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Cytospora Canker Research: WCHC2022

Dr. Jane E. Stewart

Plant Pathology Colorado State University

Dr. Stephan Miller

Cytospora Canker

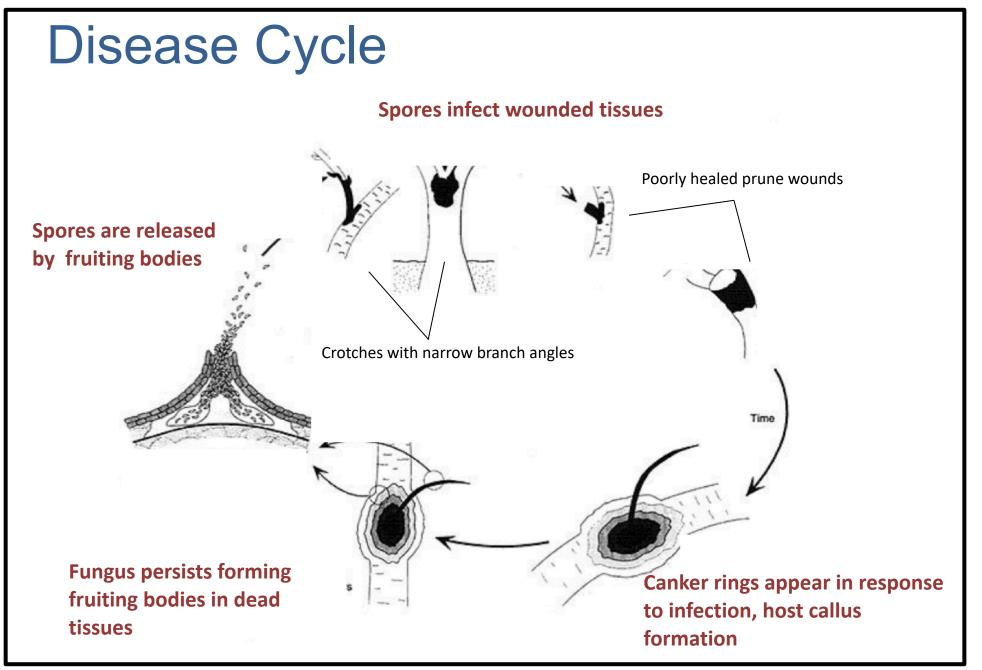
- Caused by a fungus
- Opportunist pathogen (prefers weak trees), cannot invade healthy intact bark



Pruning cut with infection

canker

canker



(Grove & Biggs 2006)

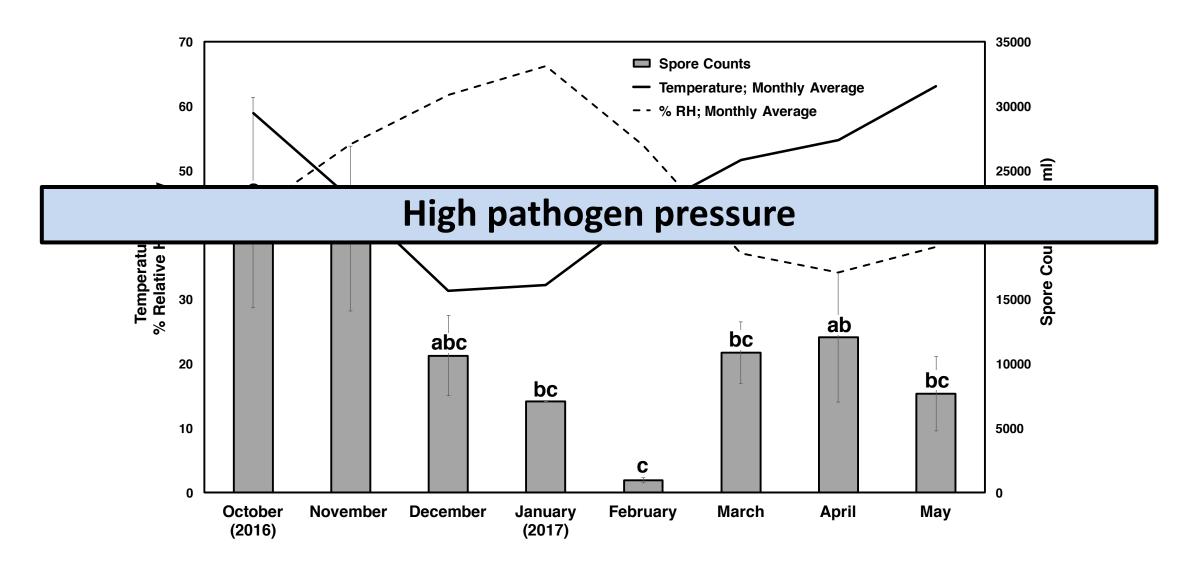
Cytospora Disease Cycle

through

- Fungus grows in bark tissue open wounds, and can can also enter buds
- Kills by girdling branches or trunk of tree
- Attacks tree when temperatures are above freezing and moisture is available
- Trees affected by drought, late spring frosts, insect and fungi defoliation, sunscald, herbicides, or mechanical injury are susceptible to Cytospora infection

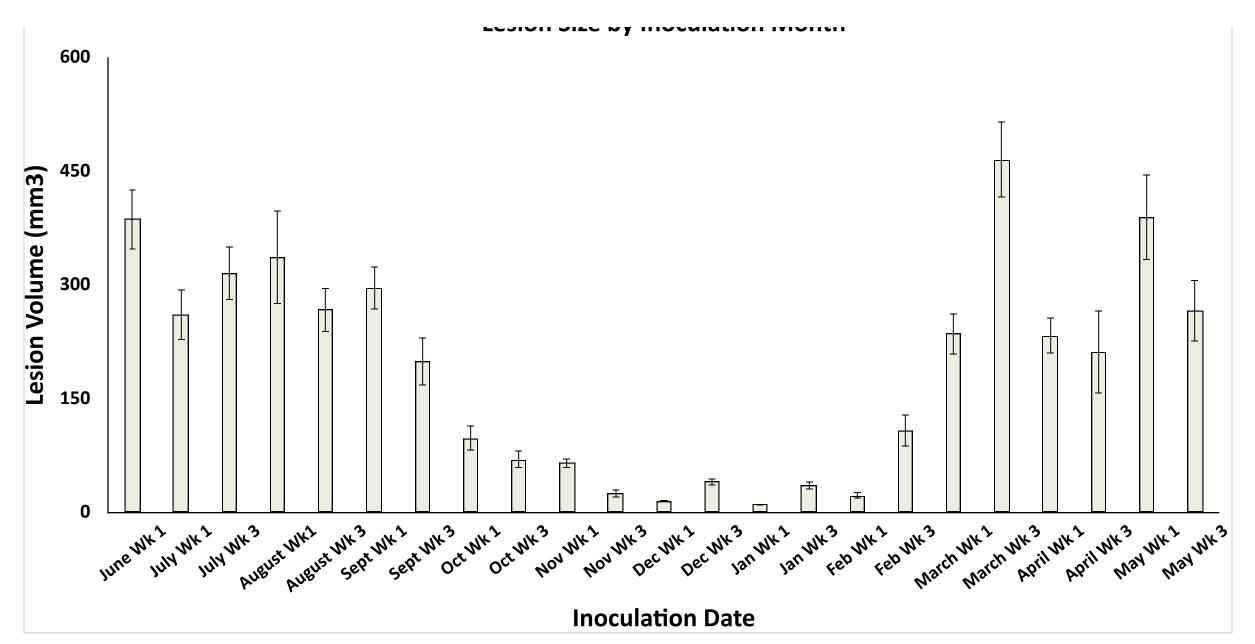
Alan Biggs

Inoculum produced throughout the year

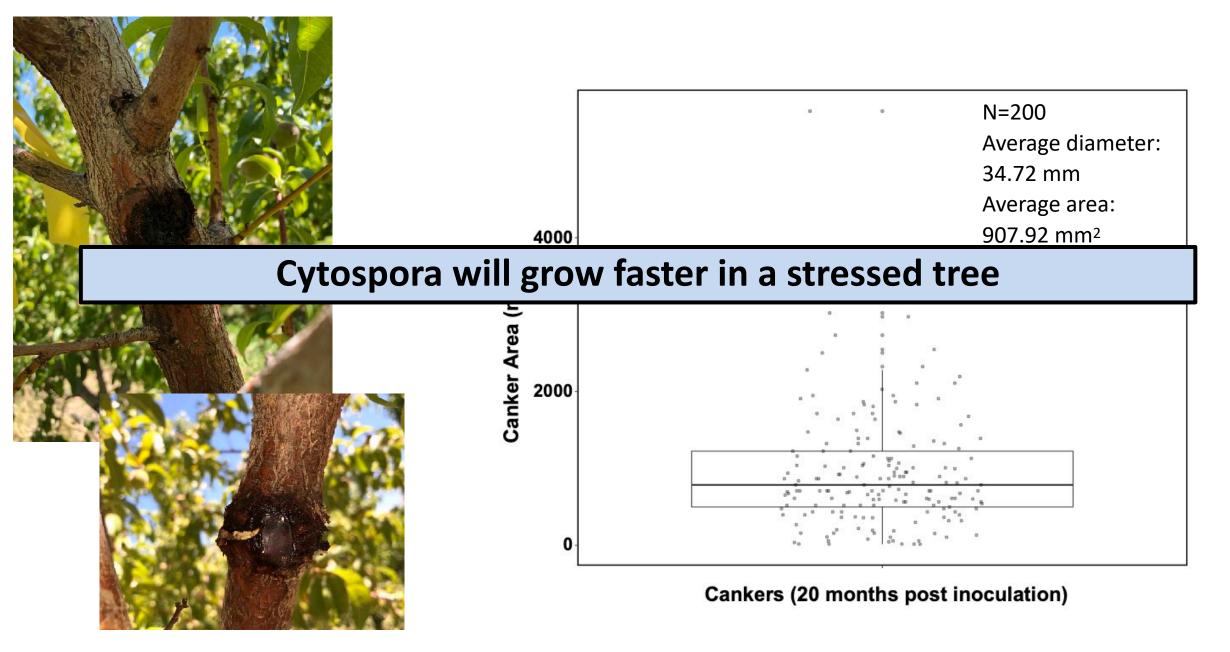


(α = 0.05) (Tukey's HSD adjusted p-values: *P* < 0.05)

Cytospora can infect peach at any season

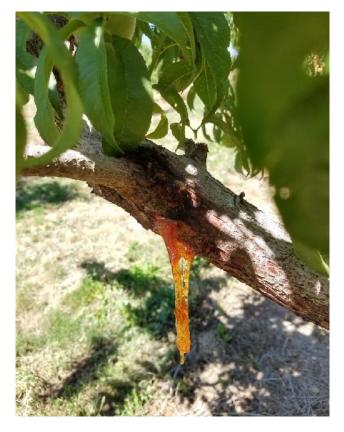


Canker growth over 20 months



Comprehensive program for Cytospora Management

- What chemicals are effective against Cytospora?
- Are cultivars equally susceptible?
- How is Cytospora moving around in orchards?
- What is the economic threshold for Cytospora canker?
- What cultural practices are associated with infection?



