Syngenta’s Citrus Soil Assay for *Phytophthora* 

Kendra McCorkle 
Last Updated: 10/02/16
Contents

- My Background
- Topic selection
- Module Contents
- Value of the learning module
- Acknowledgements
- Questions
Background Information - Personal

- Florida Native
  - Reside on FL’s East coast
- Family in the citrus industry
Background Information - Personal
Background Information - Professional

- Indian River State College (2008-2010)
  - A.A. Environmental Science

- Syngenta Internships

- University of Florida (2010-2012)
  - B.S. Environmental Management
  - Minor Soil and Water Science

- Syngenta Crop Protection
  - R&D Specialist (2012)

- Iowa State University (2014-2016)
  - M.S. Agronomy
Topic selection

- Syngenta invested in my degree
- Current role entails fungicide efficacy trials
  - Another research project?
- Passionate about citrus
- Topic: Syngenta’s Citrus Soil Assay program
  - Important piece of my history

Why a learning module?

- Create a training document for Syngenta
  - Basic introduction to the program
  - Used for internal and external customers
Module Contents

- Florida citrus
- Major diseases in Florida citrus
- Citrus *Phytophthora*
- Citrus greening
- *Phytophthora* and citrus greening interaction
- Syngenta’s citrus soil assay
Florida Citrus

● 500,000 total acres of citrus in FL
  - 453,000 acres of oranges (~90% of total)
  - 46,000 acres of grapefruit (~10% of total)

● Generates $9 billion for FL economy (Florida Citrus Mutual, 2012)

● Florida provides 80% of the orange juice produced in the U.S. (Fox-Skelley, 2015)
Devastating Freezes in FL
Citrus Canker detected in FL 1 year after eradication
Devastating Hurricanes in FL
Citrus greening detected in FL
What is Citrus *Phytophthora*?

- Soil-inhabiting *oomycetes*
- Cause diseases such as:
  - foot rot, gummosis, root rot, and brown rot of fruit
  - loss of fruit yield and tree vigor in citrus groves.
  - depletes the roots carbohydrate reserves (Graham, 2012)
- Hinders the ability of the tree to uptake water and nutrients
- Prolonged periods of wetness can lead to severe outbreaks of *Phytophthora*. 
Phytophthora Lifecycle in Citrus

Disease cycles of Phytophthora diseases affecting the roots, bark, and fruit of citrus trees.

Phytophthora symptoms

Root Rot
● Cortex of fibrous roots are infected
● Roots turn soft & water soaked
● Roots slough off cortex
  (Graham et al., 2016)

Foot Rot
● Infection of scion near ground level
● Bark lesions which extend to bud union on resistant rootstocks or to soil on susceptible rootstocks
  (Graham et al., 2016)
Phytophthora symptoms Cont.

**Crown Rot**
- Infection of the bark below the soil line
- Abundant gum exudation in bark cracks (Graham, 2012)

**Brown Rot**
- Common in early fall on early maturing varieties like Hamlin and Navels (Graham, 2012)
- Caused by spores produced on fallen fruit
- Requires extended periods of rainfall
Species of *Phytophthora* that infect citrus

- **Phytophthora nicotianae**
  - Most common species
  - Causes foot rot, gummosis, and root rot
  - Most active during warmer seasons

- **Phytophthora palmivora**
  - Not as prevalent as *P. nicotianae*
  - More aggressive than *P. nicotianae*
  - Causes severe root damage and brown rot
Phytophthora Management

Monitoring
- Soil sampling for propagule counts & root mass (1x-3x per year)

Nursery Sanitation
- Disease free nurseries
- Seed should be heat treated
- Proper site selection
- Use of registered fungicides (Revus)
  - No use of Group 4 Fungicides (Acylalanines)

New Plantings
- Resistant rootstocks
- Test nursery stock & planting sites
- Proper planting depths
- Proper drainage
- Soil treatment

Existing Plantings
- Proper drainage & irrigation management
- Regular population monitoring
- Fungicide treatment of susceptible root flushes

(Futch and Graham, 2015)
**Phytophthora Management Cont.**

**Chemical Control**

- Soil applications of Ridomil (Mefenoxam) – has activity against *Phytophthora* in the soil as well as diseases that have already invaded roots

- Rotate Ridomil with foliar applications of fungicides from a different fungicide class e.g. Aliette or Phosphite

---

Check inoculated with *Phytophthora*

Treated with [RidomilGold](#)

Photos from trial conducted by Kendra McCorkle at Syngenta in 2014
Citrus Greening

- A.K.A. Huanglongbing
- Thought to be caused by the bacterium *Candidatus liberibacter asiaticus* - Affects the vascular system of the plant
- Found in China in the 19th century (University of Florida, 2013).
- Affects the major citrus production areas - Asia, Africa, Brazil, and the United States (University of Florida, 2013).
- First found in south Florida in 2005 - can now be seen in every grove across FL
- The most destructive disease that Florida’s citrus industry has seen

Photo Taken by: Kendra McCorkle
Citrus Greening Causal Agent

- Citrus greening is vectored by the Asian citrus psyllid (ACP)
  - *Diaphorina citri*
  - Small insects (3-4 mm in length)
  - Adults can be identified by their 45 degree angled position on the plant
- Infection begins in the trees phloem and travels down to the tree’s root system then moves back up (Fox-Skelly, 2015).

