The fate of *Calonectria pseudonaviculata* during composting

John Pecchia and Rob Harvey
Department of Plant Pathology and
Environmental Microbiology
Boxwoods

• Introduced to Americas in 1652
• Three species commonly used (160 cultivars)
  • *Buxus sempervirens*
  • *Buxus microphylla*
  • *Buxus sinica var. insularis*
• Reproduction is done via propagation
• Originally few pests
  • Several insects and fungi

www.clearzonenuisery.com

Bir et al. 1997
Boxwood Blight has well established symptoms

• Leaf Spots
• Stem Lesions
• Defoliation
Outward signs are also present
Cleared Boxwood Leaves
Boxwood Blight

- 19 States with confirmed cases
- PA Included
- CT Monetary Losses $3,000,000 (LaMondia 2014)
- Boxwoods are $103 million industry

Created By Rob Harvey using Diymaps.com©
C. Pseudonaviculata has a rapid life cycle

Inoculation

Germination (3 hours)

Penetration (5 hours)

Intercellular growth (Mesophyll)

Exit stomata (2-3 days)

Sporulation (7 days)

Overwinter survival as microsclerotia?

Henricot 2006
Formation of microsclerotia

• Glucose Yeast Extract Tyrosine Agar
  • Cellophane (natural) covered petri dish
Fate in Compost Windrow?

• Variables:
  • Temperature
  • Oxygen
  • Ammonia
  • Moisture
  • pH
Bioreactor Schematic
Reactor Construction
Compost Studies

• Straw, poultry manure and distiller’s grain

• 30:1 C:N ratio

• 60-70% moisture
Experimental Set up

- **Control**: Microsclerotia being incubated at 20 °C
- Pick 3 Temperatures: 40, 50, and 60 °C
- Pick 3 Time Intervals: 24, 48, and 72 hrs
- Set each incubator to one temperature, then randomly assign reactor to pull at time point
Compost Temperatures in Reactors

Temperature (°C) vs Time Post Start (Hrs)

PennState College of Agricultural Sciences
Methodology

• **Survival assessment** – surface sterilization 10% Clorox (0.825% Sodium Hypochlorite) 30 sec, sterile water 30 sec
• Counted microsclerotia survival based on mycelium germination
• Noticed a lot of contamination
• Issues with unrefined methods still at this point
So, what qualifies as a positive result?

• Seeing sporulation either on the agar directly, or from a microsclerotia

• Mycelium with the characteristic chestnut brown color

• Instead of % germ, squares will now show a +/- for growth
Revised Wash Procedure

- Fill 30 mL of water or 1% Clorox into 50 mL tubes
- Metal pouch unwrapped on sterile petri dish lid
- Place squares in sterile petri dish bottom
- Transfer all squares into 1% Clorox, rock 5 minutes
- Quickly transfer all squares to water, rock 30 minutes
- Transfer to petri plate
### Results

<table>
<thead>
<tr>
<th></th>
<th>+ Control</th>
<th>40 °C</th>
<th>50 °C</th>
<th>60 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>48 hrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>72 hrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
### Compost

<table>
<thead>
<tr>
<th></th>
<th>+ Control</th>
<th>40 °C</th>
<th>50 °C</th>
<th>60 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RXT1503</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>48 hrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>72 hrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>RXT1504</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>48 hrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>72 hrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>RXT1601</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>48 hrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>72 hrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>RXT1602</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>48 hrs</td>
<td>*?</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>72 hrs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Heat

<table>
<thead>
<tr>
<th></th>
<th>20 °C</th>
<th>40 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hrs</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>48 hrs</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>72 hrs</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td><strong>Trial 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hrs</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>48 hrs</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>72 hrs</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>
Ammonia Impact?

- Ammonium Chloride used as the source
- MES buffered to pH of 6 (6.13 after plates poured)
- 6 treatments
  - Control – Normal GYET – no MES etc
  - 0 mM NH$_4$Cl – GYET with MES only
  - 25 mM NH$_4$Cl (NH$_3$ = 0.0189 mM)
  - 50 mM NH$_4$Cl (NH$_3$ = 0.0379 mM)
  - 75 mM NH$_4$Cl (NH$_3$ = 0.0568 mM)
  - 100 mM NH$_4$Cl (NH$_3$ = 0.0758 mM)
Mycelial growth (on plates) at different ammonia concentrations

![Graph of mycelial growth vs. ammonia concentration]

- Ammonia (mg) scale:
  - 0.0
  - 0.2
  - 0.4
  - 0.6
  - 0.8

- Growth (mm) scale:
  - 0
  - 10
  - 20
  - 30
  - 40

- Time points:
  - 5 Day
  - 10 Day
  - 15 Day

PennState
College of Agricultural Sciences
Mycelial growth on plates at different pH concentrations
Conclusions

• Temperature in combination with ammonia seem to kill *C. pseudonaviculata* microsclerotia (ms) at relatively low compost temperatures (40°C < 50°C, 24 hours)

• Increased ammonia reduced microsclerotia survival
Current and Future Work

• Verify time/temp conclusions (after bleach wash/temperature exposure confirmation)
  • 3 reactor experiments starting August

• Compare effects of ammonia on both species (Europe and US)
  • Winter

• Test survival of infected plant material in compost system
  • Spring/summer
Thank You!

- Rob Harvey – PSU PPEM
- Don Davis – PSU PPEM
- Dana Rhodes – PA Dept of Ag
- Nina Shishkoff – USDA ARS
- Joanne Crouch – USDA ARS