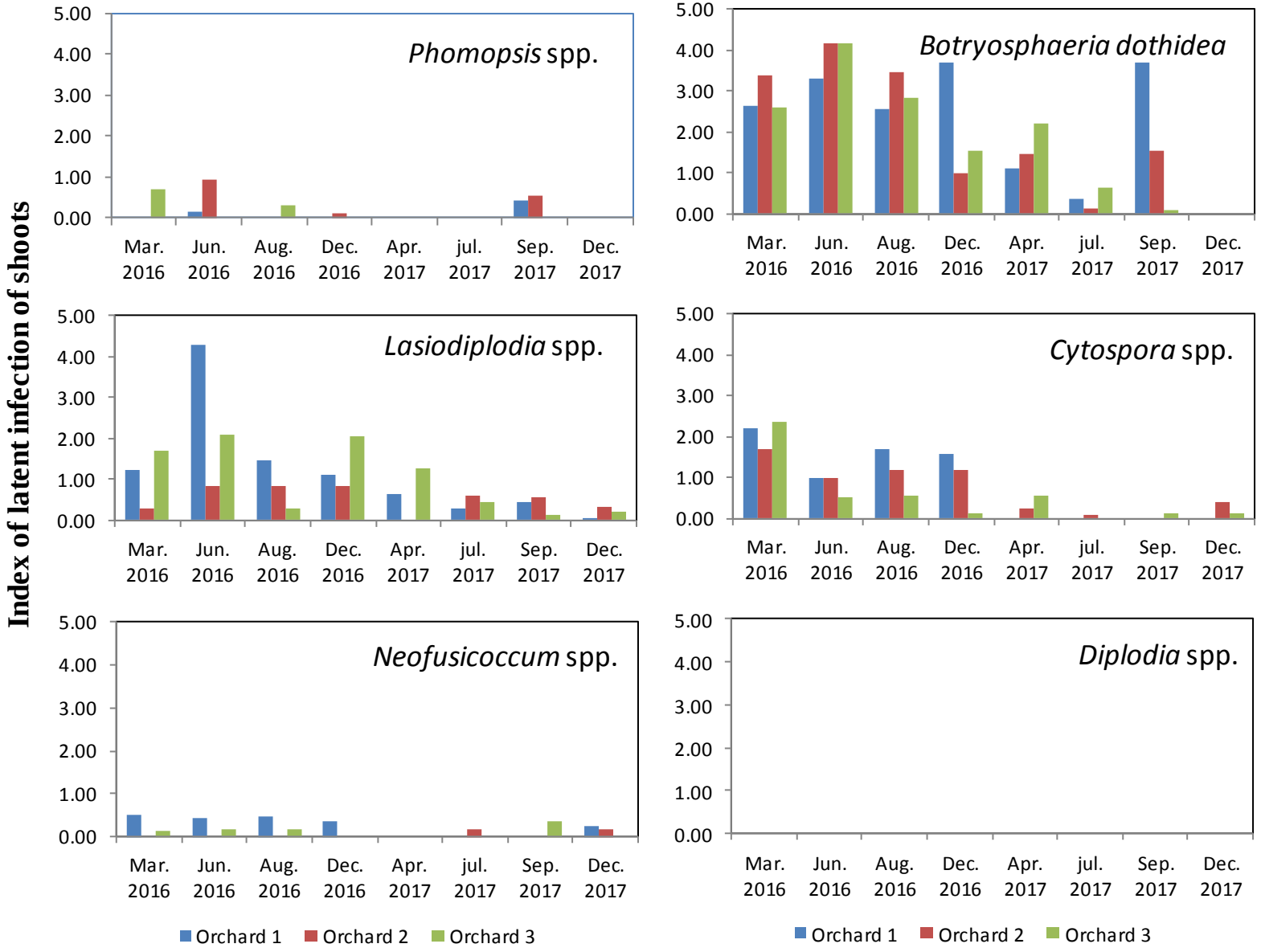
For newly-emerged shoots



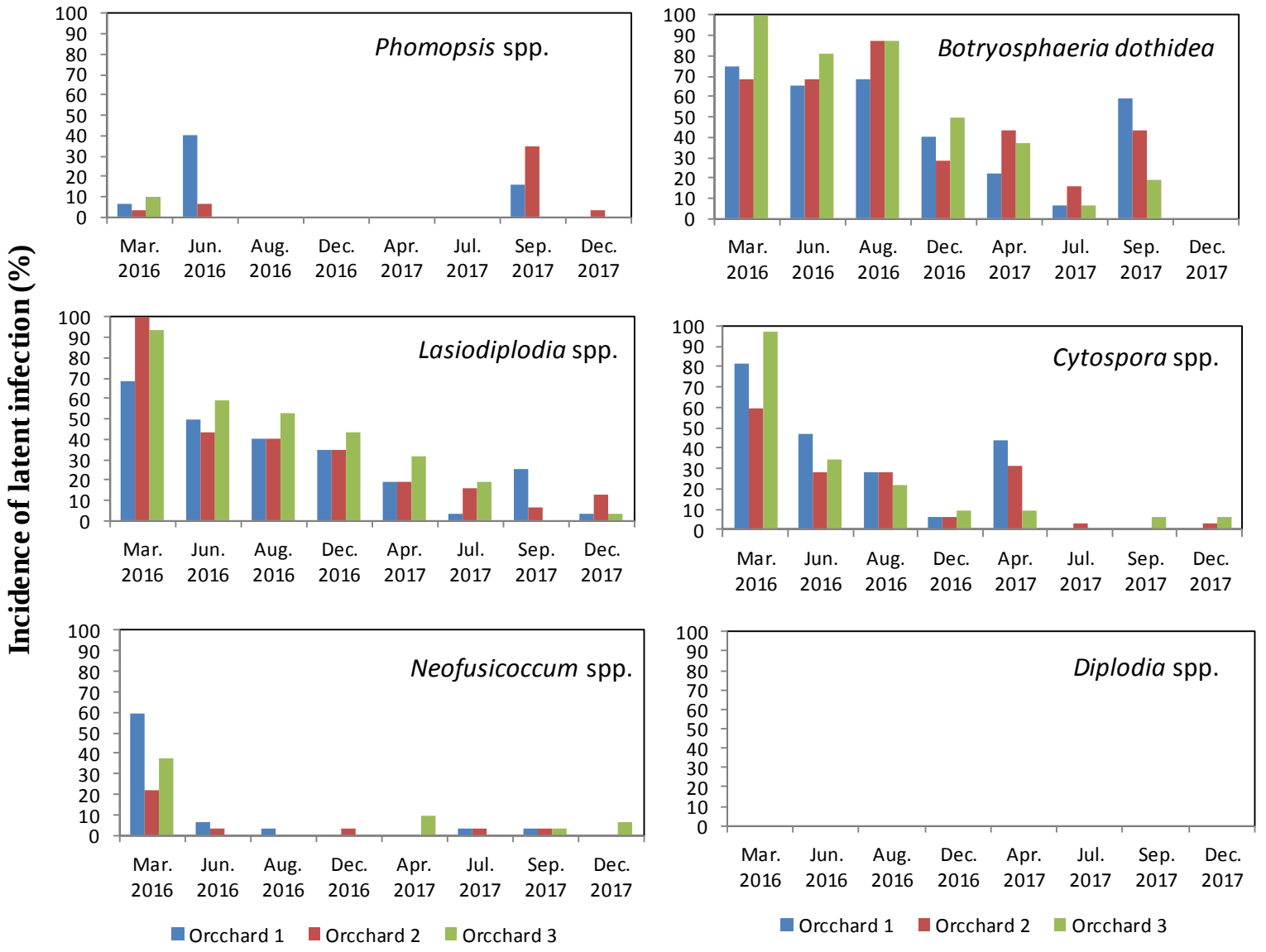
For newly-emerged shoots



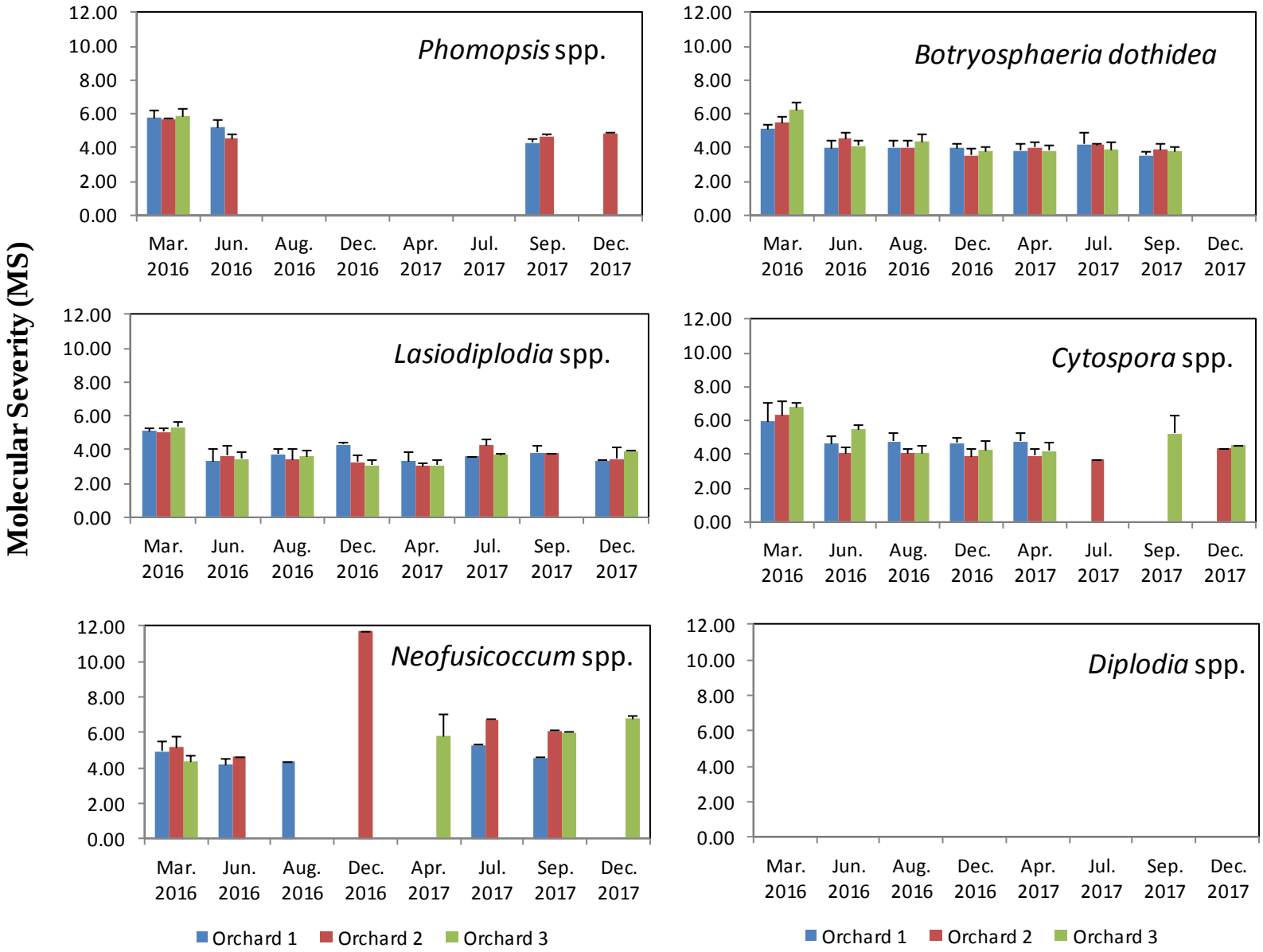
For newly-emerged shoots



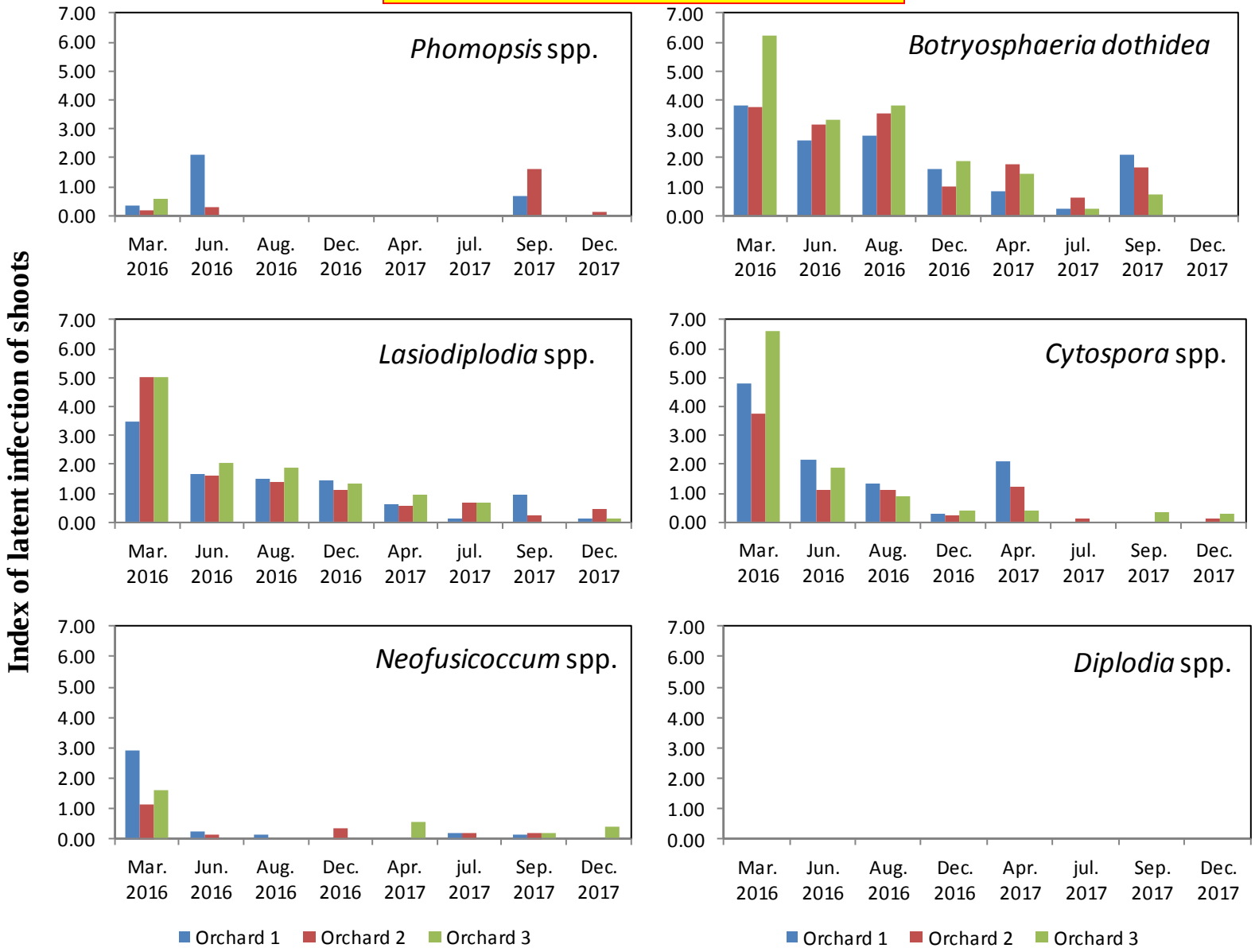
For one-year-old shoots