Source: University of Florida (http://www.crec.ifas.ufl.edu/extension/entomology/asian_citrus_psyllid.shtml)
Citrus Greening Symptoms Cont.

- Blotchy Mottle
- Oblong Misshapen Fruit
- Curved Central Core
- Twig dieback

Sources:
- University of Florida: http://www.crec.ifas.ufl.edu/extension/greening/symptoms.shtml
- University of Florida: http://edis.ifas.ufl.edu/pdffiles/PP/PP26300.pdf
- University of Florida: http://solutionsforyourlife.ufl.edu/hot_topics/agriculture/citrus_greening.shtml
- University of Florida: http://www.crec.ifas.ufl.edu/extension/greening/symptoms.shtml
Managing Citrus Greening

- Currently no cure
- Millions of dollars are being invested into research to find a cure
- Some of the management tactics that growers are using to delay the affects of HLB (Fox-Skelley, 2015):
  - Improved nutritional programs
  - Insecticides for control of the ACP
  - Pesticides to control other pests that may exacerbate the disease (such as diseases caused by Phytophthora species)
  - Removal of infected trees
  - Pruning and removal of deadwood
  - Replanting with certified budwood from the nurseries
  - Biocontrols-parasitic wasps
  - Antibiotics under section 18- crisis exemption
**Phytophthora** and Citrus Greening Interaction

- Citrus greening infection begins in the trees root system
  - *Phytophthora* can increase the effect caused by the bacterium (Graham et al. 2013)
- *Phytophthora* control is more important with the introduction of citrus greening
  - *Phytophthora* symptoms are more severe in the presence of HLB and vice versa
  - Passive management methods may be inadequate
- Citrus greening reduces the number and timing of root flushes in a season
So, why test for *Phytophthora*?

- *Phytophthora* must be managed
  - No cure for greening
  - can exacerbate greening
- *Phytophthora* diseases are variable
  - environmental conditions
  - plant susceptibility
  - soil type
- Testing for *Phytophthora*
  - At least twice a year
  - Quickly identify at risk groves
  - informs growers of the health of their groves
  - helpful management tool offered for free by Syngenta
Citrus Soil Assay Background

- Started in 1989
- Started in response to a potential resistance issue to Syngenta’s Ridomil Gold products
- Methodology developed as a collaboration between the University of Florida and Syngenta
- Program is offered for free to growers using Syngenta’s Ridomil Gold products
Citrus Soil Assay Logistics

• *In vitro* test (Controlled environment)

• Utilizes *Phytophthora*-selective medium

• Allows us to estimate the population level of *Phytophthora spp.* in the soil

• Determination of root mass along with the *Phytophthora* propagule count allows us to make predictions on the threat of the disease

• Average turn-around time for results is 7 days
How to take soil samples

- Samples are taken using a 9” soil corer
- 20 cores are taken per sample at random through a grove
- 1 sample per 20 acres
- Cores are pulled within the trees drip line (root zone)
Method for Determining *Phytophthora* Levels

1. Collect soil/root samples from grove using a 9” L x ¾’ W soil corer and transport to laboratory

2. Take a well mixed subsample, saturate with water and precondition for 2-6 days under ambient conditions

3. Suspend sample in water agar, overlay onto *Phytophthora*-selective medium in a 15 cm petri dish, and incubate 4-6 days under ambient conditions

4. Wash surface of the plate to remove soil and count the number of colonies of *Phytophthora* expressed per cm$^3$ of soil
Method for Determining *Phytophthora* Levels

Take a well mixed subsample, saturate with water and precondition for 2-6 days under ambient conditions.

- Well mixed sample to ensure its representative.
- Fill cups ¾ way full with sample.
- Double stacked cups, bottom cup has drainage holes to allow soil to reach “field capacity”.
- Fill cups with water and store in the dark.

Photos Taken by: Katherine Buxton, Syngenta
Photos Taken by: Kendra McCorkle
Method for Determining Phytophthora Levels

Suspend sample in water agar, overlay onto Phytophthora-selective medium in a 15 cm petri dish, and incubate 4-6 days under ambient conditions.