Testing Chemical Controls

Treatment name	Active ingredient	Mode of Action	Treatment name	Active ingredient	Mode of Action
CaCl	CaCl	Multi-site	Microthiol	Sulfur	Multi-site
Neem Oil		Not classified	Disperss		
	Neem Oil		Fontelis	Penthiopyrad	Respiration
Mpede	Potassium salts	Multi-site			
Kaligreen	Potassium	Not classified	Torino	Cyflufenamid	Unknown
	bicarbonate		Pristine	Pyraclostrobin & Boscalid	Respiration
Serenade	Bacillus subtilis	Lipid synthesis/ transport	Aliette WDG	Fosetyl	Unknown
NuCop WP	Copper Hydroxide	Multi-site	Topsin M WSB	Thiopthanate-methyl	Site- specific
Badge X2	Copper Hydroxide & Copper Oxychloride	Multi-site	Benlate WP	Benomyl	Cytoskeleton/ motor proteins
ZnSO4	ZnSO4	Multi-site		N-Trichloromethylthio-4-	
Lime sulfur	Calcium polysulfide	Multi-site	Captan	,	Multi-site
				dicarboximide	
				Difencoconazole &	
			Inspire Super	Cyprodinil	Protein synthesis
			Ziram	Zinc dimethyldithiocarbamate	Multi-site

Testing chemicals as laboratory tests

Laboratory Trials:

 Testing chemicals in the lab on petri dishes

Effective:

- Aliette, Topsin, Benlate, Captan Inspire, Mpede, Kaligreen, Serenade, NuCop, Badge, ZnSO4, Lime Sulfur
- Testing chemicals in the lab on detached branches

Effective:

Topsin, Captan, NuCop, Lime
 Sulfur





Fungicide/ Sealant Trials (2018/2019)

Novel Treatment Combinations	% Active Ingredient in Aqueous Solution
VitiSeal	10%
VitiSeal + Thiophanate-methyl	10%, 0.053%
Latex Paint	70%
Latex Paint + Thiophanate-methyl	70%, 0.053%
JMS Oil + Lime Sulfur BSP	90%, 3%
VitiSeal + Lime Sulfur BSP	10%, 3%
Nu-Film + Lime Sulfur BSP	10%, 3%
Latex + Lime Sulfur BSP	70%, 3%

Fungicide/ Sealant Trials (2018/2019)

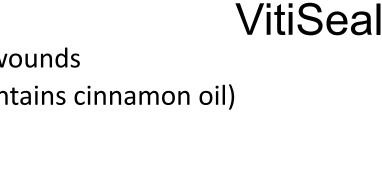
- Sealant barrier for plant wounds
- Co-polymer emulsion (contains cinnamon oil)
- Was not OMRI approved

JMS Stylet-Oil

- OMRI approved
- Mineral oil

Nu- Film

- OMRI approved
- Spreader/ sticker derived from pine





Methods: Fungicide Trials (2018/2019)

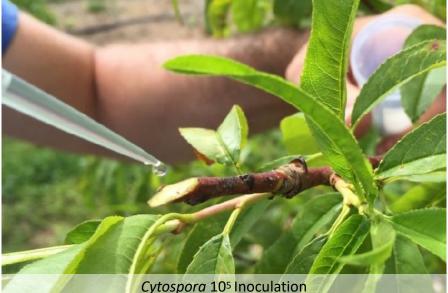
- 1. Wounds were made on 1-year old tree shoots and sprayed with label mid-rate applications of fungicides
 - Random complete block design with 20 tree blocks
- 2. 10⁵ spore suspension inoculations made on branches and wrapped in Parafilm
- Reanches were harvested 3 months post inoculations and assessed





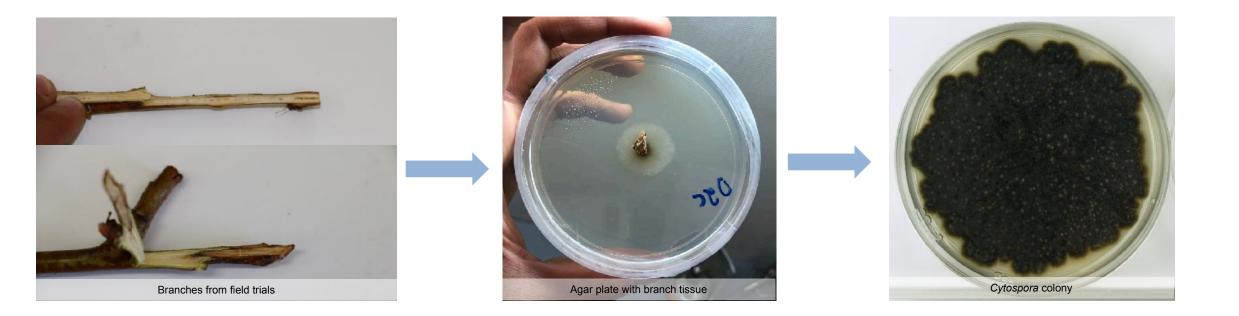


David Sterle

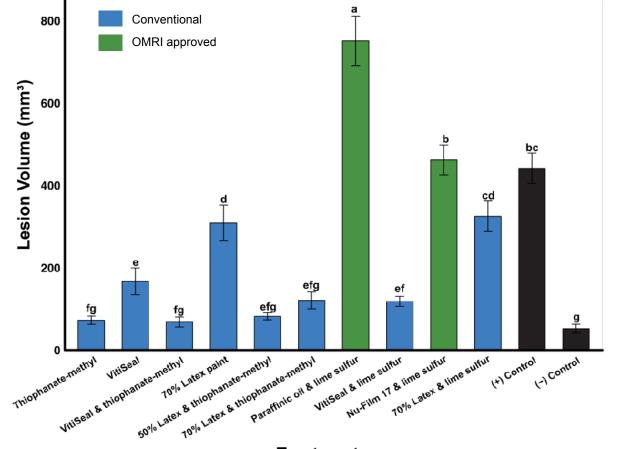


Are Spores Present if Lesions are Not?

- Isolated tissue from inoculation area
- Placed plant tissue on petri dish to see if fungus was there
- n= 20 per treatment



Results: Fungicide Efficacy (2018/2019)

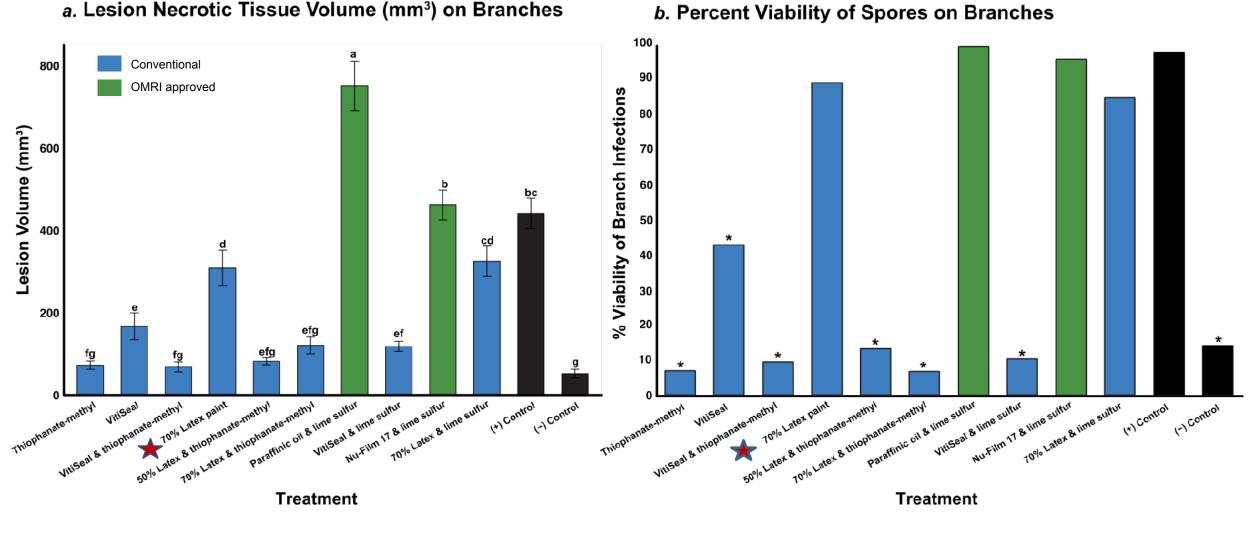


a. Lesion Necrotic Tissue Volume (mm³) on Branches

Treatment

(α = 0.05) Tukey's HSD adjusted p-values: *P* < 0.05 n= 20 replicates per treatment/ bar $(\alpha = 0.01)$ Fisher's Exact Test for Contingency Tables n= 20 replicates per treatment/ bar

Results: Fungicide Efficacy (2018/2019)



(α = 0.05) Tukey's HSD adjusted p-values: *P* < 0.05 n= 20 replicates per treatment/ bar $(\alpha = 0.01)$ Fisher's Exact Test for Contingency Tables n= 20 replicates per treatment/ bar

Miller, Sterle, Minas, Stewart. 2020

Most Effective Preventive Treatments

Effective Chemicals:

• Topsin, Captan, Vitiseal, Lime Sulfur and maybe Cueva

Effective Chemical Mixtures:

 Latex+Topsin, Latex+Captan, Vitiseal+Topsin, Vitiseal+Lime Sulfur

Topsin is high risk for resistance development



Preventive Control for Cytospora Canker on Peach

Fact Sheet No. 2.954

Crop Series | Diseases

by Jane E. Stewart¹, Stephan T. Miller¹, Ioannis S. Minas^{2,3}

Are some cultivars more tolerant to Cytospora?





