Cytospora Canker on Colorado Peaches: Current Research

Jane E. Stewart
Plant Pathologist
Colorado State University
Bioagricultural Sciences and Pest Management

Cytospora canker

- Caused by fungal species *Cytospora*
- Opportunist pathogens, cannot invade healthy intact bark

Pruning cut with infection

canker

canker

Disease cycle

Crotches with narrow branch angles

Unhealed pruning stub

Shaded winter damaged twig

Leaf scars, damaged buds

Rain

Humid, wet

Conidia spread by rain; germinate and infect injured and dead tissues, expands as cankers

Poorly healed wound

Time

Canker rings formed by infection and host callus formation

Fungus persists and forms fruiting structures in dead wood and cankers

Biggs and Grove 2006. Plant Health Instructor

Cytospora presence in Colorado

Surveyed

- 200 acres
- 42 varieties
- 2-32 year old orchards (mean 11 years)

Results

- **100% of orchards surveyed infected** (mean 75% infected)
- **Ave number of infections per tree was 5.2** (range 0-27)
- Currently analyzing relationships between practices and infection

Questions

- How many *Cytospora* species cause infections on peaches ?
- What chemicals are effective against Cytospora?
  - Preventative vs. suppressive?
- Are trees susceptible year around?

Species differ biologically

- Three species have been identified on peach in other peach growing regions:
  - *Cytospora leucostoma*
  - *Cytospora paraleucostoma*
  - *Cytospora cincta*

- Variation in occurrence and virulence on different peach varieties

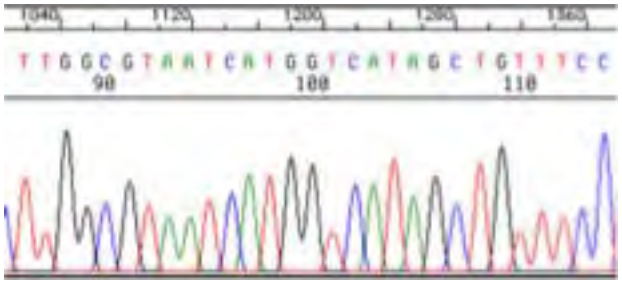
- Found on different locations of the tree

What species are found at Orchard Mesa and Rogers Mesa?

- Collections made in July-August 2015
  - 135 isolates were recovered at different elevations
  - Identified species based on DNA sequencing

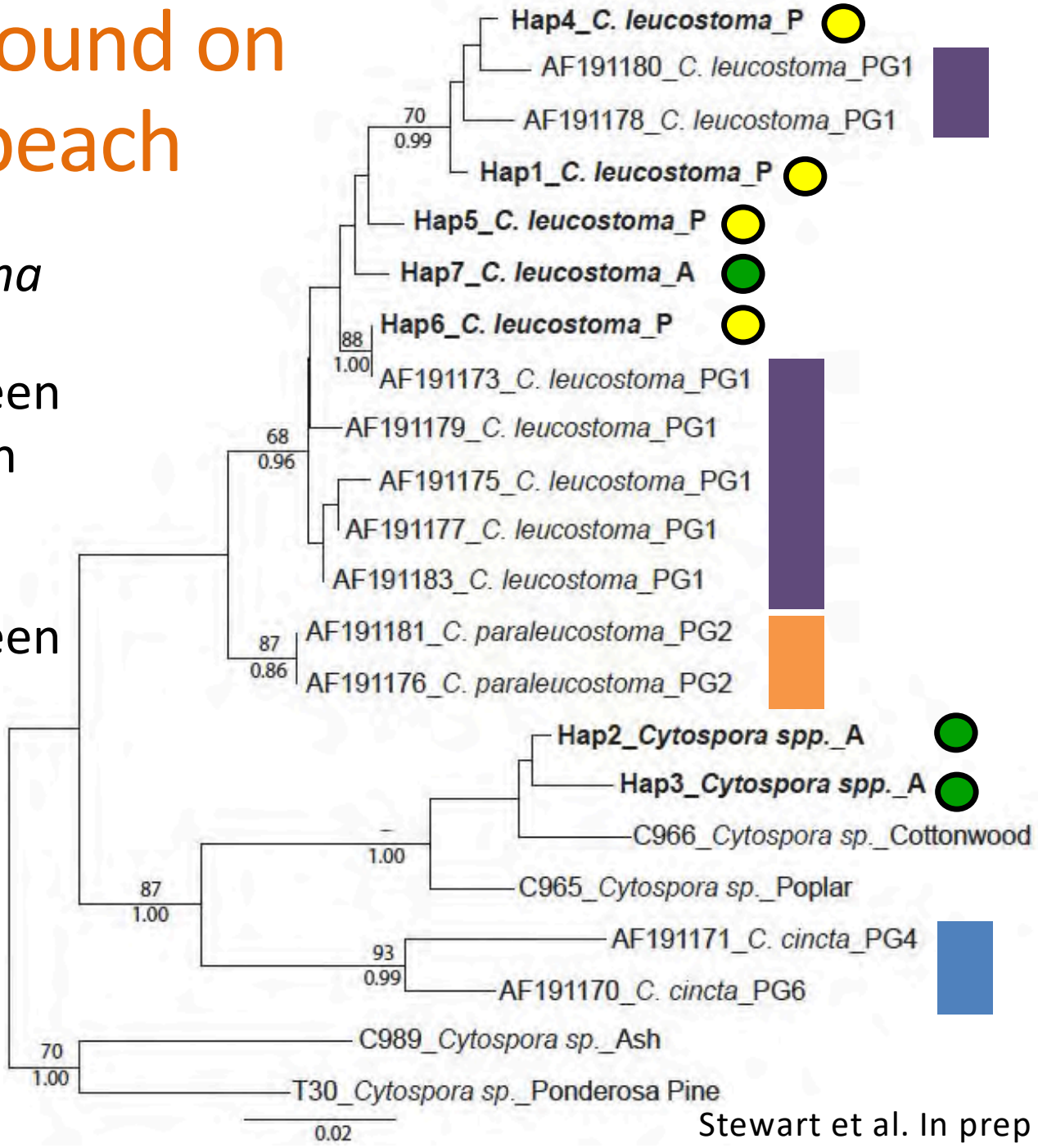
K. Kimbrough

H. Larsen



One species found on Colorado peach

- *Cytospora leucostoma*
- No difference between orchards low vs. high elevation
- No difference between winter/summer isolates
- Apple and peach isolates genetically distinct



Stewart et al. In prep

Risks of other species introduced into Colorado

- Virulence
  - Difference among *C. leucostoma, C. paraleucostoma*, *C. cincta*
- Growth at different temperatures
- Fungicide sensitivity
- Sporulation
- Differences with peach varieties

Questions

- How many *Cytospora* species cause cankers and gummosis ?
- What chemicals are effective against Cytospora?
  - Preventative vs. suppressive?
- When are trees susceptible?

What chemicals are effective against Cytospora?

- Evaluate the efficacy of conventional and organic fungicides for *Cytospora leucostoma* control
- Test wound sealing alternatives to develop preventive and spore suppressive approaches in existing orchards

Stephan Miller

Infected prune wound

C. leucostoma canker

Miller et al. Plant Disease. submitted

Chemical Testing Phases

Laboratory Assay:

- Testing chemicals *in vitro* on plates
- Testing chemicals on detached branches

Field Trials:

- Testing chemical sprays as preventive measure on branches
- Testing chemicals embedded in paints as preventive measure on branches
- Testing chemicals on existing cankers for spore suppression

Conventional Chemicals Evaluated

Treatment name	Active ingredient	Label rate (per 200 gal.)	Rate chosen	Mode of Action
Microthiol Disperss	Sulfur	10-20 lb	15 lb	Multi-site
Fontelis	Penthiopyrad	14-20 oz	17 oz	Respiration
Torino	Cyflufenamid	3.4 oz	3.4 oz	Unknown
Pristine	Pyraclostrobin & Boscalid	10.5-14.5 oz	12 oz	Respiration
Aliette WDG	Fosetyl	10 lb	10 lb	Unknown
Topsin M WSB	Thiopthanate-methyl	1-1.5 lb	1.25 lb	Cytoskeleton/ motor proteins
Benlate WP	Benomyl	24-32 oz	28 oz	Cytoskeleton/ motor proteins
Captan	N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide	3-4 qt	3.5 qt	Multi-site
Inspire Super	Difencoconazole & Cyprodinil	16-20 oz	18 oz	Protein synthesis
Ziram	Zinc dimethyldithiocarbamate	3 - 5.3 lb	1.15 lb	Multi-site