20 cc’s of representative sample suspended in water agar to help it bind to the petri dish

5 mL aliquot of soil and water agar mix

Overlay mix onto PARPH media

Photos Taken by: Katherine Buxton, Syngenta
PARPH Medium (*Phytophthora* selective)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Type of Product</th>
<th>Main Purpose in Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pimaricin</td>
<td>Fungicide</td>
<td><em>Fusarium oxysporum</em> control</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Antibiotic</td>
<td>Broad Spectrum</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Antibiotic</td>
<td>Ansamycin antibiotic</td>
</tr>
<tr>
<td>PCNB</td>
<td>Fungicide</td>
<td><em>Rhizoctonia solani</em> control</td>
</tr>
<tr>
<td>Hymexazol</td>
<td>Fungicide</td>
<td><em>Fusarium and Pythium</em> control</td>
</tr>
<tr>
<td>V8 Juice (Filtered)</td>
<td>Vegetable Juice</td>
<td>Food Source for <em>Phytophthora</em></td>
</tr>
<tr>
<td>Agar</td>
<td>Solidifying agent</td>
<td>Harden Mixture</td>
</tr>
</tbody>
</table>
Method for Determining *Phytophthora* Levels

Wash surface of the plate to remove soil and count the number of colonies of *Phytophthora* expressed per cm$^3$ of soil.

After 7 days, the soil is removed from the plate. The colonies are exposed by placing on light source.

Photos Taken by: Katherine Buxton, Syngenta
Reading the Samples

- Visually we differentiate between the 2 species by their different morphological features

- **P. palmivora**: Smaller colony, denser mycelium
  - Visually relate it to a dandelion

- **P. nicotianae**: Larger colony, thinner mycelium
  - Visually relate it to a snowflake

Photo Taken by: Kendra McCorkle
Phytophthora Damage Threshold

- Day 7: the total number of colonies are counted
- Growers can compare their total propagule count to the damage threshold developed by the University of Florida (shown below)
- If a sample has 10 or more colonies, a treatment is advised
- If a sample has 20 or more colonies, a treatment at a higher rate is advised

<table>
<thead>
<tr>
<th>Number of Propagules per cm$^3$ of soil</th>
<th>Disease Threat</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9</td>
<td>Minimal</td>
</tr>
<tr>
<td>10-20</td>
<td>Moderate</td>
</tr>
<tr>
<td>&gt;20</td>
<td>High</td>
</tr>
</tbody>
</table>

Photo Taken by: Kendra McCorkle
Why determine root mass?

- Fibrous roots are the foundation for citrus tree health and productivity
  - Important for water and nutrient uptake
- Provides growers with a clearer picture of what is occurring below ground
- Can assess the total “disease load” of the tree
  - Compare *Phytophthora* propagules to fibrous root mass
- 1 out of every 5\textsuperscript{th} soil sample (or 1 per grove) collected is processed for root mass determination
  - Every sample isn’t processed due to the amount of resources required
For 1 of every 5 soil samples collected use a #10 sieve on a soil catch pan to collect roots. Then place in labeled Ziploc bags and deliver samples to the lab.

Using a hose or faucet rinse the soil from the sample leaving only roots, small rock, etc.

Using tweezers carefully pick out the citrus fibrous roots smaller than 2.0 mm in diameter and place them in a coin envelope.

Place the envelope in the oven at 70°C for 24 hours.

Weigh the dried roots and record each sample. Root weights will be evaluated in combination with Phytophthora propagule counts to give the grower a better understanding of Phytophthora disease threat.
Method for Determining Root Mass From Soil Samples

A sieve is used to separate the roots from the soil

The soil and roots are sent in to be processed separately for efficiency

Photos Taken by: John Taylor, Syngenta
Method for Determining Root Mass From Soil Samples

Once the sample arrives, it is rinsed through a #10 sieve.
The sieve is then flipped over onto a tray and the fibrous roots are picked out.

Photos Taken by: Kevin Langdon, Syngenta
Summary

● The citrus soil assay for *Phytophthora*
  - great tool for growers to evaluate their trees root system and make critical decisions on whether to make an application
  - Important tool for Syngenta as it allows us to understand the issues growers are facing and provide solutions where possible

● Improvements are made every year to the process
  - Addition of root mass in 2013 on every 5th sample
  - Ridomil Gold (Mefenofoxam) resistance monitoring began in 2011

● In the future, we will attempt to run root mass samples on every sample to better represent the disease threat

● This program continues to be unique to Syngenta
The value of the learning module

- Training document for Syngenta
  - Basic introduction to the program
    - Current training from SOP and myself
  - Internal and external customers
- Module Provides information on:
  - Florida citrus industry
  - Current issues in the industry
  - Importance of the program
  - Explains processes involved
Acknowledgements

- Committee Members
  - Alison Robertson
  - Kenneth Moore
  - Andrew Lenssen
- Dawn Miller
- Gretchen Anderson
- Program Professors
- Fellow students
- Syngenta
- Family

Thank you so much!
Questions?
References


References Continued