Are some cultivars more tolerant to Cytospora canker?

1. Investigate Host Susceptibility

• Are there peach cultivars that are less susceptible to *C. plurivora*?

2. Investigate Environmental Conditions Favoring Disease

- Does environmental stress increase disease severity?
 - 1. Water deficit?
 - 2. Increased soil pH?



Exp. 1 Greenhouse Trials:

Evaluating cultivars under controlled conditions



Exp. 2 Field Trials:

Evaluating cultivars in production setting



Field Trials

Greenhouse Trials

Methods: Greenhouse Trials

Cultivars

1. Glohaven/Lovell Michigan

2.Glowingstar/Lovell

3.Blushingstar/Lovell

4.Starfire/Lovell

5.Newhaven/Lovell

6.Flamin Fury PF 19-007/Lovell

7.Flamin Fury PF 23/Lovell

8.Flamin Fury PF 24/Lovell

9.Red Haven/Lovell

10.0'Henry/Lovell California

11.Angelus/Lovell

12.Suncrest/Lovell

13.Cresthaven/ Lovell

Design

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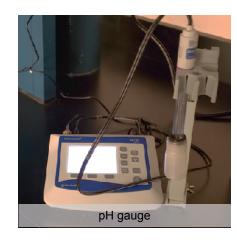
- 5 tree rep. per cult. per treatment
- Trees established for 2 months
- Trees stressed for 2 months
- Inoculated after 2 months of treatments

Treatments

- 1. Control
 - 100% pot capacity
 - pH 7
- 2. Deficit-Irrigation
- 60% pot capacity
- pH 7
- **3**. High-pH
- 100% pot capacity
- рН 9

100% pot capacity determined by weight

- by weight
- Trees watered at 60% pot capacity for two months
- pH adjustments made through irrigation water, through addition of sodium carbonate and bicarbonate





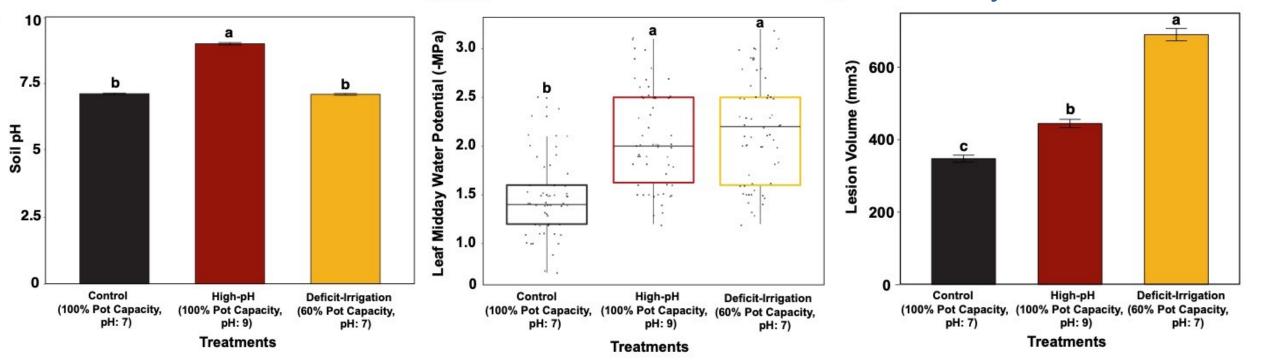
- Leaf water potential (LWP) measurements taken at solar mid-day
- Soil slurries evaluated for pH
- Lesion volume mm³ measured 8 days post inoculation



Pressure bomb for measuring LWP

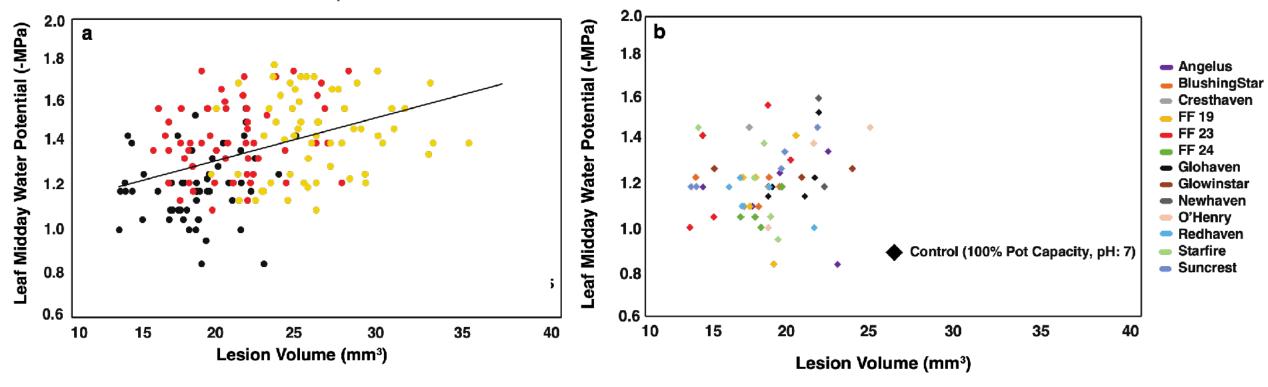
pH and deficit-irrigation differences between treatments

Differences in lesion sizes by treatment



(α = 0.05) Tukey's HSD adjusted p-values: *P* < 0.05 n = 65 observations per treatment

Relationship between lesion volume and leaf water potential

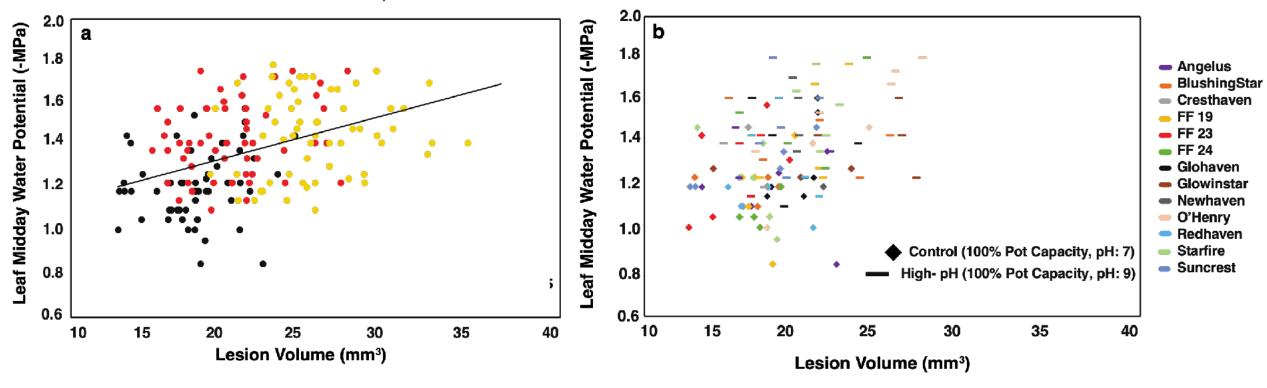


Control (100% Pot Capacity, pH: 7)

High- pH (100% Pot Capacity, pH: 9)

• Deficit- Irrigation (60% Pot Capacity, pH: 7)

Positive Relationship between lesion volume and leaf water potential

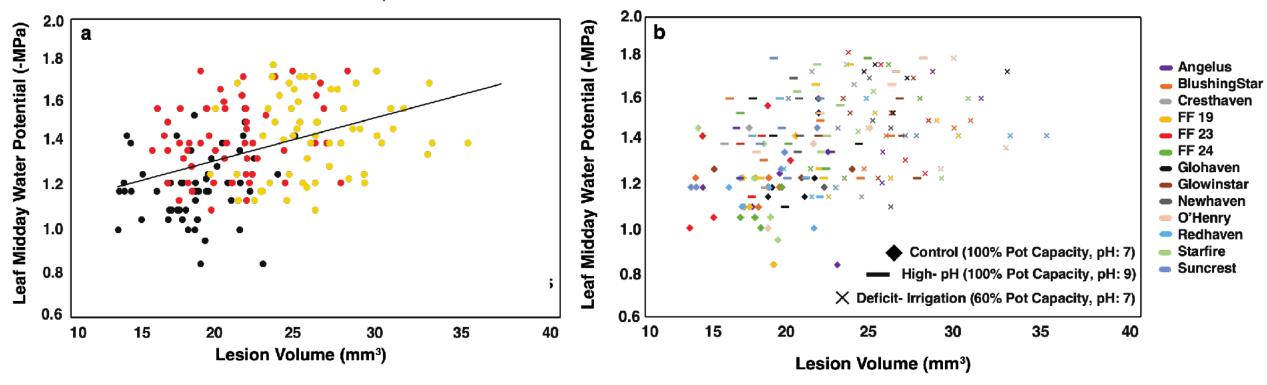


Control (100% Pot Capacity, pH: 7)

High- pH (100% Pot Capacity, pH: 9)

Deficit- Irrigation (60% Pot Capacity, pH: 7)

Positive Relationship between lesion volume and leaf water potential



No cultivar trends

High- pH (100% Pot Capacity, pH: 9)

• Deficit- Irrigation (60% Pot Capacity, pH: 7)

Control (100% Pot Capacity, pH: 7)

Field Trials: Evaluating cultivars in production setting

Cultivars

1. Glohaven/Lovell	Michigan
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- 2.Glowingstar/Lovell
- 3.Blushingstar/Lovell
- 4.Starfire/Lovell
- 5.Newhaven/Lovell
- 6.Flamin Fury PF 19-007/Lovell
- 7.Flamin Fury PF 23/Lovell
- 8.Flamin Fury PF 24 Cold Hardy/ Lovell
- 9.Red Haven/Lovell
- 10.0'Henry/Lovell California