For one-year-old shoots



For one-year-old shoots



Conclusions: For shoots without any symptoms

- Five of the 6 canker-pathogen groups were detected in newly- emerged and 1-year-old shoots, suggesting that they can cause latent infection ("endophytic" phase).
- *Cytospora, Botryosphaeria dothidea*, and *Lasiodiplodia* species were the 3 predominant species causing latent infection.
- *Phomopsis & Neofusicoccum* species occurred infrequently in shoots.
- *Diplodia* species were not detected in any of the shoot samples.
- In general, incidences of latent infection and molecular severity were higher in the spring.

Band canker of almond

Year 2

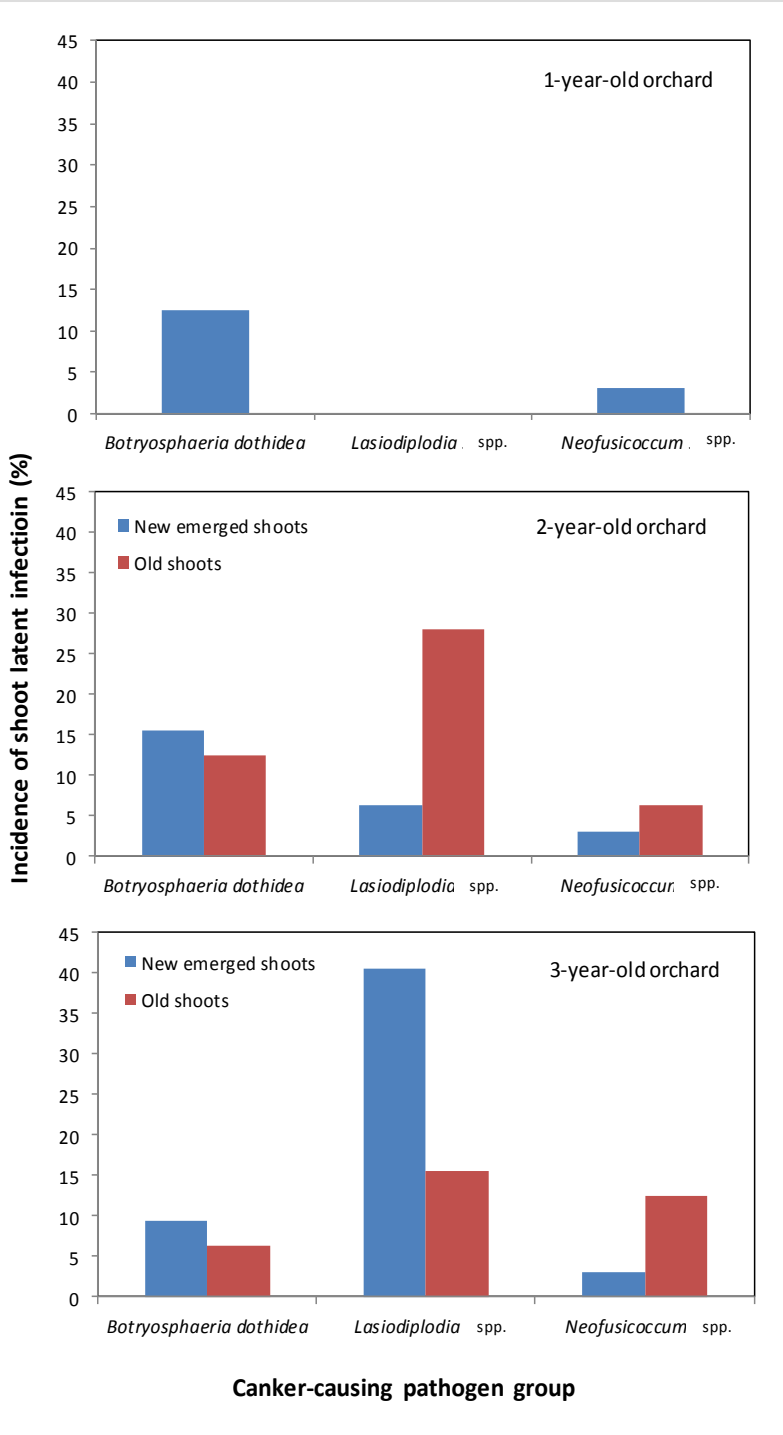
double band canker

Year 1

A young (3rd-leaf) almond orchard with uniformly – spread gaps due to Band canker