Organic Chemicals Evaluated

Treatment name	Active ingredient	Label rate (per 200 gal.)	Rate chosen	Mode of Action
CaCl	CaCl	48 oz	48 oz	Multi-site
Neem Oil	Neem Oil	3 qt	3 qt	Not classified
Mpede	Potassium salts	2-4 gal	3 gal.	Multi-site
Kaligreen	Potassium bicarbonate	2.5-3 lb	2.75 lb	Not classified
Serenade	Bacillus subtilis	14-20 oz	17 oz	Lipid synthesis/ transport
NuCop WP	Copper Hydroxide	8-20 lb	10 lb	Multi-site
Badge X2	Copper Hydroxide & Copper Oxychloride	3.5-5.25 lb	4.25 lb	Multi-site
ZnSO4	ZnSO4	4-6 lb	5 lb	Multi-site
Lime sulfur	Calcium polysulfide	20-24 gal.	22 gal.	Multi-site

Methods

In vitro chemical plates

1. Chemical treatments amended in agar solution, at commercial mid-rate

2. Isolates inoculated onto plates

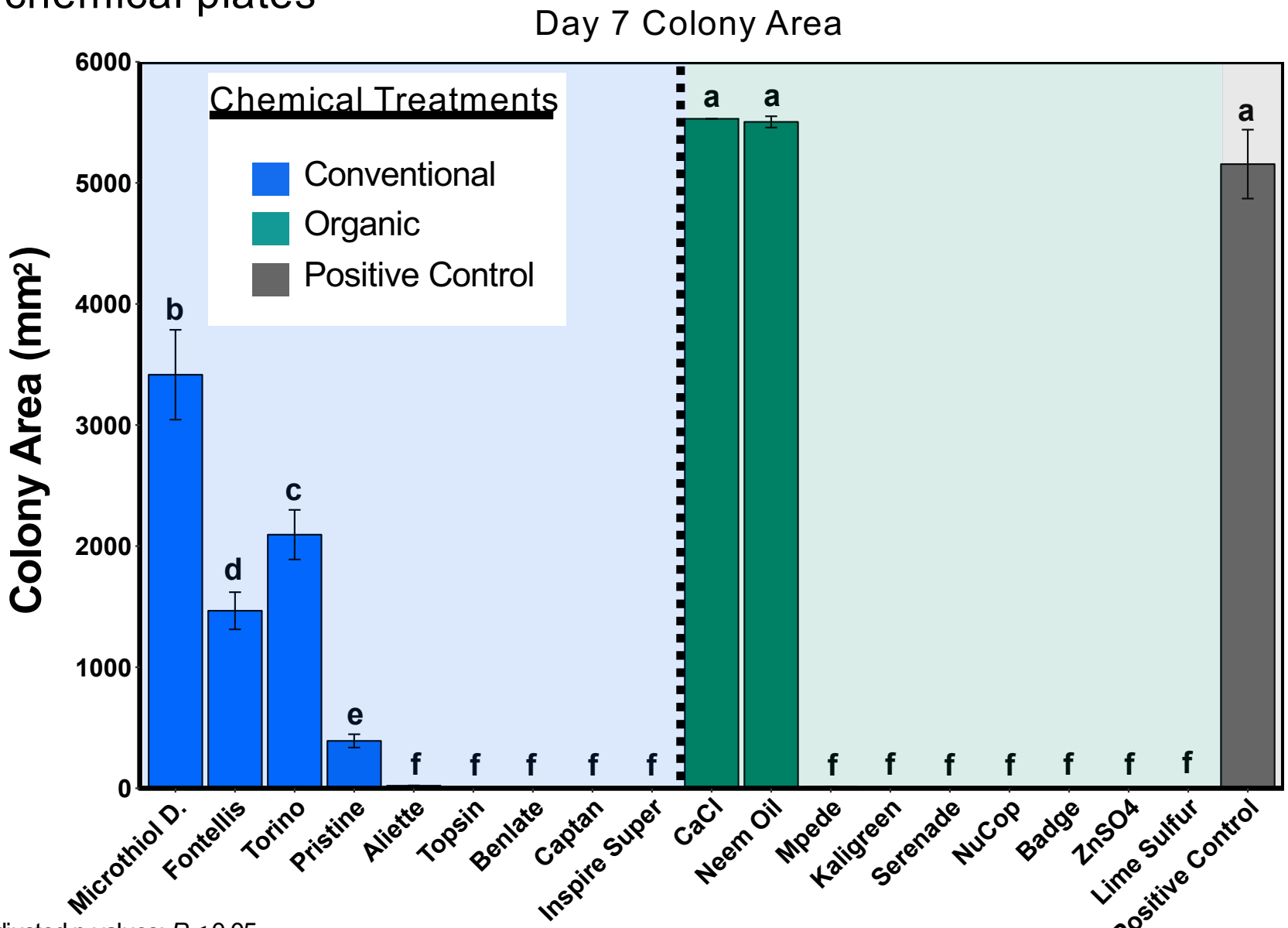
3. Inoculated plates incubated at 25°C for 7 days

4. Colony areas assessed every 24 hours

***C. leucostoma* growth in chemically amended plates**

Several Effective Conventional + Organic Chemicals

In vitro chemical plates



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

Methods

Detached branches, chemically submerged

1. Detached branches wounded then submerged in mid-rate chemical solutions for 5 minutes

Conventional chemicals:

Aliette, Topsin, Benlate, Captan, Inspire, Ziram

Organic chemicals:

Neem oil, Mpede, Kaligreen, Serenade, NuCop, Badge, ZnSO4, lime sulfur

2. *Cytospora* inoculated onto wounded branches

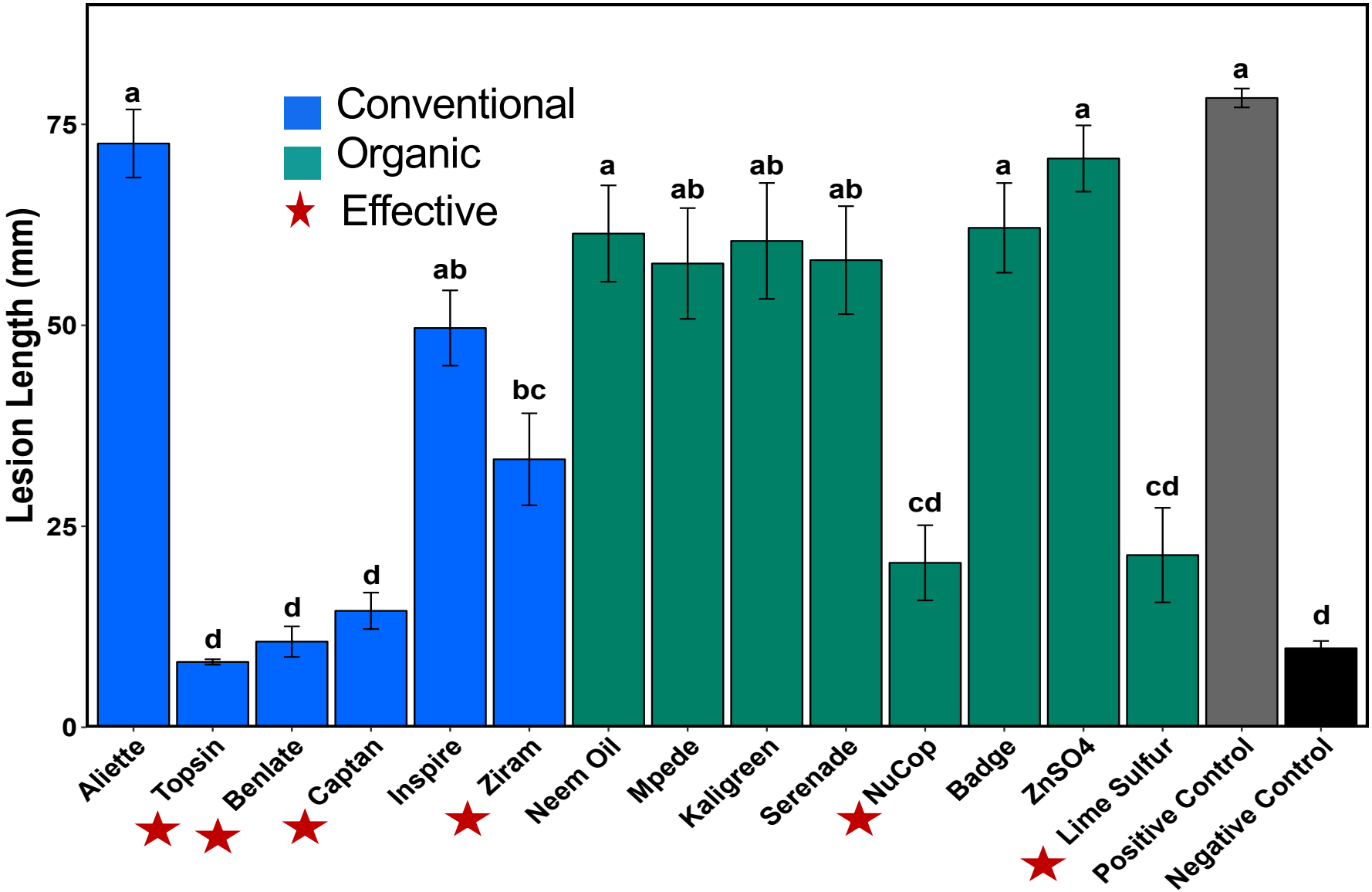
3. Lesion lengths assessed 8 days post inoculation