11.Angelus/Lovell

12.Suncrest/Lovell

Design

5 tree repetitions per cultivar per treatment

Trees established from 2018-2020

Treatments

- **1.**Full-irrigation row
- RAW of clay-loam soil
 - 2.1 acre/inch water

2. Deficit- irrigation row

60% of RAW = 1.26 acre/inches





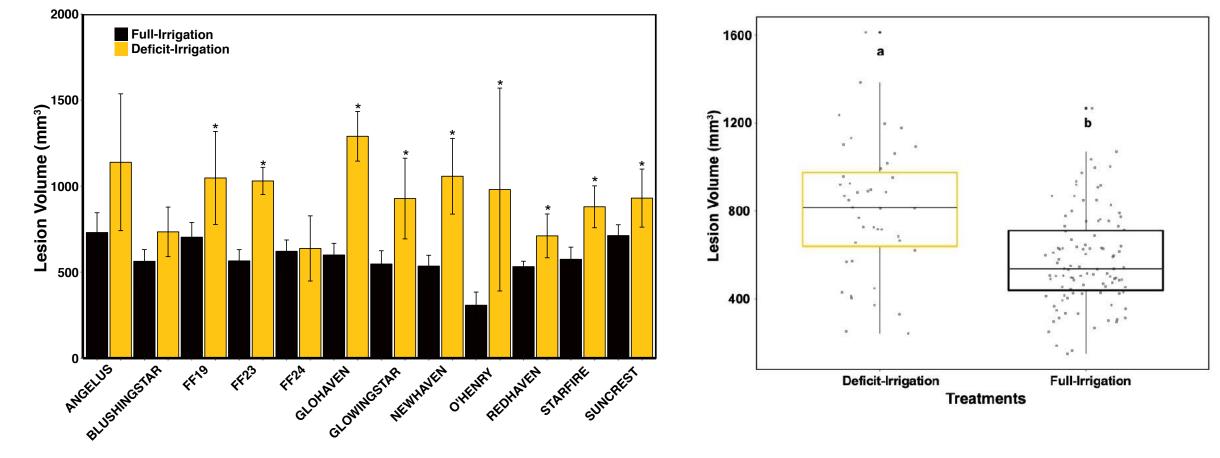
Dr. Greg Litus

Sean Wright

- Pre-dawn WP taken b/t 0300HR and 0600HR
 - Taken once a day for an entire irrigation period
- Trees Inoculated after 3 months of treatments
- Lesion volume mm³ measured 8 days post inoculation



Increased lesion size under deficit-irrigation





(α = 0.05) Tukey's HSD adjusted p-values: *P* < 0.05 n= 25 replicates per bar, 5 averaged observations per bar Are some cultivars more tolerant to Cytospora canker?

Are there peach cultivars that are less susceptible to Cytospora canker?

- PF24 showed evidence of tolerance in all trials
- Little differences across scion tissues

Does environmental stress increase disease severity?

• Water deficit + increased pH/ salinity increases disease severity on host and decreases tree water potential.



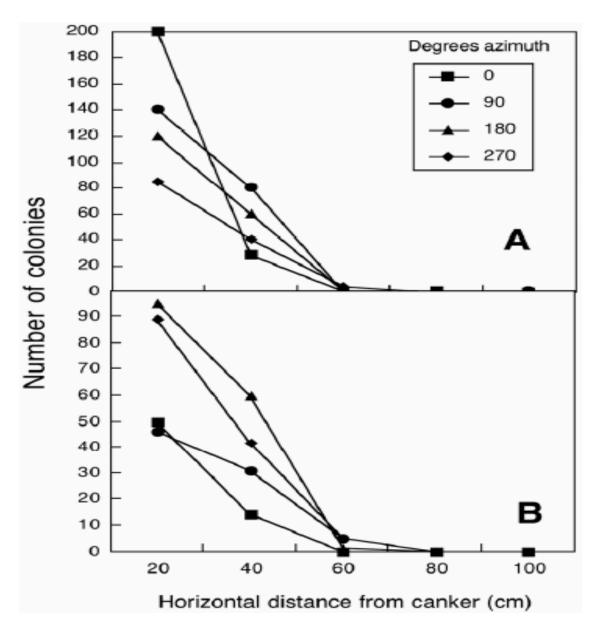
Comprehensive program for Cytospora Management

- What chemicals are effective against Cytospora?
- Are cultivars equally susceptible?
- How is Cytospora moving around in orchards?
- What is the economic threshold for Cytospora?
- What cultural practices are associated with infection?



Rain splash is important for inoculum spread

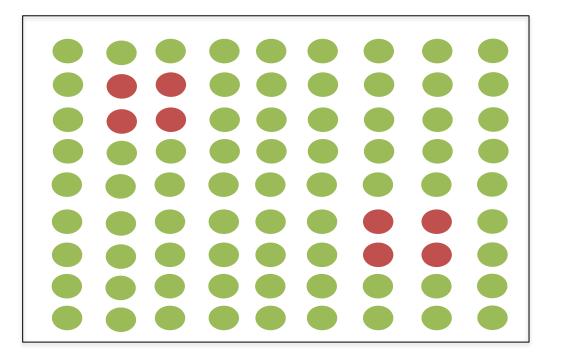
- Maximum spread 2 ft from rain splash
- More inoculum further at angles higher than 90 degrees from canker



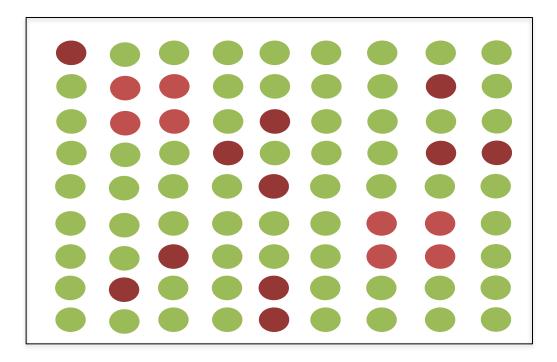
Grove and Biggs 2006. Plant Disease

Is inoculum spread similarly in Colorado?

Pattern of disease if spread only by rain splash



Patterns we observe in orchards in Colorado



What are ways spores travel long distances? Wind, insects, humans?

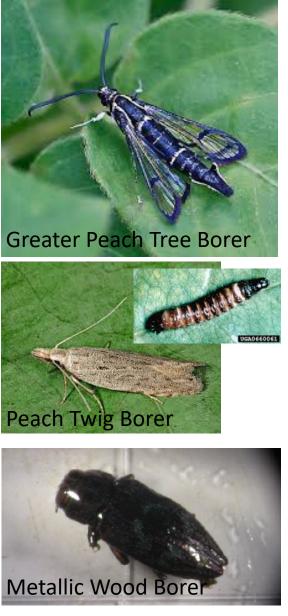
Marker development as an epidemiology tool

- A digital drop molecular assay was developed for and tested against closely-related species of *Cytospora*
- Collect aerial samples
- Collect insects and test for vectors
- Test nursery stock





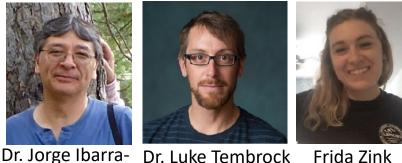




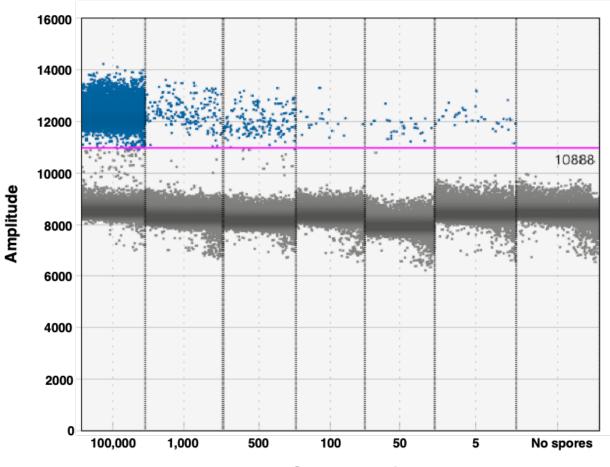
Droplet Digital PCR – detection method

- C. plurivora specific molecular assay developed
- PCR performed in 20,000 droplets (1nl) = 20ul reaction
- If target sequence is present in droplet it is amplified, and a reporter dye emits a fluorescent signal
- Fraction of positive to negative droplets determines concentration of target DNA





Dr. Jorge Ibarra- Dr. Luke Tembrock Fric Caballero



Stewart et al. 2021

Spores per ml

How is *Cytospora* moving around in orchards?

