Integrated Disease Management in Spinach Seed Crops in the USA

2008 Danish Spinach Field Day

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Developing an integrated disease management program for spinach seed crops in the PNW

Crop Profile for Spinach Seed Crops in Washington State
http://www.ipmcenters.org/cropprofiles/docs/waspinachseed.html
Economically significant pathogens of spinach seed crops in WA

Leaf spot fungi:
- *Cladosporium variabile*
- *Stemphylium botryosum*

Vascular wilt fungi:
- *Fusarium oxysporum f. sp. spinaciae*
- *Verticillium dahliae*

Virus:
- *Cucumber mosaic virus* (CMV)

“Others”:
- Downy mildew, *Beet western yellows virus*,
- *Beet curly top virus*, Seedling blights
Leaf spot fungi of spinach seed crops in WA


Photo by M.L. Derie

Cladosporium variabile

Stemphylium botryosum
Spinach co-infected with *Stemphylium botryosum* & *Cladosporium variabile*

- Overlap of lesions caused by *S. botryosum* and *C. variabile*
- Small, distinct lesions caused by *C. variabile*
- Rapidly-expanding, diffuse lesions caused by *S. botryosum*
Influence of pollen on spinach leaf spot


Impact: Timing foliar fungicide applications based on anthesis!
Spinach leaf spot fungicide & yield loss trials
Spinach seed crop foliar fungicide trials


<table>
<thead>
<tr>
<th>2003 Fungicide trial</th>
<th>Severity of leaf spot (3 Sep)</th>
<th>Seed yield (lb/37 ft²)</th>
<th>% Seed infected Stemph. botryosum</th>
<th>% Seed infected Cladosp. variabile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (water)</td>
<td>19.4 a</td>
<td>1.8 a</td>
<td>23.6 a</td>
<td>9.0 a</td>
</tr>
<tr>
<td>Topsin M 70WP (thiophanate-methyl)</td>
<td>12.0 b</td>
<td>1.7 a</td>
<td>19.6 a</td>
<td>0.6 bc</td>
</tr>
<tr>
<td>Manex (maneb)</td>
<td>7.7 c</td>
<td>2.1 a</td>
<td>11.6 b</td>
<td>3.8 b</td>
</tr>
<tr>
<td>Bravo WeatherStik (chlorothalonil)</td>
<td>5.5 d</td>
<td>1.7 a</td>
<td>11.2 b</td>
<td>2.8 bc</td>
</tr>
<tr>
<td>Dithane DF Rainshield (mancozeb)</td>
<td>5.3 d</td>
<td>1.8 a</td>
<td>8.8 bcd</td>
<td>3.8 b</td>
</tr>
<tr>
<td>Rovral 4F (iprodione)</td>
<td>2.1 e</td>
<td>1.6 a</td>
<td>6.2 bcd</td>
<td>3.8 b</td>
</tr>
<tr>
<td>Amistar (azoxystrobin)</td>
<td>1.9 e</td>
<td>1.7 a</td>
<td>4.4 d</td>
<td>0.2 c</td>
</tr>
<tr>
<td>Sovran (kresoxim-methyl)</td>
<td>1.3 e</td>
<td>1.4 a</td>
<td>3.6 d</td>
<td>1.8 bc</td>
</tr>
<tr>
<td>Cabrio EG (pyraclostrobin)</td>
<td>1.3 e</td>
<td>2.3 a</td>
<td>6.0 cd</td>
<td>0.4 c</td>
</tr>
<tr>
<td>Pristine EG (pyraclostrobin+boscalid)</td>
<td>1.0 e</td>
<td>1.9 a</td>
<td>5.8 cd</td>
<td>0.2 c</td>
</tr>
</tbody>
</table>

- No treatment affected seed germination
- Correlation of leaf spot severity with % seedborne

\[ S. botryosum = 0.81 & C. variabile = 0.57 \]
Overwintering of spinach leaf spot fungi


Cladosporium variabile on volunteer spinach

Stemphylium botryosum on spinach seed stalk debris
Pleospora herbarum
= sexual stage of Stemphylium botryosum
Seedborne *Stemphylium botryosum* & *Cladosporium variabile* in spinach


