Efficacy of organic sulfur compounds from garlic/onion on white rot Sclerotia germination

Michael Qian, Oregon State University, Corvallis, OR 97330

Jeremiah Dung, Central Oregon Agricultural Research Center, Oregon State University, 850 NW Dogwood Lane, Madras, OR 97741

Feb. 12, 2018
White rot disease

*Schlerotium cepivorum*

- Few sclerotia in a liter of field soil potentially can result in crop failure
- Long dormant period in the absence of allium hosts
- Once white rot is identified in a field, growers are not able to grow allium crops.

Garlic with white rot disease
Hypothesis and approach

• If we can stimulate the germination without the presence of host plant, sclerotia will die

Objectives

• Develop a fast laboratory approach to stimulate sclerotia germination
• Use laboratory approach to screen bio-stimulants
• Provide bio-stimulant input for field trials
Previous Experiment-Sclerotia isolation (Dung Jeremiah)

- Sclerotia isolation
- Sclerotia preparation and activation
- Sclerotia viability
Previous experimental set-up

I-Chem Certified pre-cleaned vial, 20 mL

Filter paper, maintain moisture

Teflone lined Silicone Septa

When add PDA agar to the vial, achieve germination

When just filter paper, no germination

Sclerotia
• **Germination Results -- Set A**

(a) Control  
Filter paper, 10 sclerotia, 100 μl H₂O, 10 μl methanol in 20 mL vial  
No germination

(b) DADS stimulant (5 ppm in headspace)  
Filter paper, 10 sclerotia, 100 μl H₂O, 10 μl 1% (10000 ppm) DADS in 20 mL vial  
Germination level: (+++)

**Figure 4. Germination Result of Set A**

• *S. cepivorum* were provided by Dr. Dung; +++ represent the germination level
However, we have difficulties to germinate the sclerotia consistently

• Sclerotia should not geminate without stimulants
• Keep stimulants in a defined container
  • Sulfur compounds are volatile
  • Escape
  • Cross-contamination
• Need best germination environment
  • Temperature, moisture, oxygen
Modifications for experimental set-up

Filter paper, maintain moisture

Sclerotia

PDA agar could be added to the bottom
Many, Many trials...

- **Problems**
  - Molds grew easily on PDA agar
  - Germination was not reproducible due to uncontrollable temperature and moisture
New Experimental Design

- Evaluate field soil as germination media
- Introduce incubation oven to achieve better environment control (temperature, moisture)
Sieve field soil through #10 and #20 sieves

30 cc soil and 13.5 ml water in petri dish

Sclerotia plating

Inspect sclerotia germination using a dissecting microscope

Seal with tape

Apply sulfur standards
Concentrations of sulfur standards

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (gal/acre)</th>
<th>Applied amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dially disulfide,</td>
<td>0.1</td>
<td>7 µL 10 % solution</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7 µL pure solution</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>70 µL pure solution</td>
</tr>
</tbody>
</table>

Triplicate for each treatment
Dose Response of DADA on Germination

Warning: DADS was added on the top of soil, effective dosage will be different if added in the soil due to interaction with soil matrix
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (gal/acre)</th>
<th>Applied amount</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dimethyl disulfide (DMDS)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethyl disulfide (DMDS)</td>
<td>0.1</td>
<td>7 µL 10% solution</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7 µL pure solution</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>70 µL pure solution</td>
</tr>
<tr>
<td><strong>Sulfur powder</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfur powder</td>
<td>0.01</td>
<td>10 mg sulfur powder</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>100 mg sulfur powder</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1000 mg sulfur powder</td>
</tr>
<tr>
<td><strong>Dimethyl trisulfide (DMTS)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diethyl disulfide (DEDS), diisopropyl disulfide (DIPDS) Dipropyl disulfide(DPDS)</td>
<td>0.01</td>
<td>7 µL 1% solution</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>7 µL 10% solution</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7 µL pure solution</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>70 µL pure solution</td>
</tr>
</tbody>
</table>
Other Organic Sulfur on Germination

DMDS

\[
H_3C-S\cdot S\cdot CH_3
\]

Germinated sclerotia vs. Concentration (gal/acre)

DMTS

\[
H_3C-S\cdot S\cdot S\cdot CH_3
\]

Germinated sclerotia vs. Concentration (gal/acre)
Short summary

1. In terms of DADS #1, DADS #2, DMTS, IPDS and sulfur powder treatments, lower concentrations (0.01, 0.1 gal/acre) had a better stimulant effect on schlerotia germination compared to higher concentrations (1, 10 gal/acre), leading to higher amount of germinated schletotia.

2. For DMDS, DMDS and DPDS, treatment with higher concentrations had increased amount of germinated schlerotia. 10 gal/acre of DMDS had the largest amount of germinated schlerotia among all treatments.

3. Absence of germinated schlerotia in control treatments indicated little cross-contamination during incubation.
Garlic oil distillation

• 400g garlic (California early/California late)
• 1.5 L Milli-Q water
• Blend garlic with water, and let it sit in the hood for 2 hrs
• Distillation and collect the oil phase
Garlic Oil (1 gal/acre) on Sclerotia Germination

Treatments:
- DI water control
- DADS
- California early garlic oil
- California late garlic oil

Germinated sclerotia
Garlic Juice On Sclerotia Germination

Germinated sclerotia

- DI water control
- DADS
- California early garlic oil
- California late garlic oil
- Garlic juice (unknown source)
Garlic Powder on Sclerotia Germination

- DI water control
- DADS
- California early garlic oil
- California late garlic oil
- Garlic juice (unknown source)
- Garlic powder (Sensient)
Raw Onion on Sclerotia Germination

Germinated sclerotia

Treatments

- DI water control
- DADS
- California early garlic oil
- California late garlic oil
- Garlic juice (unknown source)
- Garlic powder (Sensient)
- Raw onion

Oregon State University 22
Volatile Compounds in Cabbage

1-Butene, 4-isothiocyanato-

Allyl isothiocyanate
Germination results

Germinated sclerotia

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI water control</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>DADS</td>
<td>3.8±0.2</td>
</tr>
<tr>
<td>California early garlic oil</td>
<td>3.2±0.2</td>
</tr>
<tr>
<td>California late garlic oil</td>
<td>3.0±0.2</td>
</tr>
<tr>
<td>Garlic juice (unknown source)</td>
<td>8.5±0.3</td>
</tr>
<tr>
<td>Garlic powder (Sensient)</td>
<td>9.2±0.4</td>
</tr>
<tr>
<td>Raw onion</td>
<td>10.0±0.5</td>
</tr>
<tr>
<td>Cabbage</td>
<td>9.8±0.4</td>
</tr>
</tbody>
</table>
Germination results

DI water control
DADS
California early garlic oil
California late garlic oil
Garlic juice (unknown source)
Garlic powder (Sensient)
Raw onion
Cabbage
Chinese cabbage

Germinated schlerotia
Summary

- Soil-based germination seems more reliable
- Germination dose-response correlation was observed
- Even very low application rate of DADS can stimulate the germination, although types of soil may affect the effective dosage
- High dosage can inhibit sclerotia germination
- In addition to DADS, many other sulfur organic compounds can also stimulate germination
- Garlic oil, garlic juice, garlic powder all can stimulate germination
- Cabbage can also stimulate the sclerotia germination