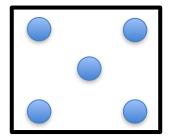
Collection Type

1. Insect Collections

- 3 conventional, 3 organic
- 10 weeks of collection
- **MWB** (Chrysobothris mali)
- GPTB (Synanthedon exitiosa)
- PTB (Anarsia lineatella)

2. Aerial Collections

- 3 conventional, 3 organic
- 10 weeks of collection



- 1 trap per species in each circle
- Insects collected individually
- Pooled by trap location, orchard, and collection date
- 5 mature cankers flagged
- Collections made 0.5m from canker
- Rotary vane sampling pump 20 l/min for 5 mins at each canker
- Pumped onto agar plate
- Plate effluent collected in 15ml tubes
- Pooled by orchard and collection date

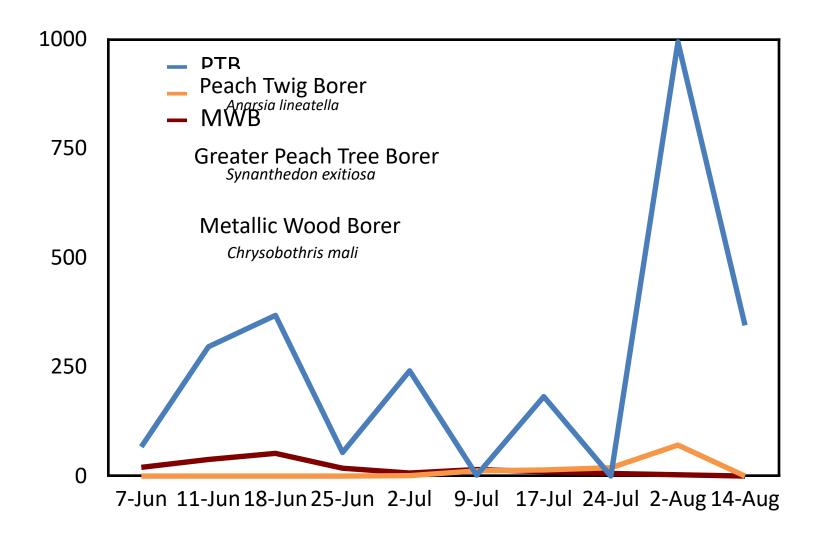




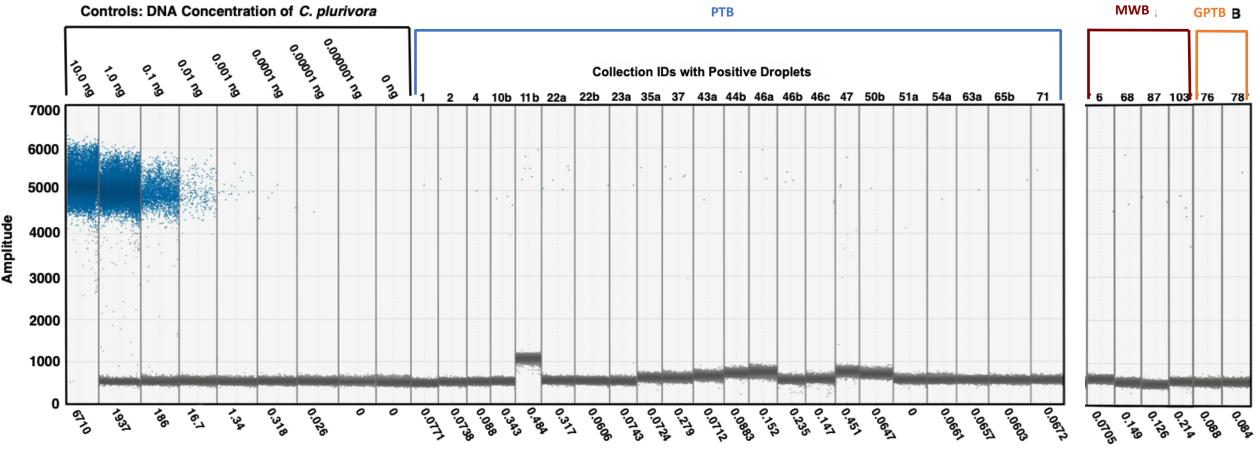
Total numbers of insects collected

Total insects: 2,917

- Peach Twig Borer: 2,628
- Greater Peach Tree Borer: 119
- Metallic Wood Borer: 170



Insect dissemination possible, but rare



Target DNA Molecule Concentration per uL (Copies/uL)

Total fraction of pooled samples with positive amplification

22 / 119 (18.4%)

PTB

4 / 31 (12.9%)

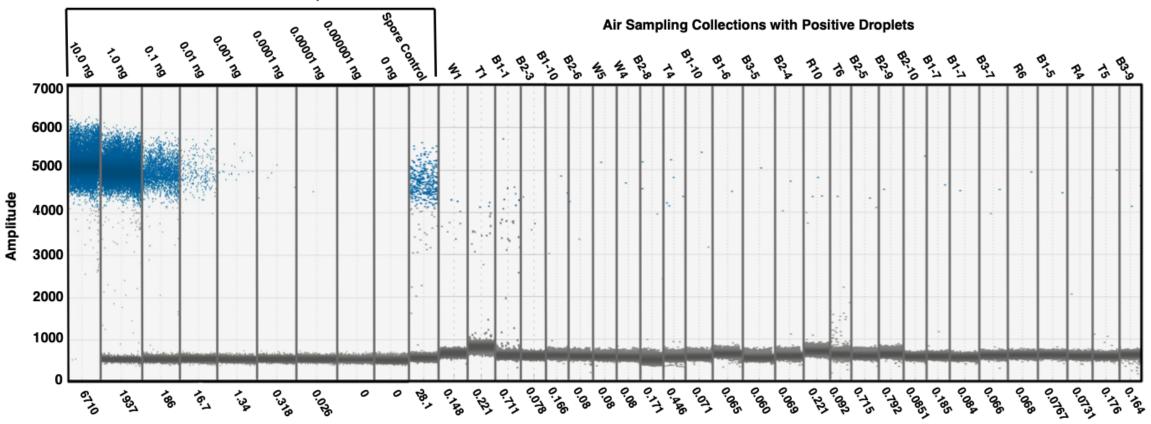
MWB

2 / 19 (10.5%)

GPTB

Peach Twig Borer Greater Peach Tree Borer Metallic Wood Borer

Aerial dissemination possible, but...



Controls: DNA Concentration of C. plurivora

Target DNA Molecule Concentration per uL (Copies/uL)

Total fraction of pooled samples with positive amplification

27 / 64 (42.1%)

Movement of *Cytospora* on Nursery stock?

- Studies have categorized *Cytospora* species as potential endophytes
- Is there a latency period which requires stress before symptoms are present?
 - Nursery to field?

Sampled a variety of scion / root stocks from three nurseries at two stages:

1. Upon arrival at CSU (Asymptomatic)

2. Potted and maintained throughout summer (Symptomatic)





Movement of Cytospora on Nursery stock?

Scion / Rootstock	Nursery
Cresthaven / Krymsk 1	Nursery 1
Blushingstar FA18 / Krymsk 1	Nursery 1
Reliance / Krymsk 1	Nursery 1
Contender / Krymsk 86	Nursery 1
Blazingstar / Krymsk 86	Nursery 1
PF Lucky 13 / Krymsk 86	Nursery 1
Coralstar / Krymsk 86	Nursery 1
All-star / Krymsk 86	Nursery 1
O'Henry / Krymsk 86	Nursery 1
Glohaven / Krymsk 86	Nursery 1
Redhaven / Krymsk 86	Nursery 1
Flamin' Fury PF17 / Krymsk 86	Nursery 1
Glowingstar / Krymsk 86	Nursery 1
Flamin' Fury PF 28-007 / Krymsk 86	Nursery 1
Angelus / Krymsk 8	Nursery 1
Cresthaven / Halford	Nursery 2
Glohaven / Halford	Nursery 2
Glowingstar / Halford	Nursery 2
Starfire / Halford	Nursery 2
Redhaven / Halford	Nursery 2
Suncrest / Halford	Nursery 2
Flamin' Fury PF23 / Lovell	Nursery 3

Design

- 22 different scion / rootstock combinations
- 25 tree replicates per cultivar / rootstock

Sampling

- 1. Asymptomatic
- 10 trees per cultivar sampled
- Total: 220 trees
- Sampled immediately upon arrival

2. Symptomatic

- 15 trees per cultivar sampled
- Total: 330 trees
- Sampled over four months



Symptomatic Sample

- 3 tissue samples removed from the mainstem and from branches of each tree
- Surface sterilized and plated on nutrient agar
- Assessed daily for *C. plurivora* features
- Colonies morphologically similar to C. plurivora were sequenced (ITS)
- Planted in 57.8- liter pots in shade house
- Evaluated weekly for symptoms
- 361 symptomatic isolations made on nutrient agar
- Colonies morphologically similar to C. plurivora were sequenced (ITS)

No Cytospora spp. were identified on nursery stock

Are infections originating from nurseries?

- No evidence of *Cytospora* after 922 tissue isolations on 330 trees
 - No signs (fruiting bodies) observed at any point
- No Cytospora was confirmed in any of the samples
- Top fungi isolated (based in ITS sequencing)

Evaluate dissemination mechanisms

- Can insects disseminate spores? Yes, but likely rare
- Can spores be aerially dispersed? Yes, but likely rare
- Is Cytospora present on nursery stock? No

Nursery	Fungal Identity
N2	Botrytis cinerea
N2	Epicoccum nigrum
N2	Rhizoctonia sp.
N1	Phoma sp.
N3	Phoma sp.
N2	Botrytis cinerea
N3	Alternaria infectoria
N1	Alternaria sp.
N3	Alternaria arborescens
N2	Epicoccum nigrum
N2	Rhizoctonia alpina
N2	Alternaria sp.

Comprehensive program for Cytospora Management

- What chemicals are effective against Cytospora?
- Are cultivars equally susceptible?
- How is Cytospora moving around in orchards?
- What is the economic threshold for Cytospora?
- What cultural practices are associated with infection?



