Managing Sclerotinia sclerotiorum in high tunnels with biofumigation and solarization

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Outline

• High tunnels
• Sclerotinia sclerotiorum and Phytophthora capsici
• Biofumigation and solarization
• Experiments
  – Glucosinolate production
    • Effects of genetics, growing conditions, planting date
  – Bioassays
    • Petri dish, soil-based
  – High tunnel field study
High Tunnels

- Unheated greenhouses
- Metal quonset frame
- Plastic cover
- Passive ventilation
- Soil-based production
- Simple
- Cheap
Frame: $2,000

Plastic: $650

Hardware: $850

Cost: $3,500+
**Sclerotinia sclerotiorum**

- Thrives in cool, moist conditions
- Persists in soil as sclerotia
- White mold of lettuce
- Broad host range
- Problem in high tunnels
White Mold Life Cycle

Spring and Early Summer
- Sclerotia germinate producing apothecia.

Summer
- Infection of plant at soil line
- Sclerotia may germinate directly, producing mycelia that infect and colonize neighboring healthy tissue.
- Infected tissue decays leaving sclerotia inside and outside, and stems are hollowed and bleached.

Fall and Winter
- Fungus overwinters as sclerotia in plant debris and soil.
- Fungus grows out of senescing tissue onto healthy stems and leaves.

The fungus can colonize healthy tissue rapidly producing water soaked lesions and may produce white mold growth depending on conditions.

Ascospores are trapped by flowers of the first full bloom.
- Ascospores germinate and infect senescing petals as they fall to the ground or are trapped in canopy.

Windborne ascospores, forcibly ejected
- Windblown ascospores, forcibly ejected

Apothecium cross section
- Apothecium cross section

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Phytophthora capsici

- soil-borne fungal pathogen of peppers, tomatoes, melons, cucumbers, and squash
- severe losses
- recent problem in Kentucky field vegetable production
Phytophthora capsici

- Grows best between 25-30°C
- Forms microscopic spores called sporangia
- Forms thick-walled over-wintering oospores after mating
Organic management options

• Biofumigation
  – General: Use of volatile chemicals produced by plants to control or suppress soil-borne pests and diseases
  – Specific: Soil incorporation of brassicas, which contain glucosinolates that break down into volatile isothiocyanates

• Solarization
  – Using clear plastic over the soil surface to capture solar energy, heat soil, and kill weed seeds and soil-borne pests and diseases
Glucosinolate production

- **Objective**
  - Identify suitable brassica cover crop
- **Methods**
  - Evaluated 47 brassica accessions for suitability as a cover crop in high tunnel (winter) and field (spring)
    - *Brassica juncea* (Indian mustard),
    - *B. napus* (Rape),
    - *B. carinata* (Ethiopian mustard),
    - *Eruca sativa* (Arugula)
  - Recorded survival, days to maturity, biomass production
  - Determined glucosinolate content of most promising accessions (Antonious et al 2009)
Ten accessions that demonstrated relative cold tolerance, rapid maturity, and superior biomass production were selected from 48 accessions of the National Germplasm Repository:

<table>
<thead>
<tr>
<th>Accession</th>
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<tbody>
<tr>
<td>Ames 8660</td>
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<td>Brassica juncea</td>
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<tr>
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<td>Ida gold</td>
<td>Brassica campestris</td>
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Materials & Methods

- Greenhouse
- High Tunnels
- Field

Indian mustard
Oil seed rape
Arugula
Field mustard
Analysis of Glucosinolates

- Extraction using boiling methanol
- Clean-up using celite column
- Separation by anion-exchange resin “Sephadex”
- Hydrolysis with Thioglucosidase
- Quantification
Glucosinolates

- Greenhouse
- Tunnels
- Field

Species:
- Brassica juncea
- Brassica napus
- Eruca sativa
- Brassica campestris

Plot details:
- Y-axis: μMoles g⁻¹ Fresh Weight
- X-axis: Varieties

Varieties:
- Ames 8660
- Ames 8674
- Ames 8709
- Ames 8887
- PI-120923
- PI-603015
- PI-169083
- PI-633215
- Pacific gold
- Ida gold

Legend:
- 'a', 'b', 'c', 'd', 'e', 'f', 'g', 'h'

Additional notes:
- Pacific gold and Ida gold are distinct from the other varieties.
- PI-120923 and PI-603015 show similar trends to Ames 8674 and Ames 8887, respectively.