Inoculated detached branches

C. leucostoma lesion

C. leucostoma lesion

Several Conventional + Organic Chemicals Effective



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

Methods

Chemical field sprays

1. Wounds made on 1-year wood
2. Label mid-rate chemical sprays applied after wounding
   - Conventional: Topsin and Captan
   - Organic: Lime Sulfur and NuCop
3. Inoculation (Summer, Fall, Spring)
4. Branches harvested

Cytospora Inoculation

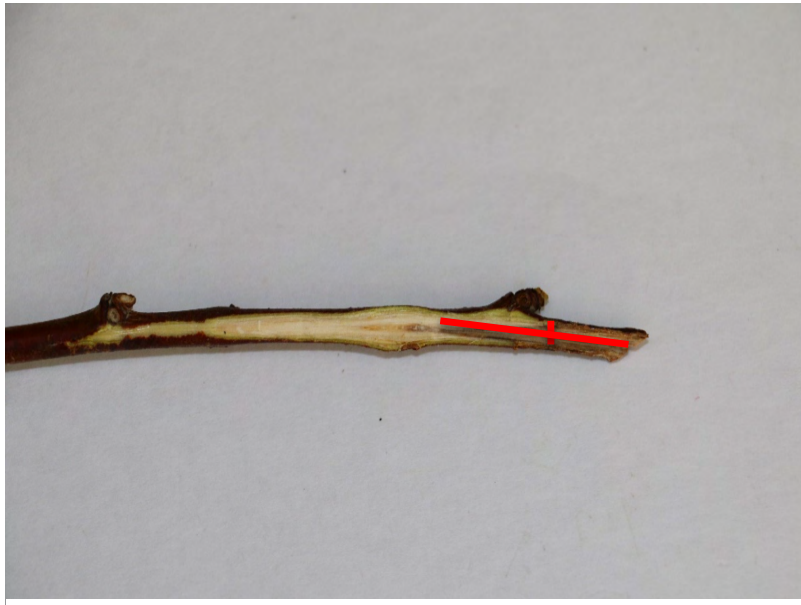
Cytospora lesion

Prune wound on 1
peach shoo

Chemical applications

Inoculation on peach
shoot pruning cut

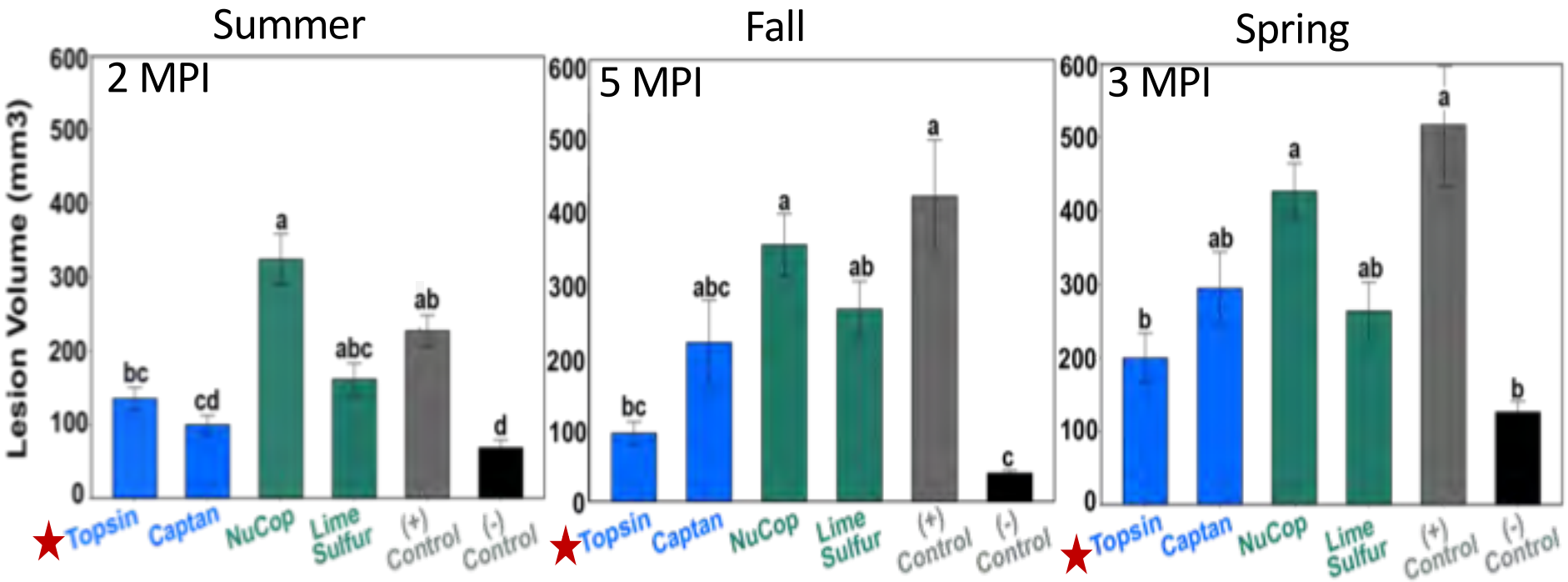
Opening *Cytospora* infected branch



Cytospora lesion in prune wound

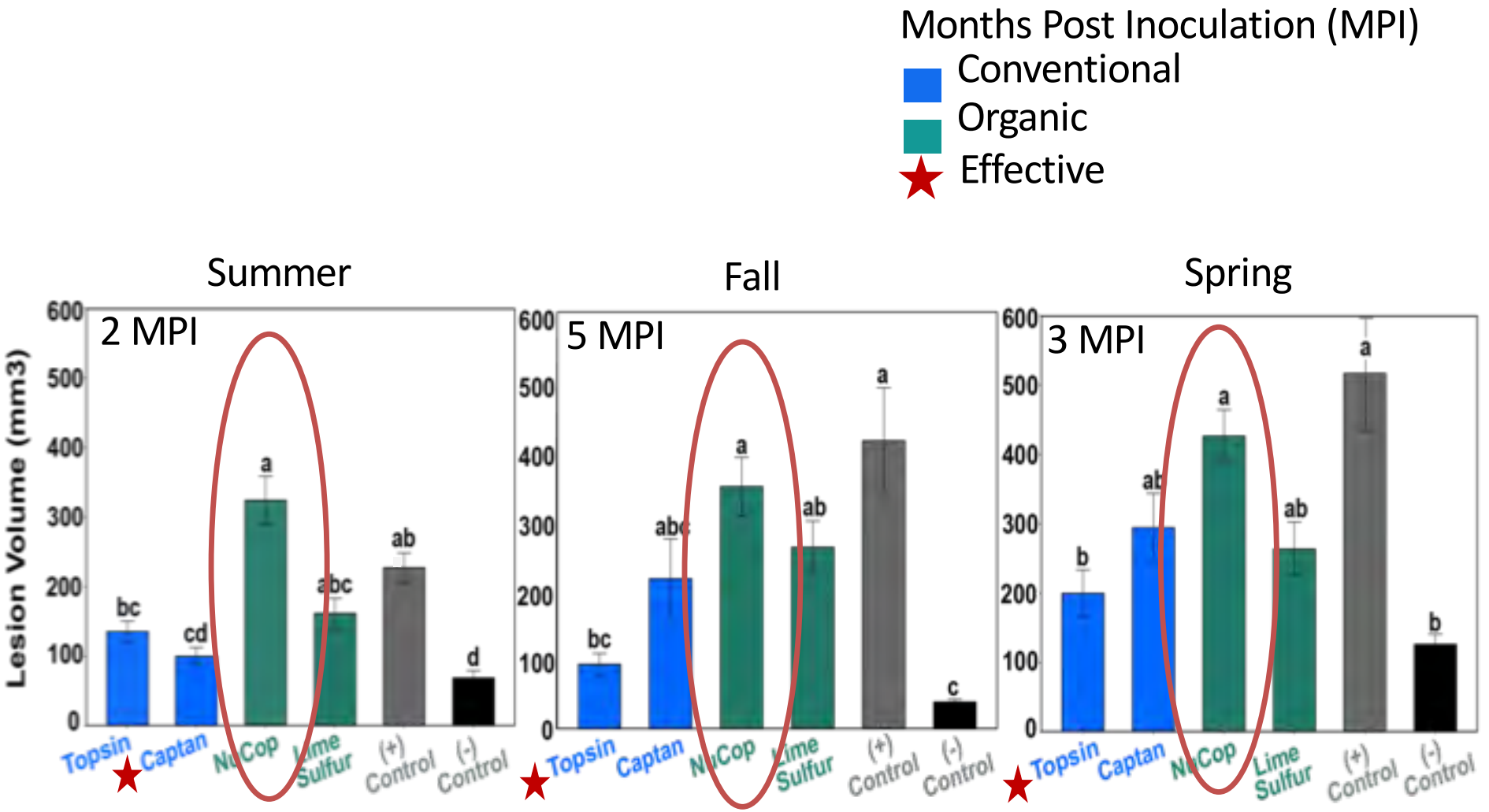
Topsin and Captan Seasonal Efficacy

Months Post Inoculation (MPI)
- Conventional
- Organic
- ★ Effective



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

NuCop Potential Phytotoxicity to Peach



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

Methods

Chemical paint field sprays

1. Wounds made on 1-year wood

2. Label mid-rate chemical sprays applied after wounding
   - Conventional: Latex, Topsin and Captan
   - Organic: kaolin clay (Surround), Lime Sulfur and NuCop