Seed transmission

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. variabile</em></td>
<td>5.1 (18.1)</td>
<td>0.1 (0.4)</td>
</tr>
<tr>
<td><em>S. botryosum</em></td>
<td>9.1 (10.3)</td>
<td>3.3 (3.7)</td>
</tr>
</tbody>
</table>
Hot water seed treatment

Trial 1

Incidence (%) of seed with Cladosporium variabile

Seed germination (%)

Trial 2

Incidence (%) of seed with Stemphylium botryosum

Duration (min)

Trial 1

Trial 2
Evaluation of fungicide seed treatments for seedborne *Stemphylium botryosum*

(du Toit et al., 2007. Plant Disease Management Reports 1:ST003)
Seedborne wilt fungi of spinach

*Fusarium oxysporum f. sp. spinaciae*

*Verticillium dahliae*
Fusarium Wilt in Spinach Seed Crops

Fusarium oxysporum f. sp. spinaciae
Fusarium Wilt in Spinach Seed Crops
Resistance (susceptibility) of parent lines
Seedborne *Verticillium dahliae* in spinach
Verticillium dahliae associated with spinach seed in the Pacific Northwest USA

1. Prevalence in commercial spinach seed lots
2. Pathogenicity of spinach seed isolates
3. Seed-to-seed transmission of V. dahliae

Pathogenicity of *Verticillium dahliae* isolates from spinach seed

Symptoms only observed after bolting is initiated.
Verticillium wilt of spinach

- Non-inoculated
- Inoculated

Photo by M.L. Derie.
Verticillium wilt vs. Fusarium wilt of spinach
Fusarium wilt  Verticillium wilt  Control
Seed treatment trial for leaf spot fungi:
(du Toit et al., 2007. Plant Disease Management Reports 1:ST003)

% Seedborne *Verticillium* spp.
Seed treatment trial for leaf spot fungi:
(du Toit et al., 2007. Plant Disease Management Reports 1:ST003)

% Seedborne *Fusarium* spp.
Significance of *Verticillium* in spinach?

- Symptoms develop > initiation of bolting - explains lack of symptoms in fresh market/processing crops
- Fusarium wilt vs. *Verticillium* wilt
  - reddening of lower stem
  - black vs. light vascular discoloration
  - pre- vs. post-bolting symptoms
  - general wilting vs. interveinal chlorosis
  - microsclerotia, early dying
- Highly systemic, high rate of seed transmission
- Phytosanitary certificate for Mexico: <10% *Verticillium*
- 48/50 spinach isolates from Holland, Denmark & US: VCG 2B, 4B. Host range? Potato, radish, lettuce, ...?
Screening for resistance to *V. dahliae*


- Screened 130 *Spinacia* genotypes: none immune
- No qualitative or major gene resistance
- Low disease severity: 6 genotypes
- *V. dahliae* accelerated senescence, reduced vigor
- Severity ratings complicated by:
  - *Natural senescence mimics Vert. wilt symptoms*
  - *Genotypes vary in day-length response to bolting*
  - *Male plants senesce faster than female plants*
- *V. dahliae* isolates (radish, potato, spinach, mint & peppermint), *V. albo-atrum* (alfalfa), & *V. nigrescens* (spinach) all pathogenic on spinach
Screening for resistance to *V. dahliae*

6 plant introductions with partial resistance?

<table>
<thead>
<tr>
<th>Spinach PI</th>
<th>Country of origin</th>
<th>Disease severity (%) 2006</th>
<th>Disease severity (%) 2007</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inoculated</td>
<td>Non-inoculated</td>
<td>Inoculated</td>
</tr>
<tr>
<td>Ames 26243</td>
<td>China</td>
<td>10.7</td>
<td>5.5</td>
</tr>
<tr>
<td>PI 261789</td>
<td>France</td>
<td>12.5</td>
<td>6.7</td>
</tr>
<tr>
<td>PI 604789</td>
<td>Afghanistan</td>
<td>10.7</td>
<td>5.6</td>
</tr>
<tr>
<td>PI 175931</td>
<td>Turkey</td>
<td>25.5</td>
<td>5.5</td>
</tr>
<tr>
<td>PI 163309</td>
<td>India</td>
<td>20.2</td>
<td>6.9</td>
</tr>
<tr>
<td>PI 494751</td>
<td>Uzbekistan</td>
<td>20.8</td>
<td>5.6</td>
</tr>
</tbody>
</table>
Management of vascular wilt diseases in spinach seed crops using cover crops & soil amendments
Evaluation of mustard vs. winter wheat cover crops in spinach seed production
Evaluation of mustard vs. winter wheat cover crops for control of Fusarium wilt in spinach seed production


