Cytospora Canker of Dried Plum

Latent (endophytic) infection and prospects for management

Yong Luo, Franz Niederholzer, Dan Felts, & Themis J. Michailides

University of California, Davis
Kearney Agricultural Research and Extension Center

23 Feb 2018 - Red Bluff, CA
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 purpurencens*

2014
- **Cytospora leucostoma***
- Lasiodiplodia theobromae**
- Botryosphaeria dothidea*
- Diplodia seriatta**
- Nattrassia mangifera*,
- Phomopsis species*
- Paecilomyces variotii?*
- Fusarium species*

2014
- 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
- Diplodia seriata
- Fusarium sp.
- Nattrassia mangiferae
- Phomopsis sp.
- Phoma species
- Botryosphaeria dothidea
- Neofusicoccum mediterraneum
- Lasiodiplodia citricola
- Diplodia seriata
- Fusarium sp.
- Nattrassia mangiferae
- Phomopsis sp.
- Phoma species
Cytospora in dried plum
Inoculum sources for *Cytospora* canker:

pycnidia
Spores in pycnidia

Ascospores in perithecia

Airborne ascospores

Water-splashed spores
Spores ooze from pycnidia
Cytospora chrysosperma on pistachio

Spores ooze from pycnidia
Killed peach trees because of *Cytospora leucostoma* in Colorado (Grand Junction area)
Cytospora canker symptoms on peach pruning wounds
Pruning wound infection in dried plum
Susceptibility of pruning wounds to *Cytospora leucostoma* (2014/2015)

Days after pruning:
- 0 days
- 3 days
- 7 days
- 15 days
- 30 days

Canker length (cm):
- 0
- 5
- 10
- 15
- 20
- 25

Legend:
- abc
- ab
- a
- C
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 qPCR 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.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5<code>-3</code>)</th>
<th>Target species</th>
<th>size (bp)</th>
<th>temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhBT-F1</td>
<td>CATCGTTACTGACCTCGACTTT</td>
<td>Phomopsis spp.</td>
<td>102</td>
<td>82.5</td>
</tr>
<tr>
<td>PhBT-R1</td>
<td>ACGAGATTGAAGACAGGGAATAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BdF</td>
<td>CAGCGTGGGAGAACACATCAA</td>
<td>Botryosphaeria dothidea</td>
<td>103</td>
<td>81.5</td>
</tr>
<tr>
<td>BdR</td>
<td>GTGAGAGAGTACCTCGTTGAATAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LcBT-F2</td>
<td>CTGCTTTCTGTTTGTGCC/</td>
<td>Lasiodiplodia spp.</td>
<td>128</td>
<td>86</td>
</tr>
<tr>
<td>LcBT-R2</td>
<td>GAGAAGGCACACTTTACA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CtBTFF1</td>
<td>GAGCGCATGAACGTCTACTT/</td>
<td>Cytospora spp.</td>
<td>106</td>
<td>82.6</td>
</tr>
<tr>
<td>CtBtFR1</td>
<td>GGAAGAAAGCGCTACCTTTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NpBT-F2</td>
<td>ACCACAGGCAAGACCATTTTC/</td>
<td>Neofusicoccum spp.</td>
<td>118</td>
<td>86.4</td>
</tr>
<tr>
<td>NpBT-R2</td>
<td>GTCGGAGGTGCCATTGTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DpF</td>
<td>GTGTAAGTGGCGCTGCTTTG/</td>
<td>Diplodia spp.</td>
<td>118</td>
<td>84.8</td>
</tr>
<tr>
<td>DpR</td>
<td>GTAGAGAGTACCTCGTTGAATAG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
How our system works

**a) Sample collection and processing**

**b) Grinding and DNA extraction of samples**

**c) Real-time PCR assay**

**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):** 
   \[ MS = \log_{10}(\frac{P}{H}) \]
   \( P = \) 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

Incidence of latent infection (%)

Date


Phomopsis spp.