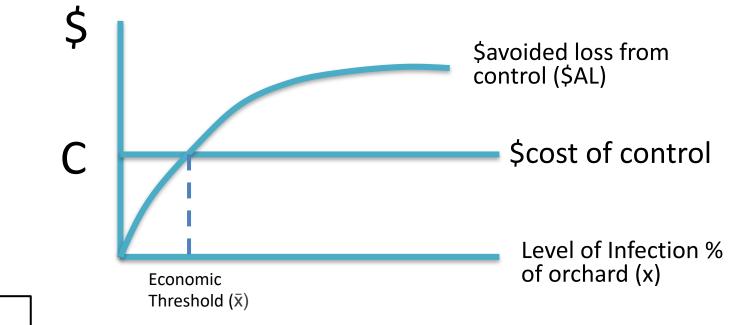
Cytospora economic thresholds

Economics of Cytospora

- Threshold for disease vs. cost of management
 - Working with Dr. Dana Hoag from CSU, Dept. of Ag Economics



Dr. Dana Hoag





Avoided losses (AL) from Cytospora Management

\$Yield *with* control **—** \$Yield *without* control



Avoided losses (AL) from Cytospora Management

\$Yield *with* control \$Yield *without* control



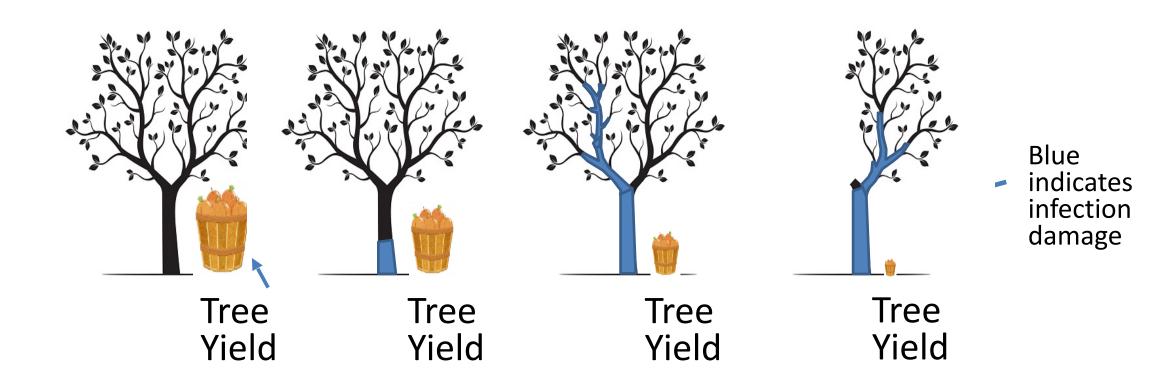
1 year 18,000x\$1.1 = 19,800 - 15,000x\$1.1 = 16,500 = \$3,300

\$Yield savings can include quantity and quality

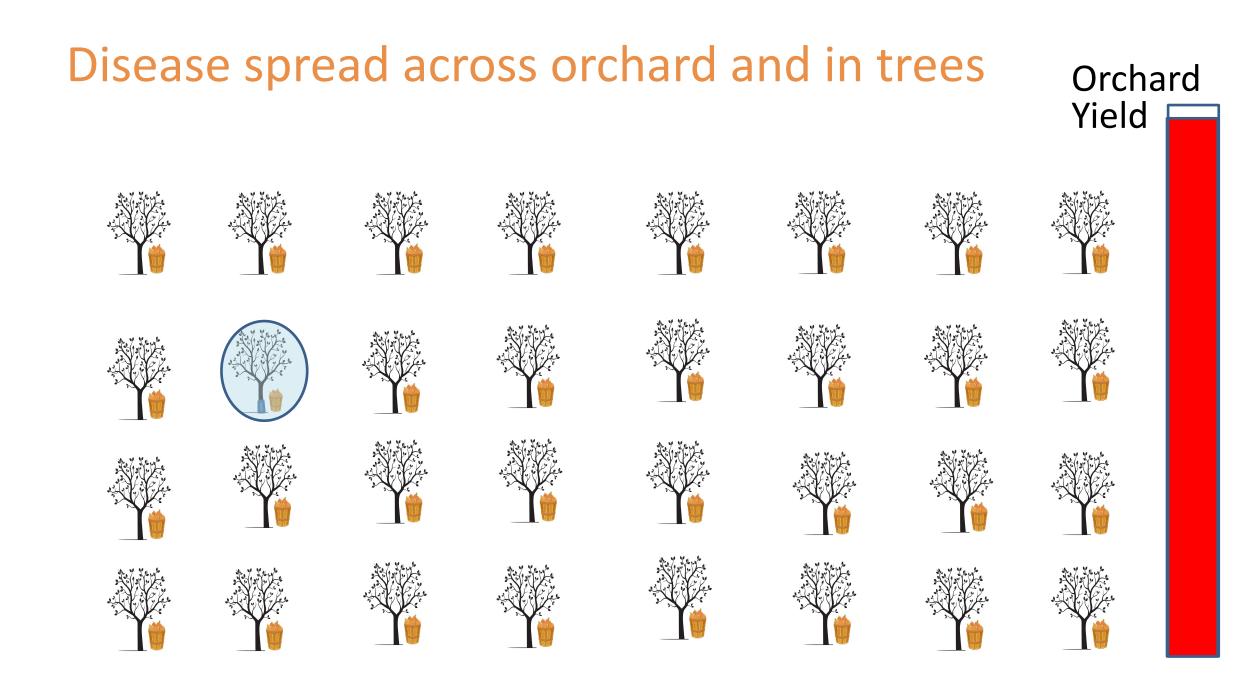
Avoided losses (AL) from Cytospora Management

\$Yield <i>with</i> cont Within tree + acro replanting	_	\$Yield <i>without</i> control Within tree + across orchard + replanting	\$ AL
1 year	18,000x\$1.1 =	19,800 - 15,000x\$1.1 = 16,500	= \$3,300
Future 10 years	In infected tree + New trees inf + Worsening in		= \$63,000