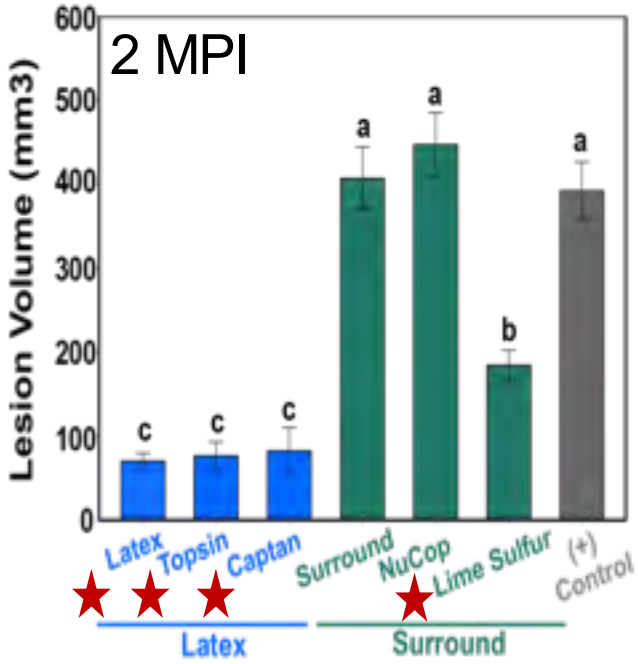
3. Inoculation (Summer, Fall, Spring)

4. Branches harvested

Latex Combinations and Lime Sulfur Show Evidence of Seasonal Efficacy

Months Post Inoculation (MPI)
- Conventional
- Organic
- ★ Effective

Summer



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

Infected Prune Wound on Peach Tree

Most Effective **Preventive** Treatment?

- Conventional treatments:
  - **Topsin**
  - **Captan**
  - **50% Latex** (Combinations)
- Organic treatments:
  - **Lime Sulfur**

Chemical Testing Phases

Laboratory Assay:
Testing chemicals *in vitro*

Laboratory Assay:
Testing chemicals on detached branches

Field Trials:
- Testing chemical sprays as preventive measure on branches
- Testing chemicals embedded in paints as preventive measure on branches
- Testing chemicals on existing cankers for spore suppression

Methods

Painting existing cankers

1. *Cytospora* cankers selected randomly in field

2. 10 ml of water (spore effluent) collected from cankers for pre-treatment measurements

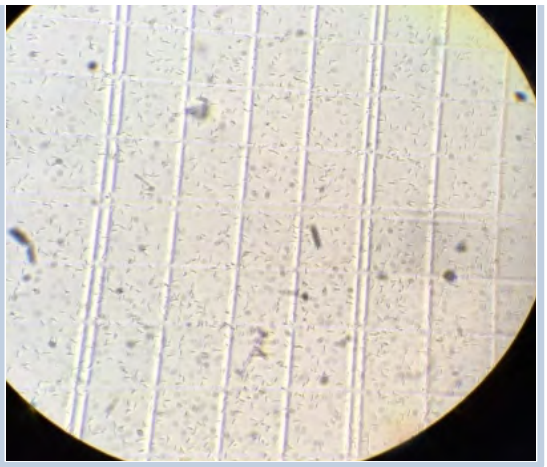
3. Chemicals paint combinations applied to cankers:

- **Conventional Chemicals:** Topsin + latex, Captan + latex, and latex
- **Organic Chemicals:** Lime sulfur + Surround, NuCop + Surround and Surround