Botryosphaeria dothidea

Lasiodiplodia spp.

Cytospora spp.

Neofusicoccum spp.

Diplodia spp.

Orcchard 1 Orcchard 2 Orcchard 3

Incidence of latent infection (%)
For newly-emerged shoots

- **Phomopsis spp.**
- **Botryosphaeria dothidea**
- **Lasiodiplodia spp.**
- **Cytospora spp.**
- **Neofusicoccum spp.**
- **Diplodia spp.**
For newly-emerged shoots

- **Phomopsis spp.**
- **Botryosphaeria dothidea**
- **Lasiodiplodia spp.**
- **Cytospora spp.**
- **Neofusicoccum spp.**
- **Diplodia spp.**
For one-year-old shoots

- **Phomopsis spp.**
- **Botryosphaeria dothidea**
- **Lasiodiplodia spp.**
- **Cytospora spp.**
- **Neofusicoccum spp.**
- **Diplodia spp.**
For one-year-old shoots

- Phomopsis spp.
- Botryosphaeria dothidea
- Lasiodiplodia spp.
- Cytospora spp.
- Neofusicoccum spp.
- Diplodia spp.
For one-year-old shoots

Phomopsis spp.

Botryosphaeria dothidea

Lasiodiplodia spp.

Cytospora spp.

Neofusicoccum spp.

Diplodia spp.
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.
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
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 qPCR 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.
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.
➤ Species composition in 2017 was similar to that in 2016 spring.

➤ In 2017, spore concentrations were significantly lower than those of 2016 spring.
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…
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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topsin (thiophanate – methyl)</td>
<td>5 g a.i.</td>
</tr>
<tr>
<td>Quilt Excel (azoxystrobin + propiconazole)</td>
<td>5 g a.i.</td>
</tr>
<tr>
<td>VitiSeal</td>
<td>1:10 dilution</td>
</tr>
<tr>
<td>Pristine + Pentra Bark</td>
<td>5 g a.i. + 1 oz</td>
</tr>
<tr>
<td>Tebuconazole</td>
<td>5 g a.i.</td>
</tr>
<tr>
<td>Pristine + VitiSeal</td>
<td>5 g a.i.+ 1:10 dilution</td>
</tr>
<tr>
<td>Untreated control</td>
<td>---</td>
</tr>
</tbody>
</table>
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)
<table>
<thead>
<tr>
<th></th>
<th>Cytospora</th>
<th>Eutypa</th>
<th>B. dothidea</th>
<th>N. parvum</th>
<th>N. mediterraneum</th>
<th>Neosc. dimidiatum</th>
<th>D. mutila</th>
<th>Avg. recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>75</td>
<td>75</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>92.9</td>
</tr>
<tr>
<td>TerraNeem</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Proud</td>
<td>0</td>
<td>0</td>
<td>75</td>
<td>0</td>
<td>25</td>
<td>0</td>
<td>50</td>
<td>21.4*</td>
</tr>
<tr>
<td>Trichoderma</td>
<td>100</td>
<td>25</td>
<td>33</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>100</td>
<td>58.3</td>
</tr>
<tr>
<td>Quash</td>
<td>100</td>
<td>75</td>
<td>75</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>32.1*</td>
</tr>
<tr>
<td>Topsin M</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>25</td>
<td>32.1*</td>
</tr>
<tr>
<td>Paint</td>
<td>75</td>
<td>50</td>
<td>75</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>85.7</td>
</tr>
<tr>
<td>CropSeal</td>
<td>100</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Rally</td>
<td>100</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>89.3</td>
</tr>
<tr>
<td>Indar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fontelis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inspire Super</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luna</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quilt Xcel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viathon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luna Experience</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bravo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quadris Top</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Merivon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pristine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abound</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ziram</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg. recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

California Dried Plum Board

UCCE
- Franz Niederholtzer
- Dani Lightle
- Rick Buchner

Various chemical companies for financial support; & dried plum growers for allowing us to work in their orchards!