Disease spread within a tree

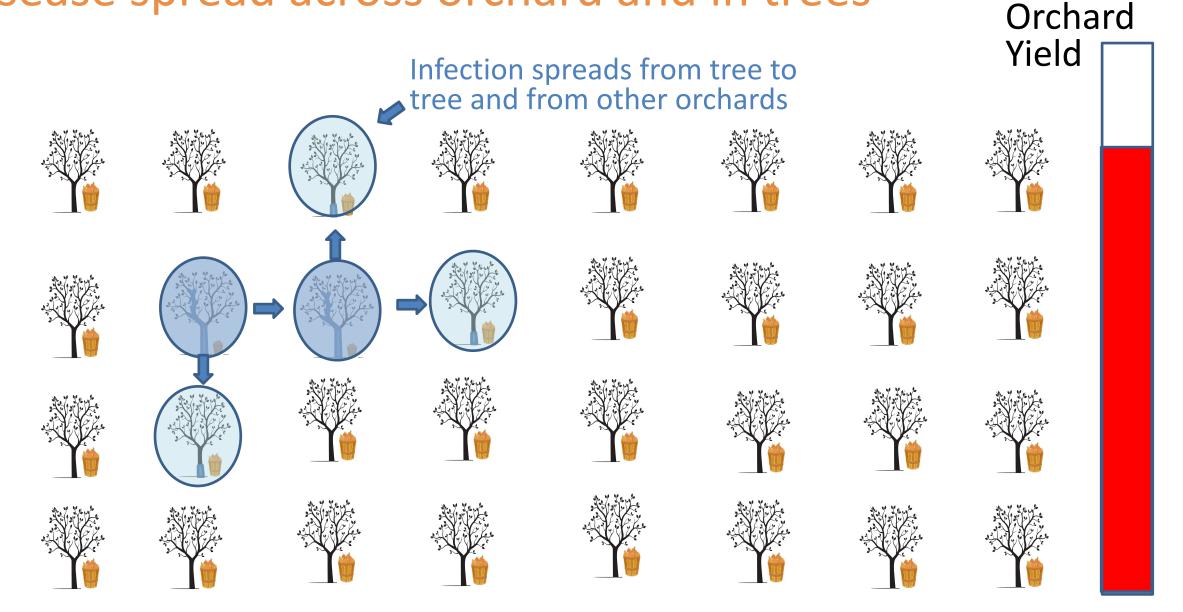


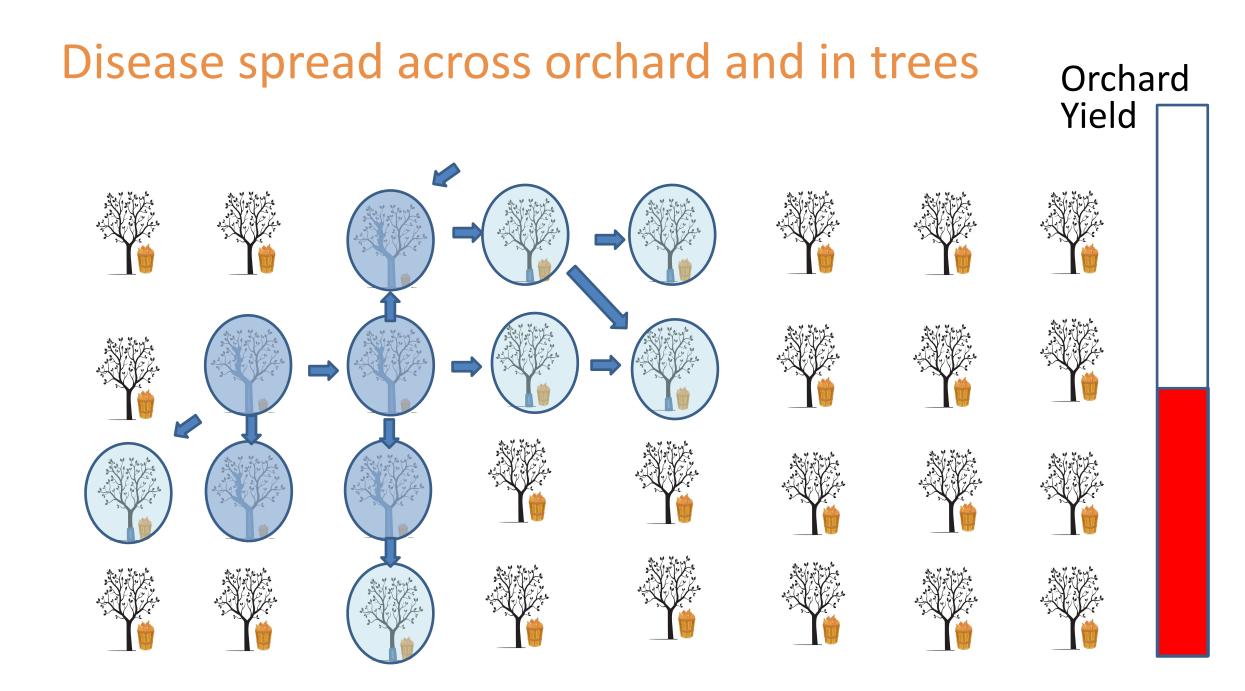


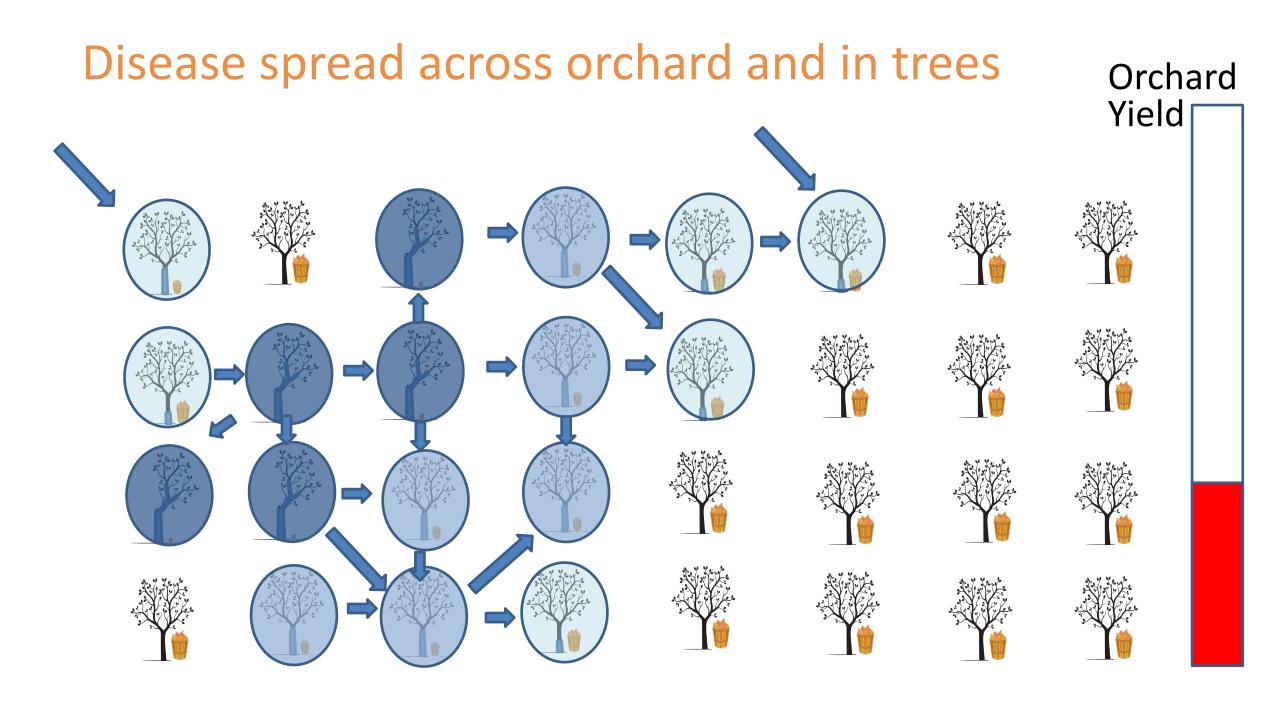


Disease spread across orchard and in trees Orchard Yield

Disease spread across orchard and in trees







Total Avoided loss

Weak Control

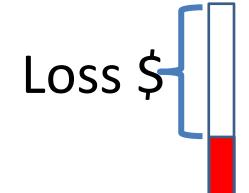
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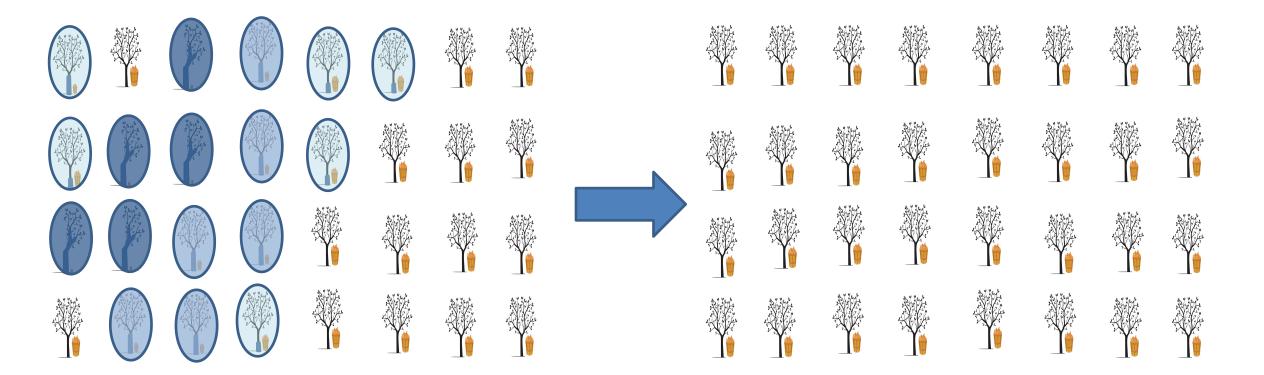
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Loss \$-

Early Replant of Orchard



Control Options and Costs

• Light control = pruning and flailing branches; canopy sprays 1 per year

Cost estimate: Additional pruning and flailing 6 hours/acre (\$16.50/hr), Canopy spray \$50/ac = \$149/ac

 Medium = pruning and flailing branches; canopy sprays 2 per year; chemical controls on pruned branches; removing highly infected trees

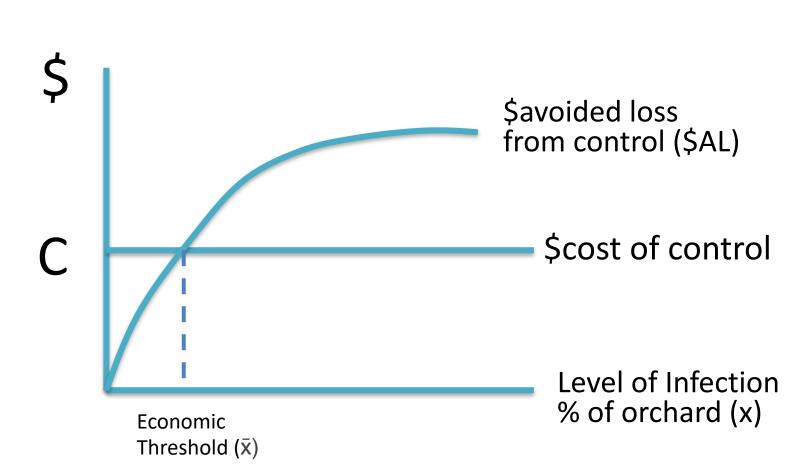
Cost estimate: Same as light control, but takes 10 hours labor plus chemicals (\$65/ ac), plus 2 sprays = \$330

 High = Pruning and removing (burning) infected branches; second pass to prune and flailing non-infected branches; canopy sprays 2 per year; chemical controls on pruned branches; well-watered trees – reduce tree stress.

Cost estimate: Same as medium control, but add 6 hours for second pass, 4 for burning, and \$50/acre for more attention to water in the winter, = \$545

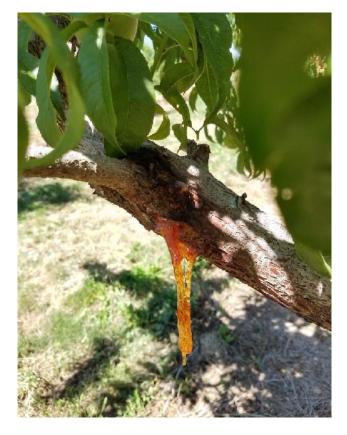
Economic threshold questions

- Infection rate from year to year
- Probability of infection
- How infection intensifies over time
- Correlation between grower practices and infection levels



Comprehensive program for Cytospora Management

- What chemicals are effective against Cytospora?
- Are cultivars equally susceptible?
- How is Cytospora moving around in orchards?
- What is the economic threshold for Cytospora canker?
- What cultural practices are associated with infection?





Sean Wright-MS student

- 1. Efficacy of chemical treatments in winter canopies over time (Sean's presentation)
- 2. Test *Cytospora* spread under micro-sprinkler and drip irrigation
- 3. Test Cytospora viability in mulch starting this winter





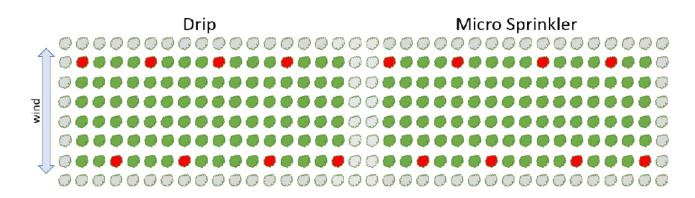




Examination of drip vs. micro irrigation on spread of *Cytospora* in orchard settings

Methods

- 2 blocks with 288 trees each
 - two treatments per block (1 drip and 1 micro sprinkler)
 - Row spacing 3.6m, tree spacing 1.8m
 - Trees trained in V-shape
- Each tree trunk painted with Topsin and latex (Miller et. al. 2019)
 - Reduce viability of *C. plurivora* and conidia extrusion
- Inoculation of *C. plurivora* on selected peach trees
 - 8 trees per treatment
- Regular watering intervals







Methods – Cont.

A State of the second s

- Buffer - Uninoculated - Inoculated

- Spore traps placed at basal area of tree, leeward side of wind
 - 6 traps per canker
 - Located at 45°, 90°, and 135° from canker
 - 90° transect traps will continue for 3 rows centered in row
 - Spore traps centered in row from inoculation
 - Total of 6 traps per inoculated tree
- Spore trap suspension collected weekly
 - ddPCR to quantify conidia collected

Sean's Current Progress

- All samples from the irrigation project have been processed and are awaiting ddPCR – this will be completed by this spring
 - Second season will begin this summer
- Second season chemical efficacy trial is underway
 - Results from first year show that efficacy decreases after 2 to 3 months, depending on chemical type

Ongoing funded projects – 2022-2024



Grace Ganter, Claudie Bertin, Sean Toporek

- 1. Survey peach, cherry, and apple orchards in CO to estimate *Cytospora* spp. incidence and severity (Grace's presentation)
- 2. Estimate *Cytospora* species distribution, genetic diversity within and among orchards, and develop an epidemiological model of the spread of the pathogen.
- 3. Perform pathogenicity assays to determine host range within fruit crops of each of the identified *Cytospora* species.