Similar situation in almond:

Latent infection in very young almond trees



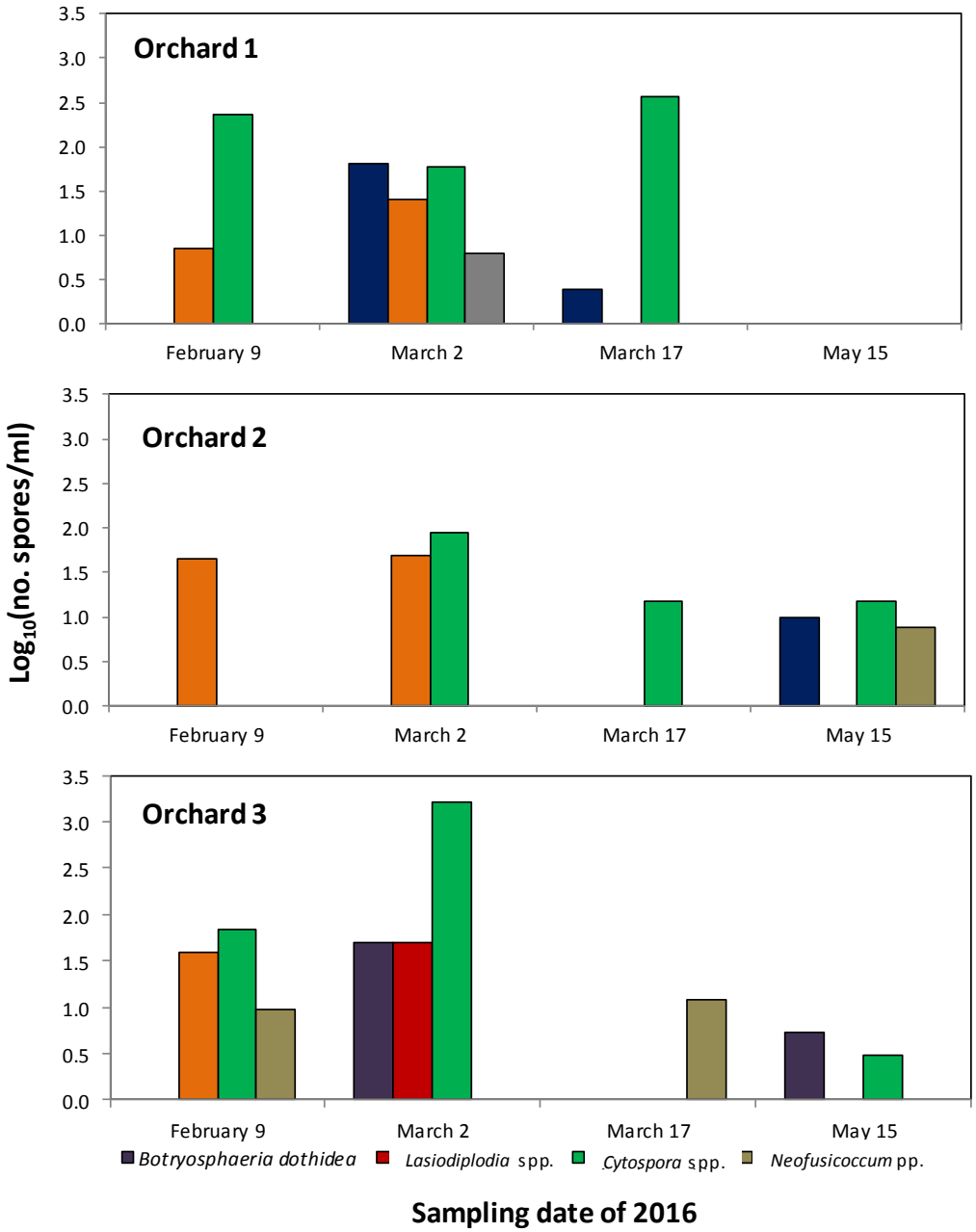
Investigations on inoculum dynamics in rain

Rain collectors

To investigate the inoculum dynamics in the rain

- Rain collectors were placed in 3 dried plum orchards.
- Rain water samples were collected periodically.
- DNA of each rain sample was extracted.
- The *q*PCR assay was applied to process the samples.
- The quantity of spores per ml for each of the 6 canker-causing pathogen group was determined for each sample.