4. Spore effluent collected from cankers after chemical applications once a month for 7 months

Spore effluent collection from canker

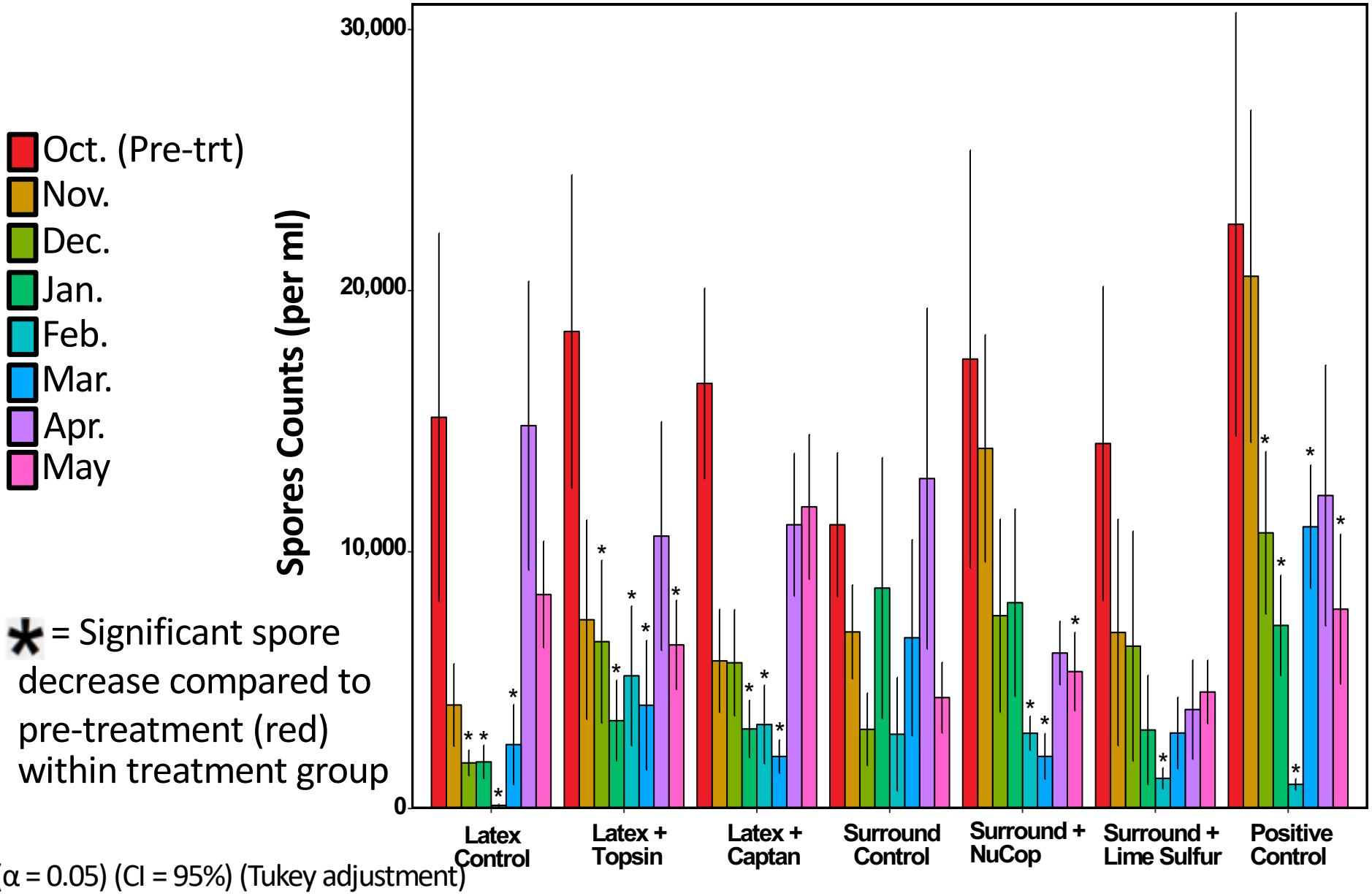
Spore counts in hemacytometer



Pre-treatment *Cytospora* canker

Latex treated *Cytospora* canker

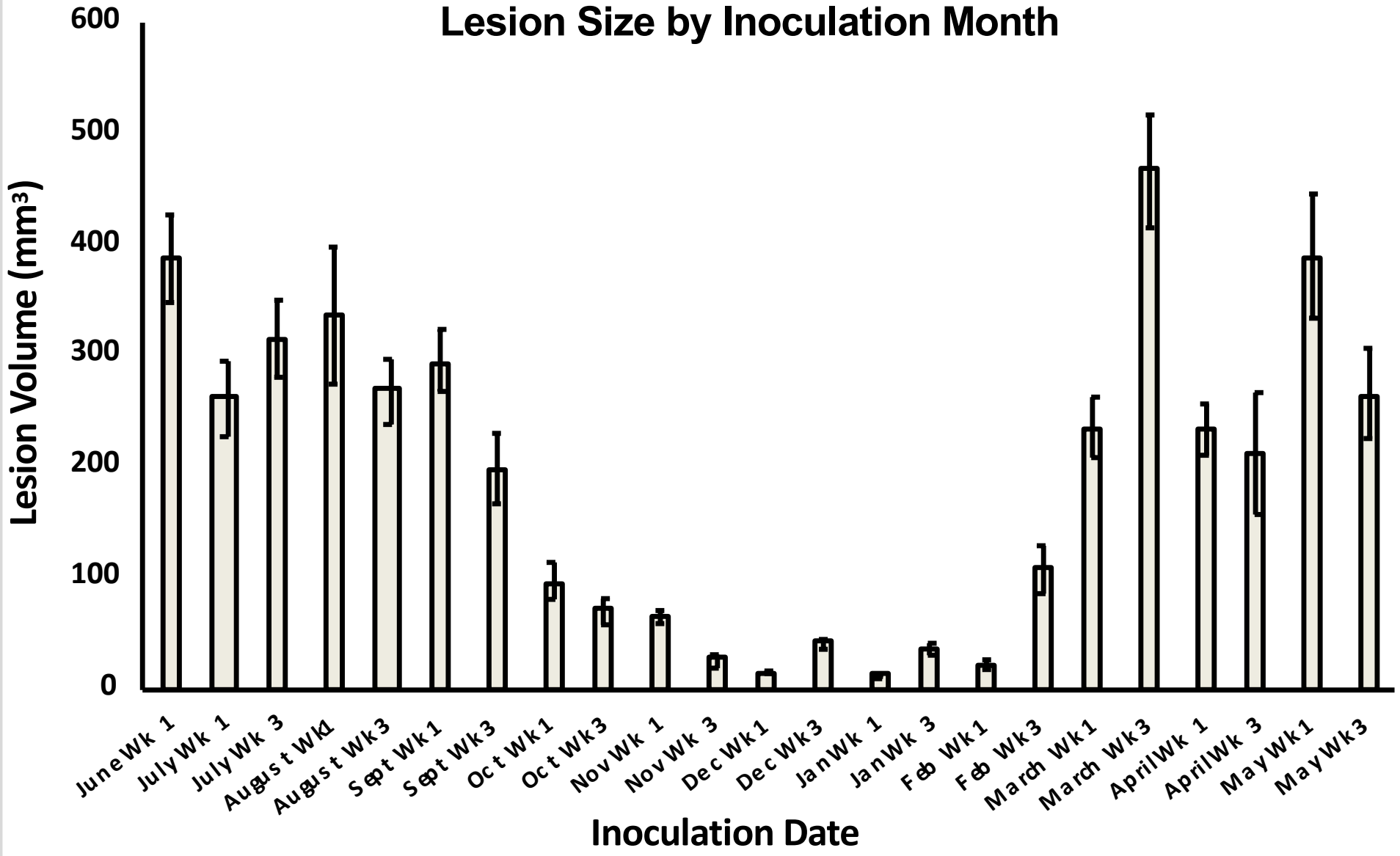
Continuous Spore Production in all Treatments



Questions

- How many *Cytospora* species cause cankers and gummosis ?
- What chemicals are effective against Cytospora?
  - Preventative vs. suppressive?
- When are trees susceptible?

Cytospora can infect peach at any season



Cytospora research summary 2016-2017

- *Cytospora leucostoma* was identified as fungal pathogen
- Chemical options for preventive and suppressive control
- Trees are susceptible year round
- The development of a molecular tool to study epidemiology
- Spores are dissemination through the year, when conditions are favorable

Most Effective **Preventive** Treatments (Conventional)

Treatment name	Active ingredient	Label rate (per 200 gal.)	Rate chosen	Mode of Action
Microthiol Disperss	Sulfur	10-20 lb	15 lb	Multi-site
Fontelis	Penthiopyrad	14-20 oz	17 oz	Respiration
Torino	Cyflufenamid	3.4 oz	3.4 oz	Unknown
Pristine	Pyraclostrobin & Boscalid	10.5-14.5 oz	12 oz	Respiration
Aliette WDG	Fosetyl	10 lb	10 lb	Unknown
Topsin M WSB	Thiopthanate-methyl	1-1.5 lb	1.25 lb	Cytoskeleton/ motor proteins
Benlate WP	Benomyl	24-32 oz	28 oz	Cytoskeleton/ motor proteins
Captan	N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide	3-4 qt	3.5 qt	Multi-site
Inspire Super	Difencoconazole & Cyprodinil	16-20 oz	18 oz	Protein synthesis
Ziram	Zinc dimethyldithiocarbamate	3 - 5.3 lb	1.15 lb	Multi-site

Most Effective **Preventive** Treatments (Organic)