United States Department of Agriculture National Institute of Food and Agriculture

Increases in Cytospora severity over 4 years

Cultivar		Cresth	esthaven Suncrest			Redhaven				Newhaven				Red Globe						
Year	2016		2020		2016		2020		2016		2020		201 6		2020		2016		202 0	
Tree Number	615		615		670		670		262		262		246		246		178		178	
Dead or Missing	6	1%	120	20%	15	2%	82	12%	0	0%	8	3%	2	1%	16	7%	7	4%	8	4%
Live trees with lost scaffolds	0	0%	161	26%	0	0%	52	8%		0%	3	1%	0	0%	2	1%	0	0%	9	5%
Live trees with observed gummosis	130	21%	517	84%	170	25%	426	64%	20	8%	60	23 %	33	13%	37	15%	54	30%	55	31 %

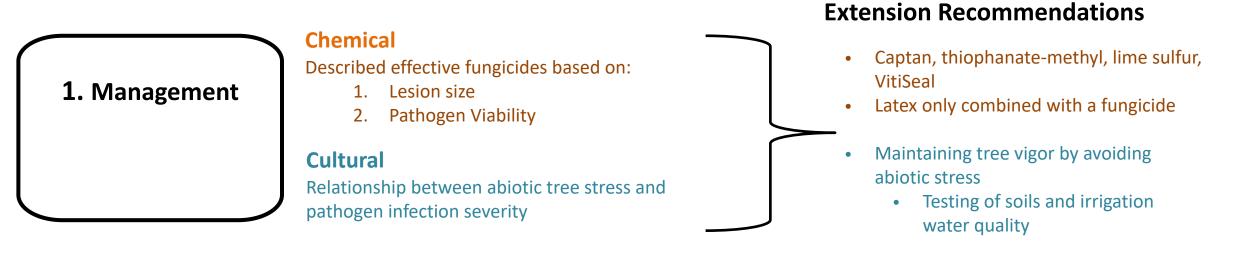
Greg Litus

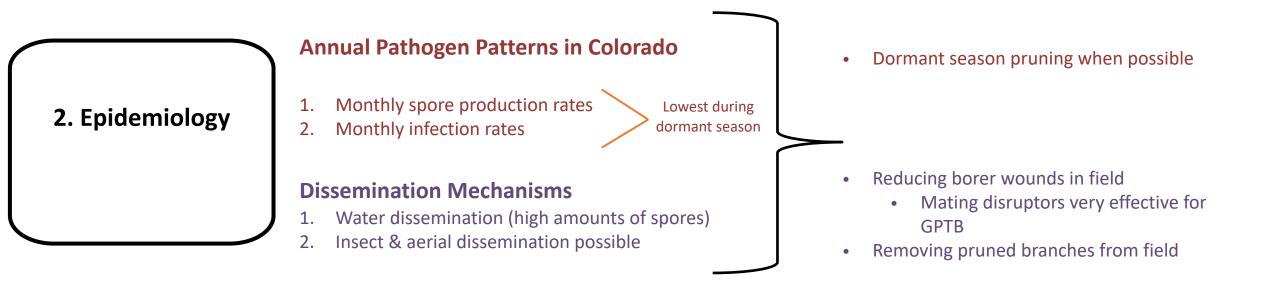
Orchard surveys and Cytospora spp. collections

- Surveyed 6 peach, 2 cherry, and 2 apple block, thus far
- Continue surveying orchards
- Collected 56 *Cytospora* isolates that represent 6 different species
- Estimate spread of *Cytospora* within and across orchards
- Perform pathogenicity assays on peach, cherry and apple

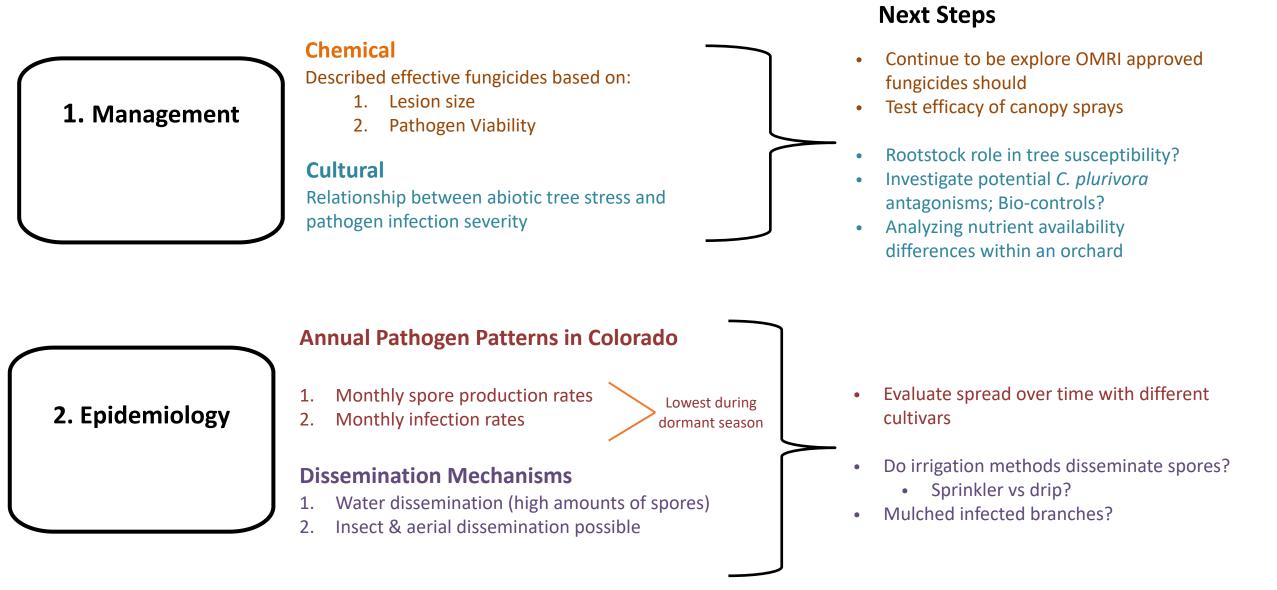


Overview of results from Cytospora program





Overview next steps of Cytospora program



Cytospora Working Group

Objectives

- Collaborate with local commercial growers to prioritize research efforts
- Prevention/Protection measures
- Disease management/spread measures
- Support funding opportunities Letters of support
- Next Meeting in Spring 2022

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Want to join? Contact:
Jane Stewart: <u>Jane.Stewart@colostate.edu</u>
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Thanks!



Jane.Stewart@colostate.edu





70+ years dedicated to the future development of agriculture in Western Colorado











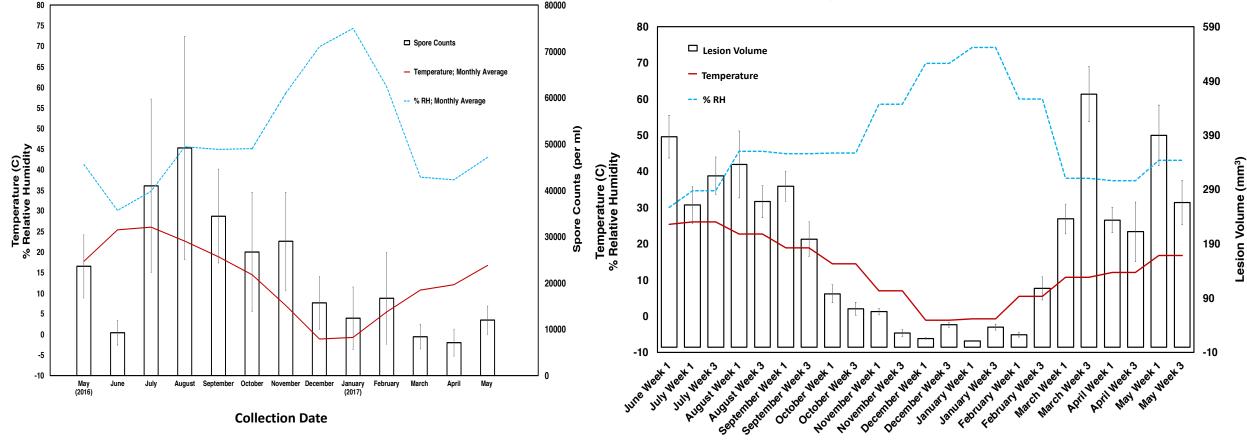
Collaborators:

Cytospora working group, CropWorx, Ioannis Minas, David Sterle, EmilyDowdy, Jeff Pieper, Bryan Braddy, Meredith Shrader, Jorge Ibarra Caballero, Luke Tembrock, Greg Litus, Frank Stonaker, Harold Larsen, Conner Henderson

Upper Grand Valley

Pest Control

District



Spore Production Occurs Year-Round

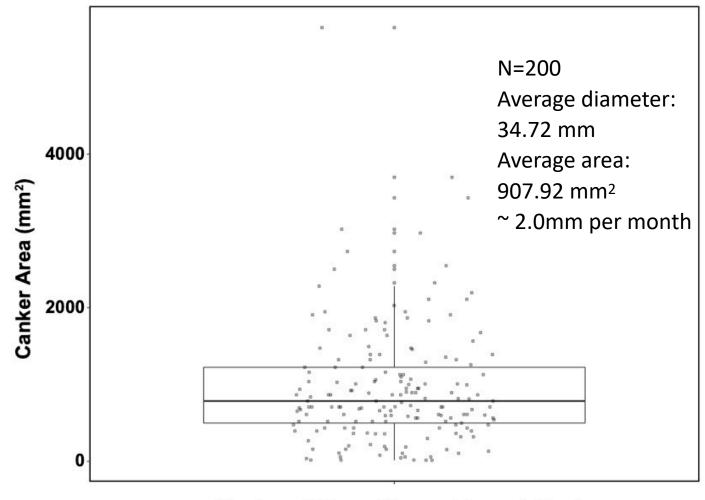
Infections Possible Year-Round; Largest Lesions >10°C

Inoculation Date

n= 25 repetitions, 5 averaged observations per date.

Canker growth over 20 months





Cankers (20 months post inoculation)

Environmental Conditions drive Cytospora canker

Year- Round Spore Production and Infection Rates Driven by Temperature