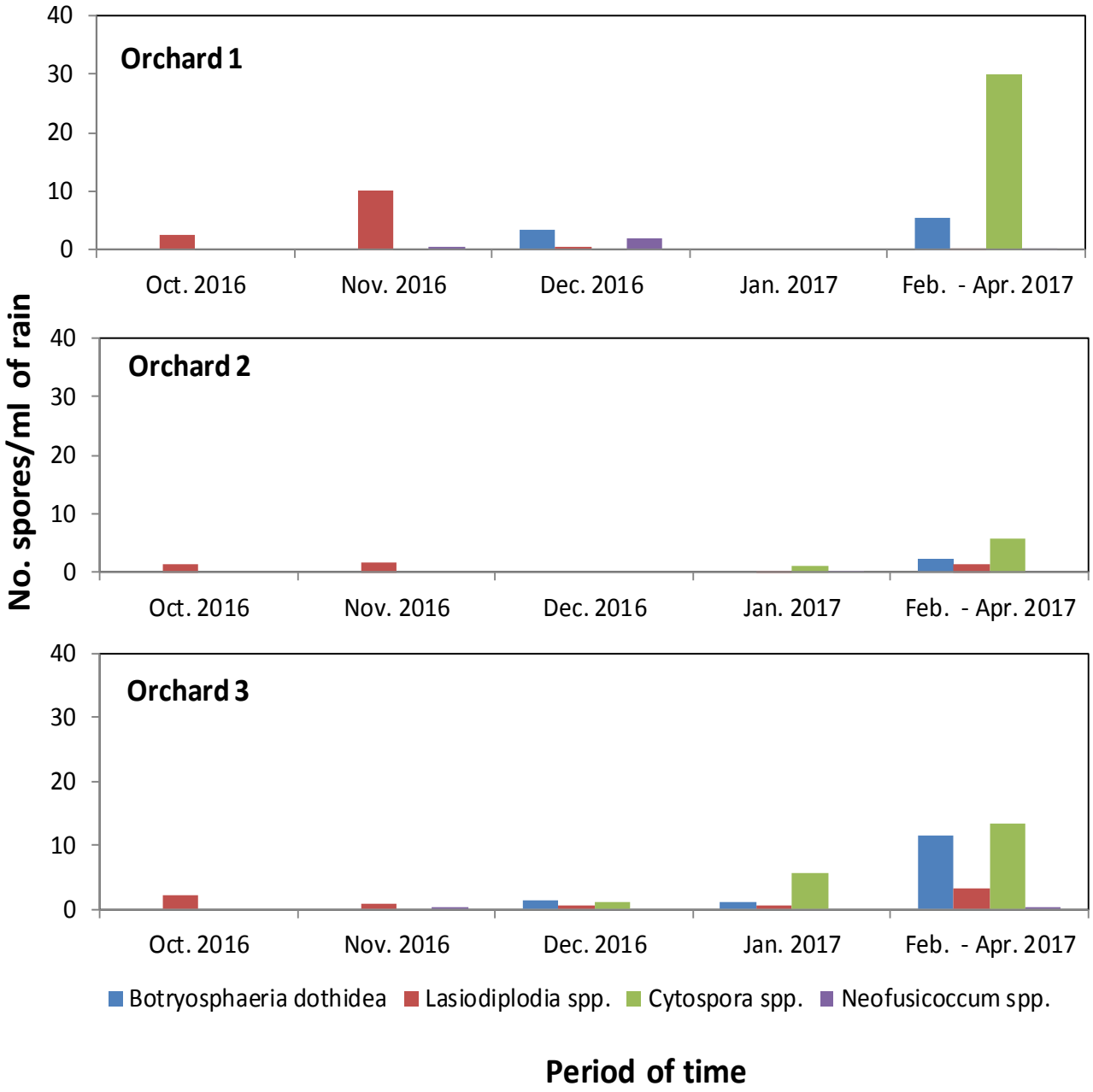
Spring 2016 to summer 2016



Conclusions

- *Cytospora* species were dominant (throughout the rainy season and at the highest densities).
- *Lasiodiplodia* species were found in early season only.
- *Botryosphaeria dothidea* and *Neofusicoccum* species were minor species in the rain water.
- *Phomopsis & Diplodia* species were not found.

Fall 2016 to spring 2017 →→ → summer 2017



- **Species composition in 2017 was similar to that in 2016 spring.**
- **In 2017, spore concentrations were significantly lower than those of 2016 spring.**

Conclusions/ Thoughts/ Ideas

Conclusions:

1. *Cytospora* species and other canker fungi establish in plant tissues very soon after the plant tissues develop.
2. *Cytospora* species are the dominant fungi in prune tissues and Cytospora canker is the dominant canker disease of prune.