Treatment name	Active ingredient	Label rate (per 200 gal.)	Rate chosen	Mode of Action
CaCl	CaCl	48 oz	48 oz	Multi-site
Neem Oil	Neem Oil	3 qt	3 qt	Not classified
Mpede	Potassium salts	2-4 gal	3 gal.	Multi-site
Kaligreen	Potassium bicarbonate	2.5-3 lb	2.75 lb	Not classified
Serenade	Bacillus subtilis	14-20 oz	17 oz	Lipid synthesis/ transport
NuCop WP	Copper Hydroxide	8-20 lb	10 lb	Multi-site
Badge X2	Copper Hydroxide & Copper Oxychloride	3.5-5.25 lb	4.25 lb	Multi-site
ZnSO4	ZnSO4	4-6 lb	5 lb	Multi-site
Lime sulfur	Calcium polysulfide*	20-24 gal.	22 gal.	Multi-site

* Rate was 3% Lime sulfur

Funding: USDA-CDA Specialty Crop February 2018 – November 2019

Project title: Cytospora management in peach orchards through cultural practices, cultivar selection, and stress mitigation

- PIs: Jane E. Stewart & Ioannis S. Minas
  - Research Associate: David Sterle
  - Student: Stephan Miller – PhD Research

CDA CO COLORADO Department of Agriculture

Project 1 - Preventive Chemical Applications

Started March 20

- Topsin
- Vitaseal
- Vitaseal + Topsin
- 70% Latex
- 50% Latex + Topsin
- 70% Latex + Topsin

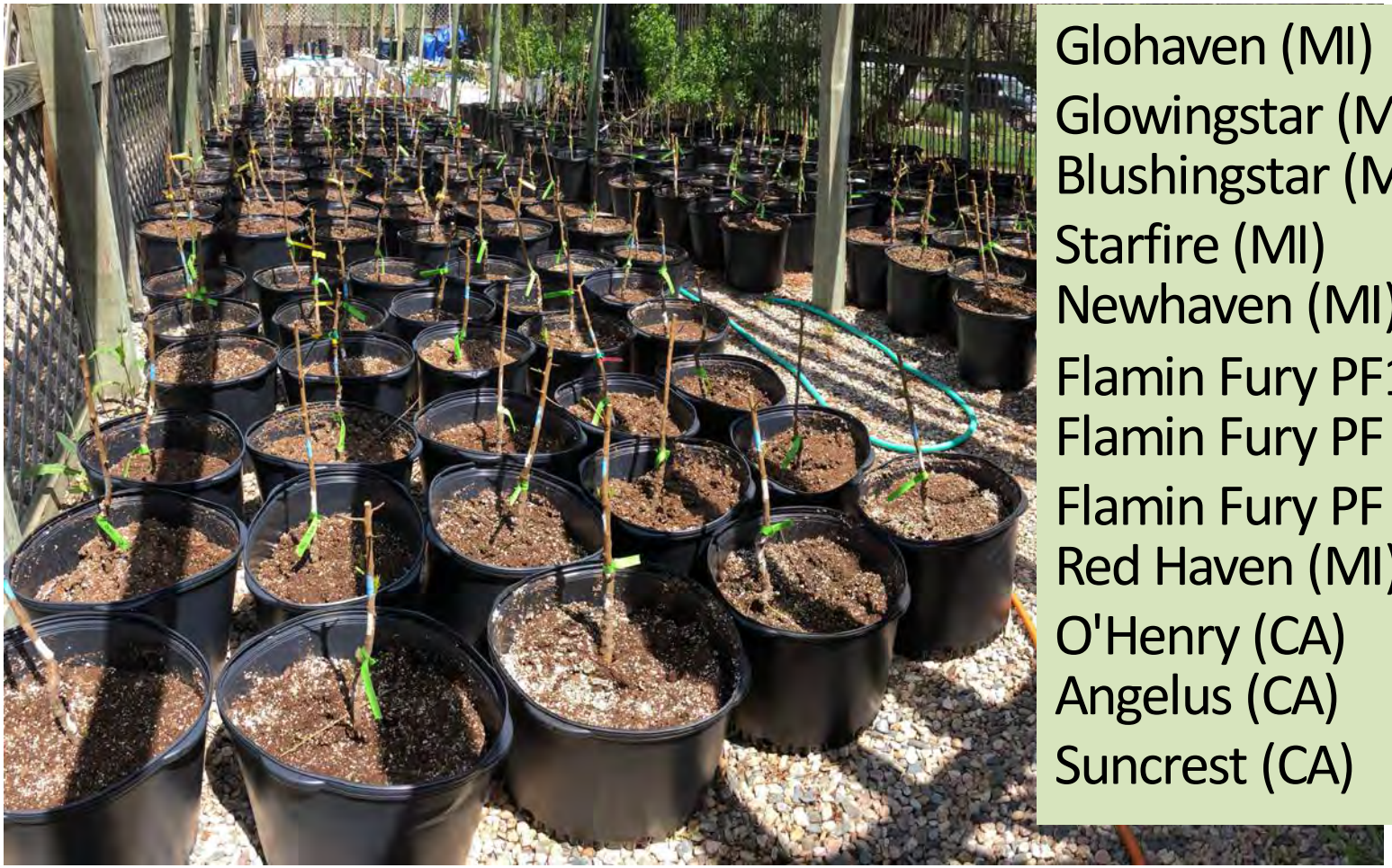
- JMS Oil + Lime Sulfur
- Nufilm + Lime Sulfur
- 70% Latex + Lime Sulfur

Project 2 - Preventive Chemical Applications

Vitaseal + Topsin
Latex + Topsin
Vitaseal + Lime Sulfur

Project 3 - Tolerance of cultivars to *Cytospora*

Tolerance under high pH and drought



Glohaven (MI)
Glowingstar (MI)
Blushingstar (MI)
Starfire (MI)
Newhaven (MI)
Flamin Fury PF19-007 (MI)
Flamin Fury PF 23 (MI)
Flamin Fury PF 24 (MI)
Red Haven (MI)
O'Henry (CA)
Angelus (CA)
Suncrest (CA)

Funding: USDA-CDA Specialty Crop February 2019 – November 2020

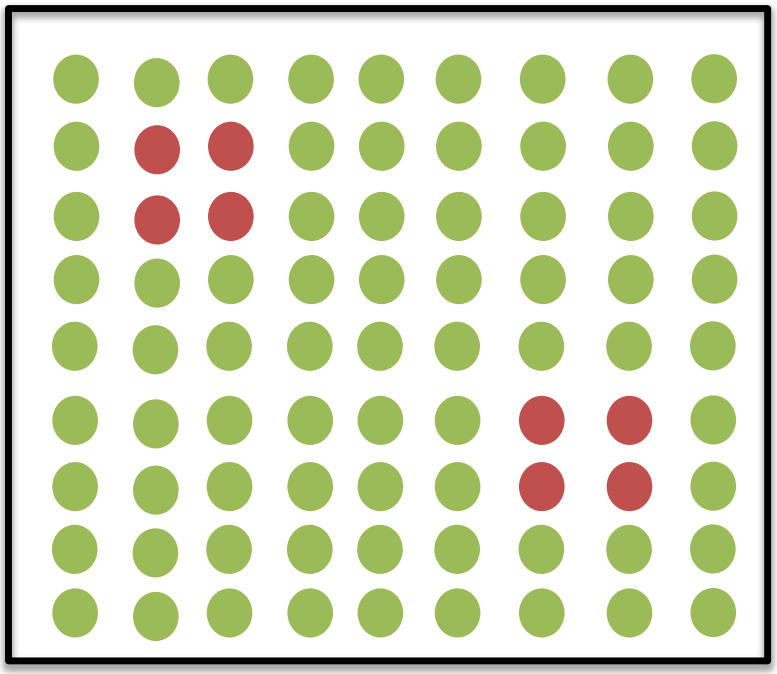
- **Project title:** Determining dispersal pathways of *Cytospora* for the development of management strategies for Cytospora canker on peaches
- PI: Jane E. Stewart
  - Student: Stephan Miller – PhD Research