Source:
- Kentucky State University
Glucosinolate production

Effect of planting date on field biomass production

- Pacific Gold mustard direct-seeded in field at weekly intervals, weather permitting
- Above-ground biomass collected and weighted at flower initiation

\[ y = -38.7x + 1916 \]
\[ R^2 = 0.9799 \]

![Graph showing the relationship between planting day and fresh weight (g/m²)](image)
Germination assay

- MeOH tissue extract
  - 0, 0.25, 0.5, 1.0, and 2.0 g f.w.
- Placed in scintillation vials for 24 h with
  - equivalent amount of myrosinase
  - 15 S. sclerotiorum sclerotia
  - sterile soil (4 mL total)
- Plated onto Petri dishes with 40 mL sterile soil for 6 wk germination at 16 °C

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*Field-grown accession tested in addition to high tunnel grown accession
Results: *S. sclerotiorum* Germination (of 15) After Exposure to Mustard Extracts
Results: *S. sclerotiorum* Inhibition

![Diagram showing inhibition of *S. sclerotiorum* with mustard dose (g fresh wt/vial). The graph plots inhibition (%) against mustard dose (g fresh wt/vial). The LD\(_{50}\) for field grown is 0.65 g/4 mL vial and for high tunnel grown is 0.163 g/mL.](image-url)
**Phytophthora capsici assay**

- MeOH tissue extract
  - 0, 0.25, 0.5, 1.0, and 2.0 g f.w.
- Placed in dark sealed culture flasks for 2 wk with
  - equivalent amount of myrosinase
  - 20 *P. capsici* oospores
  - sterile soil (4 mL total)
- Oospore germination tallied

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*Field-grown accession tested in addition to high tunnel grown accession*
Results: *P. capsici* Oospore Germination (of 20) After Exposure to Mustard Extracts
Petri dish assay conclusions

- 24 h exposure to extracts from 0.65 g mustard tissue per 4 mL vial (0.163 g/mL) reduced *S. sclerotiorum* sclerotal germination by 50%.

- ‘Pacific Gold’ gave the best reduction in *S. sclerotiorum* germination.

- *P. capsici* oospore germination was completely inhibited by exposure to extracts > 0.5 g mustard tissue / 4 mL substrate (0.12 g/mL).

- *P. capsici* oospores are more sensitive to mustard extracts than *S. sclerotiorum* sclerotia.

- More research is needed to apply these results in the field.
Greenhouse Potting Soil Assay: Methods

- Pacific Gold mustard greens and rye/vetch cover crop harvested and macerated at first flower, 06/12/08
- Cover crops separately incorporated into peat-based potting mix, 15 cm deep
  - 0, 1, 2, 4, 8 and 16 kg m\(^{-2}\)
- 3 replicates, completely randomized
- Mesh bag of 50 sclerotia buried in each pot for 4 days
- Sclerotia plated onto Petri dishes with 40 mL sterile soil for 6 wk germination at 16 °C
Greenhouse Potting Soil Assay: Results

- Low germination
- No significant effects:
  - Cover crop
  - Rate (significant if highest rate excluded)
Soil-based *P. capsici* assay: Methods

- Split plot design:
  - Main effect – Presence/Absence of Organica Plant Growth Activator
    - Blend of micro-organisms intended to promote seedling and plant establishment
  - Sub-plots – Presence/Absence of glucosinolates from green manure at 6 incorporation rates
    - Presence: Biofumigation with Pacific Gold mustard greens
    - Absence: Incorporation of rye (*Secale cereale*)/vetch (*Vicia villosa*) cover crop to add organic matter but no glucosinolates
    - Rates: 0, 13.5, 27, 54, 108 and 216 g/L (0-16 kg/m²)
Methods

- 16 liters of organic planting mix thoroughly mixed with 80 ml of *Phytophthora capsici* inoculant (0.5% volume/volume) and divided into 12 equal portions
Methods

- Green manure harvested at first flower, finely chopped and mixed into planting media at 0, 13.5, 27, 54, 108 or 216 g/L
Each mixture randomly assigned to fill two of 24 evenly spaced 65 ml cells in each of the 10 seedling plug trays

A pre-germinated yellow squash (*Cucurbita pepo*) seed planted in each cell
Methods

• Trays randomly assigned to flood irrigation with untreated tap water or tap water treated with 4.2 g/l of Organica Plant Growth Activator.
Methods

• Trays were partially submerged in the liquid to ensure thorough drenching and incubated for 5 days at 26°C.
Methods

- Seedling survival and shoot length were recorded for each treatment combination in each of 5 replicates
Results

• No significant effect:
  – Cover crop
  – Rate
Results

- **Interaction:**
  - Cover crop x Organica

- **Superior seedling growth with combination of mustard and Organica**

- **Repeatable?**
See video:

http://organic.kysu.edu/SclerotiniaVideo.shtml
Thanks
- Brian Geier
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- Southern SARE

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