Thoughts/ ideas:

1. Experimentation with fungicide sprays in late winter (late dormant) and spring to determine efficacy against the latent infection of *Cytospora* (before the appearance of any symptoms).
2. Exps. to check efficacy of fungicides that are registered for other diseases of prunes against *Cytospora* spp. (and other canker fungi (*Botryosphaeria*, *Lasiodiplodia*, etc…)
3. Open to any other thoughts, ideas, and suggestions…

The efficacy of certain fungicides to control canker disease

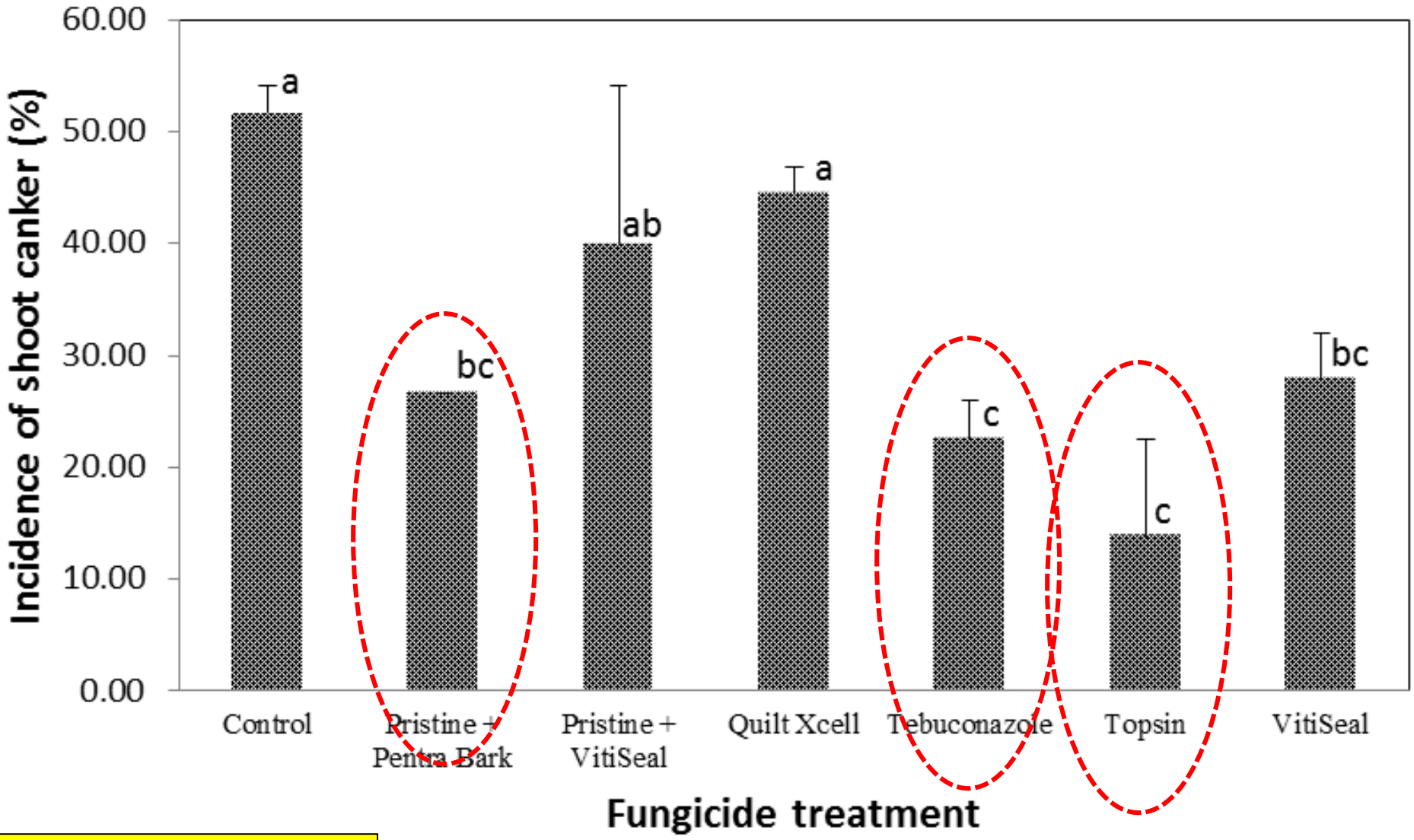
- Six fungicides were used: Topsin, Quilt Xcell, VitiSeal, Pristine + Pentra Bark, tebuconazole, Pristine + VitiSeal, plus an untreated control.
- Regular pruning was conducted in late November or early December, and fungicide treatments were conducted one day later than pruning.
- 10 wounds were used for each treatment.

- Disease was assessed on December next year.