Dispersal of spores/epidemiology
Wind, insects, pruning tools

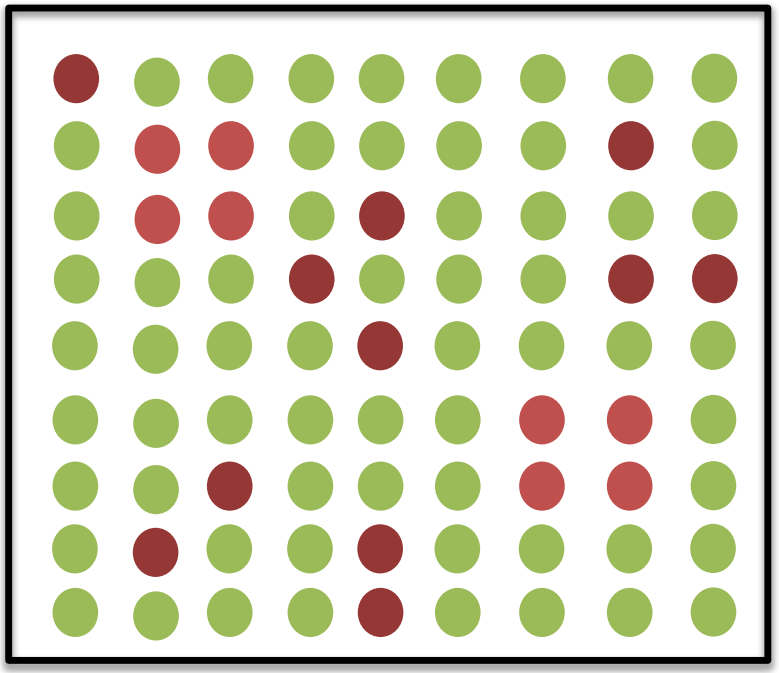
CDA CO COLORADO Department of Agriculture

Is inoculum spread similar 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?

The need for a molecular tool for *Cytospora* identification

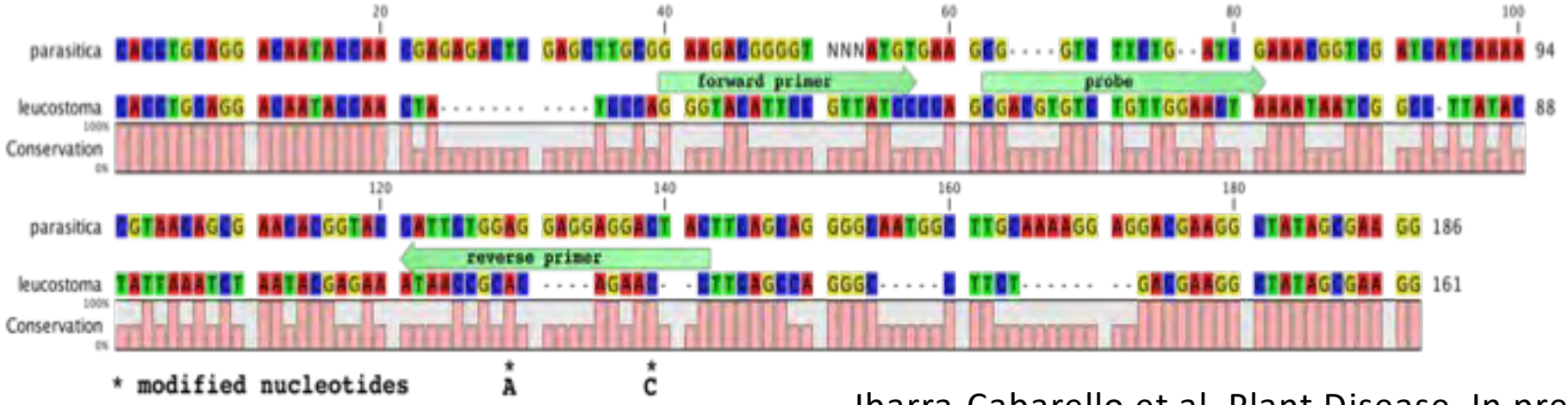
- Spores are small and difficult to differentiate
- *Cytospora* is a slow grower and is out competed by other fungi

Spores of *Cytospora leucostoma* at 40X

Marker development as an epidemiology tool

Jorge Ibarra-Cabarello

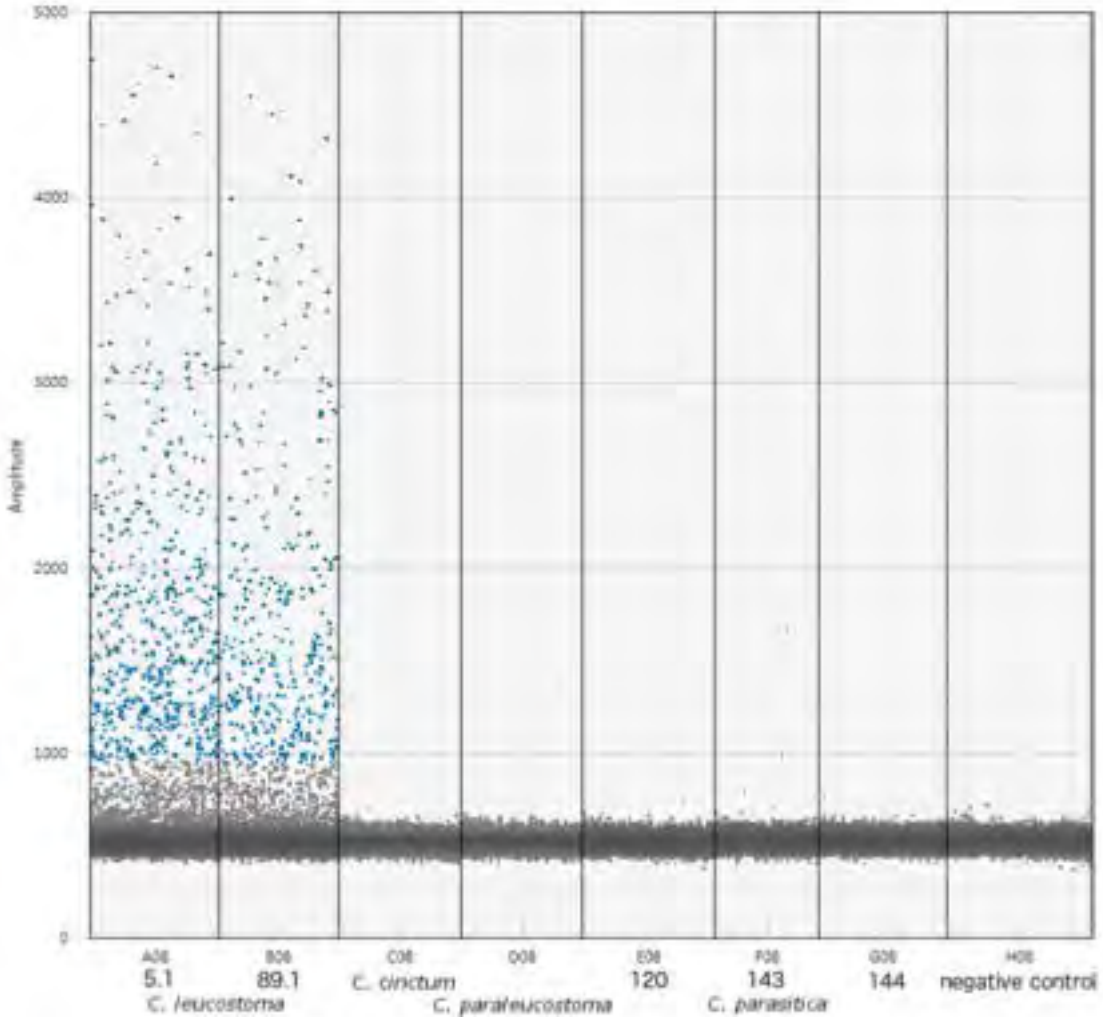
- Genomes of several Colorado isolates were used to identify unique regions in *C. leucostoma*
- A digital drop molecular assay was developed and tested against closely-related species of *Cytospora*



Ibarra-Cabarello et al. Plant Disease. In prep

Molecular assay identifies only *C. leucostoma*

- Collect insects and test for vectors
- Test pruning shears
- Test nursery stock



Thanks!

Colorado State University

Western Colorado Research Center

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

AGRICULTURAL EXPERIMENT STATION
COLORADO STATE UNIVERSITY

Upper Grand Valley Pest Control District

CO CDA
COLORADO
Department of Agriculture

Collaborators:
Greg Litus, Frank Stonaker, Harold Larsen, Jordge Lafantasie, Brady Shanahan, Anne Hess, Bruce Talbott, Cytospora working group – Larry Traubel and Steve Ela

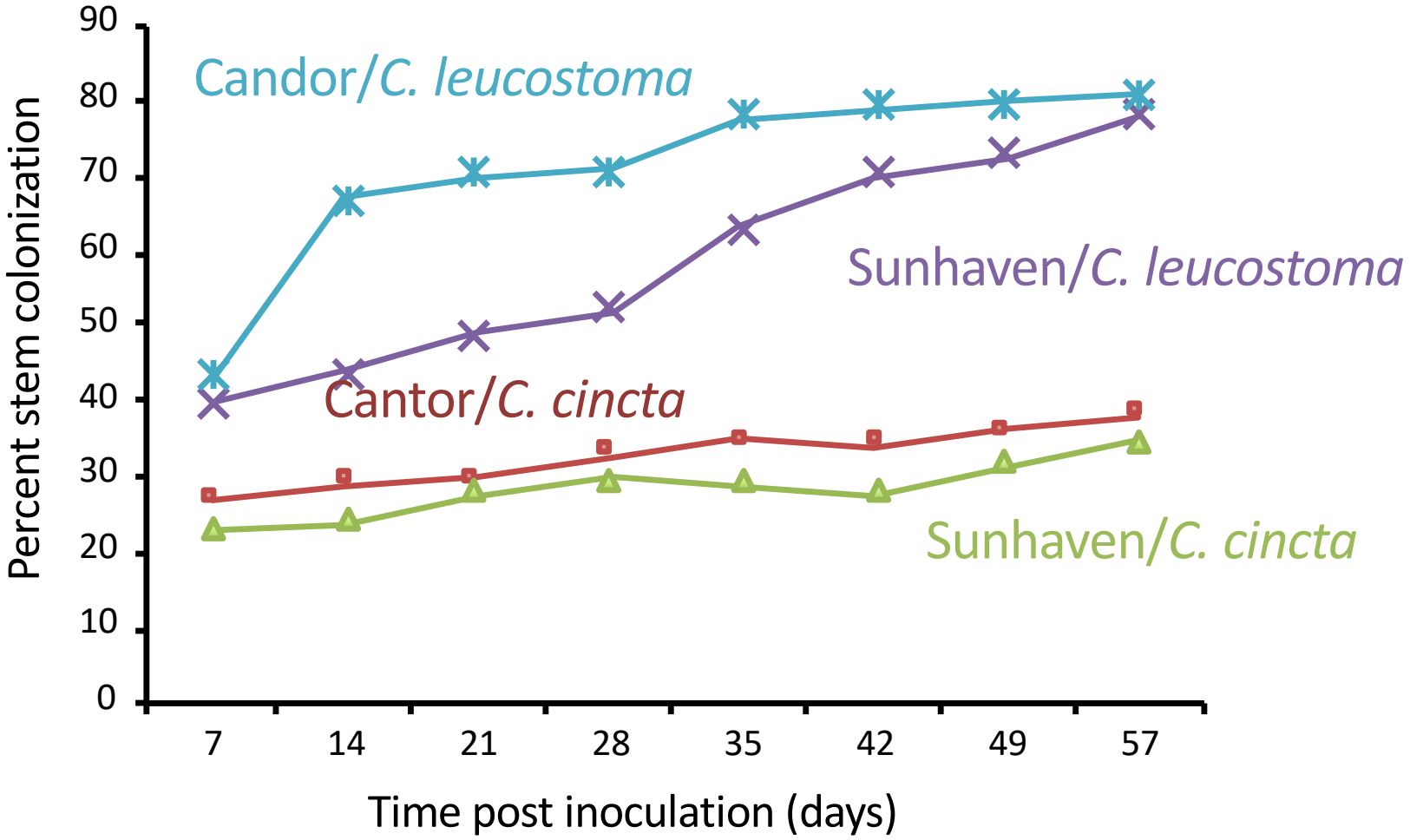
Cytospora Working Group

Objectives

- Collaborate with local commercial growers to prioritize research efforts
- Prevention/Protection measures
- Disease management/spread measures
- Support in funding opportunities

Want to join? Contact:
Jane Stewart: Jane.Stewart@colostate.edu
Ioannis Minas: Ioannis.Minas@colostate.edu

Species differ in virulence



Biggs. 1986. Phytopathology

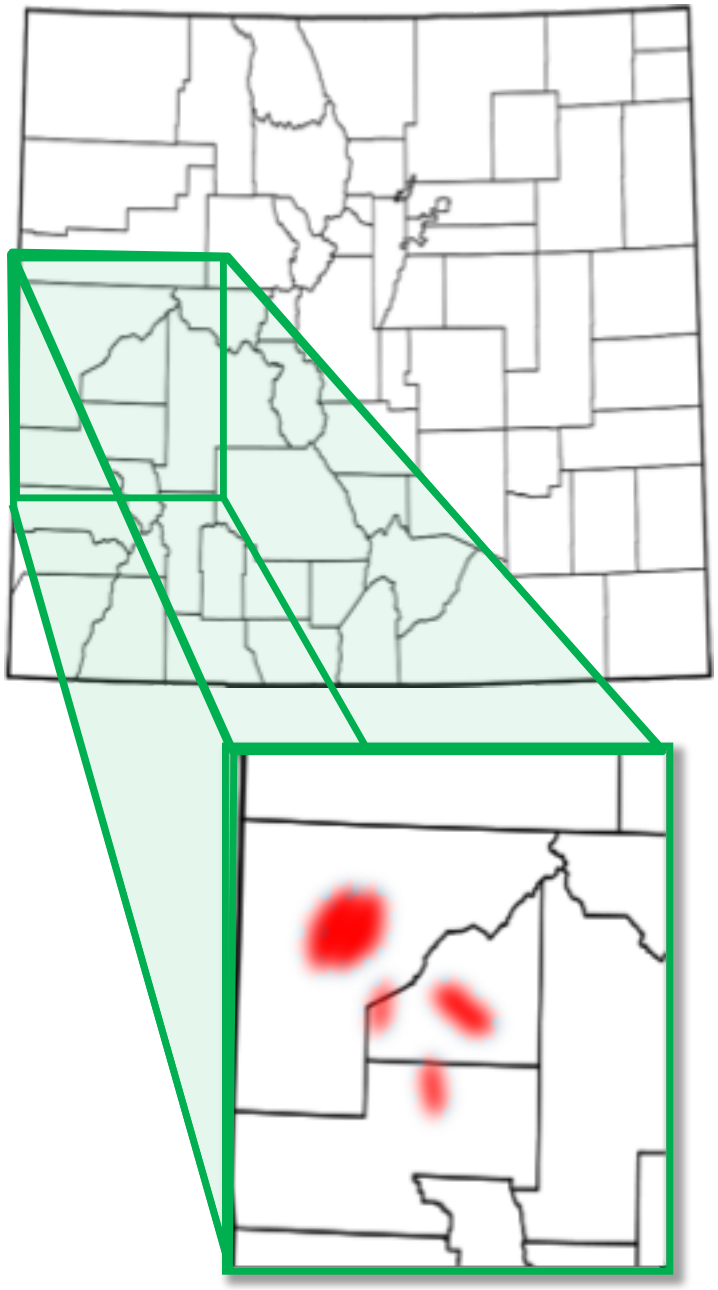
Species differ in location on tree

	Percent cankers for each causal pathogen		
Canker location	*C. leucostoma*	*C. cincta*	*C. paraleucostoma*
Branch	82.55	16.00*[c]	1.15 ns
Trunk	94.50**	4.50	0.17

Wang, Iezzoni, Adams Phytopathology.1998.

Recent Survey of Colorado Orchards

- Estimate incidence and severity of *Cytospora* in major peach production areas of Western Colorado
- March/April 2015
- Conducted in Grand Valley, North Fork and Olathe areas
  - Focus on gathering data from a widespread area and range of orchard management



Methods

- Surveyed by orchard and variety
- Recorded presence/absence for every 10th tree
- Counted and rated infection severity for every 50th tree

K. Kimbrough

- Interviewed growers
  - Irrigation
  - Orchard floor management
  - Pruning practices
  - Pre-plant practices (replant, fallow, rotation)
  - Frost protection
  - Fertilization practices
  - Pesticide application