Treatments in the field after pruning

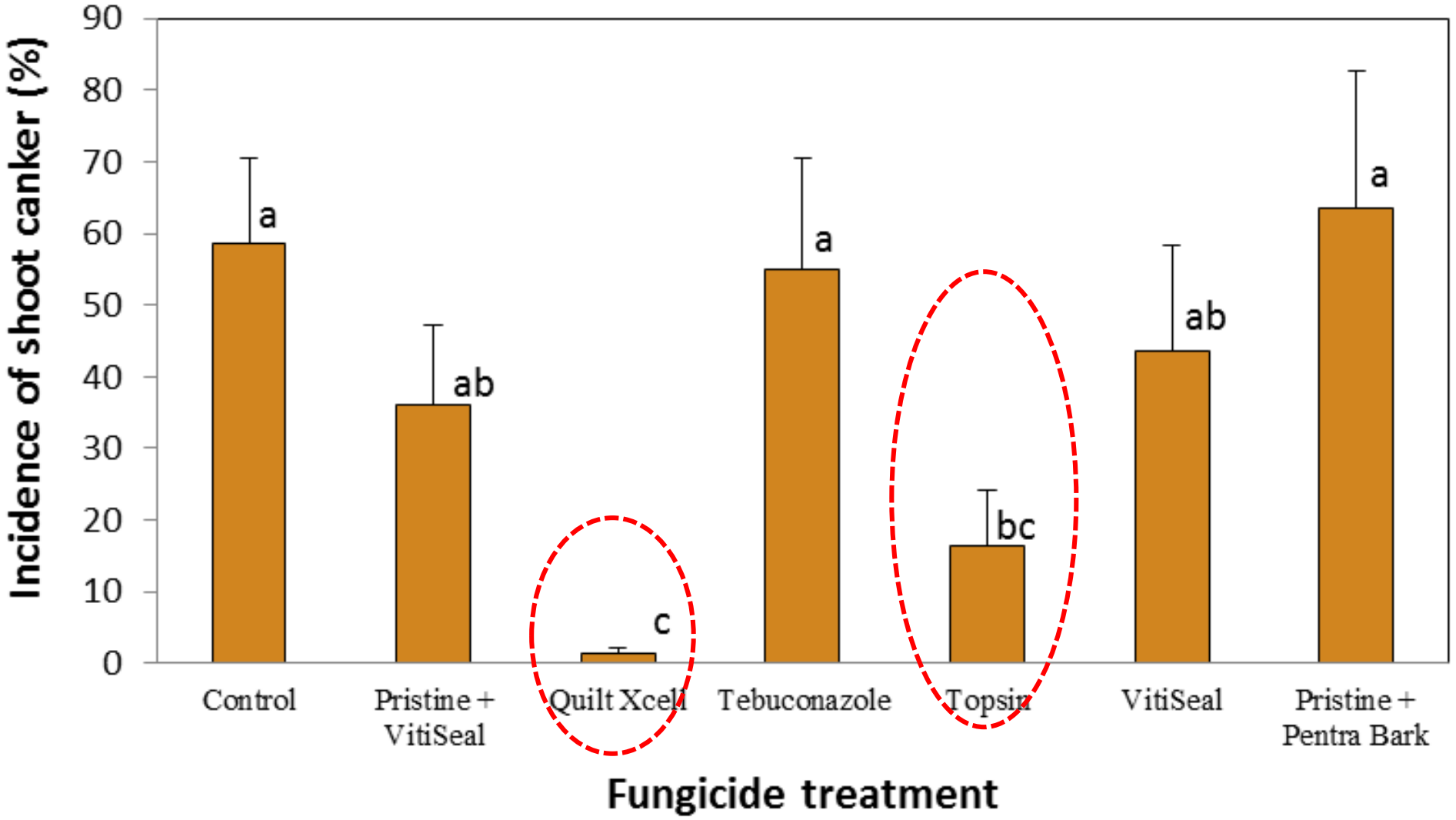
Treatment	Rate per liter
Topsin (thiophanate – methyl)	5 g a.i.
Quilt Excel (azoxystrobin + propiconazole)	5 g a.i.
VitiSeal	1:10 dilution
Pristine + Pentra Bark	5 g a.i. + 1 oz
Tebuconazole	5 g a.i.
Pristine + VitiSeal	5 g a.i.+ 1:10 dilution
Untreated control	---

Incidence of *Cytospora* infection after chemical treatment (natural infection – 2015)



Rains on 12 & 13 November

Incidence of *Cytospora* infection after chemical treatment (natural infection – 2016)



Pruning wound protection trial (c/o Dr. F. Trouillas)

	Cytospora	*Eutypa*	*B. dothidea*	*N. parvum*	*N. mediterraneum*	*Neosc. dimidiatum*	*D. mutila*	Avg. recovery
Control	75	75	100	100	100	100	100	**92.9**
TerraNeem								
Proud								
Trichoderma	0	0	75	0	25	0	50	**21.4 ***
Quash	100	25	33	0	75	75	100	**58.3**
Topsin M	50	0	50	25	25	50	25	**32.1 ***
Paint	75	50	75	100	100	100	100	**85.7**
CropSeal	100	25	100	100	50	75	75	**75**
Rally	100	75	75	75	100	100	100	**89.3**
Indar								
Fontelis								
Inspire Super								
Luna Sensation								
Quilt Xcel								
Viathon								
Luna Experience								
Bravo								
Quadris Top								
Merivon								
Pristine								
Abound								
Ziram								
Avg. recovery								

?

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Acknowledgments

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UCCE

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