The image shows a bar chart comparing the effectiveness of different crops in controlling Fusarium wilt. The crops compared are Metam sodium, B. juncea, B. juncea + B. hirta, Mustard seed meal, and Winter wheat. The chart includes data for healthy plants on 11 July and 26 July, as well as seed yield.
Association of soil properties with suppression of Fusarium wilt

• Spinach seed production in WA, USA:
  • 8-15 year rotations, Fusarium wilt prevalent
  • High rainfall, acid soils: pH 5.8-6.3

• Spinach seed production in Denmark:
  • 5 year rotations, very little Fusarium wilt
  • Alkaline, calcareous soils: pH 7.5-8.0

• Del Monte spinach nursery in Uvalde, TX
  • Spinach grown annually; alkaline, calcareous soil

• Spinach seed production in New Zealand:
  • Fusarium wilt on virgin ground; acid soils
Evaluation of limestone amendment for suppression of spinach Fusarium wilt: 2006 & 2007


4-5 April 2006
Results: 2006

Incidence of wilted/dead plants on 11 July

Incidence (%) of wilted plants on 11 July

Spinach inbred line
- Red: Male
- Blue: Susceptible female
- Black: Moderate female

Rate of limestone amendment (tons/acre)

0 0.4 2.1 2.8 3.5 4.2

0 10 20 30 40 50 60 70 80 90 100

Incidence of wilted/dead plants on 11 July
Results: 2006
Dry plant weight

Dry plant weight (lb/3.3 ft row)

Rate of limestone amendment (tons/acre)

'26 Jun
'11 Aug

Symbols:
- c
- b
- a
- ab

Legend:
- Red line: '26 Jun
- Blue line: '11 Aug
Results: 2006

Seed yield (lb/acre)

Rate of limestone amendment (tons/acre)
Results: 2006

Plant nutrient analyses (plants sampled 26 June)

<table>
<thead>
<tr>
<th>Main factor</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
<th>B</th>
<th>Zn</th>
<th>Mn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Spinach parent line</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod. female</td>
<td>5.1 b</td>
<td>1.49 a</td>
<td>0.55 b</td>
<td>0.29 b</td>
<td>26.3 b</td>
<td>33.3 b</td>
<td>42.6 a</td>
<td>7.9 b</td>
</tr>
<tr>
<td>Susc. female</td>
<td>6.4 a</td>
<td>1.41 b</td>
<td>0.63 a</td>
<td>0.35 a</td>
<td>36.4 a</td>
<td>43.5 a</td>
<td>51.9 a</td>
<td>10.4 a</td>
</tr>
<tr>
<td>Limestone rate (tons/acre)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5.5 a</td>
<td>1.15 d</td>
<td>0.60 a</td>
<td>0.31 a</td>
<td>31.9 a</td>
<td>43.6 a</td>
<td>72.5 a</td>
<td>8.3 a</td>
</tr>
<tr>
<td>1.4</td>
<td>5.5 a</td>
<td>0.39 c</td>
<td>0.57 a</td>
<td>0.31 a</td>
<td>32.0 a</td>
<td>40.7 ab</td>
<td>50.4 b</td>
<td>9.1 a</td>
</tr>
<tr>
<td>2.1</td>
<td>6.0 a</td>
<td>1.48 bc</td>
<td>0.62 a</td>
<td>0.34 a</td>
<td>32.2 a</td>
<td>39.7 bc</td>
<td>46.2 bc</td>
<td>9.7 a</td>
</tr>
<tr>
<td>2.8</td>
<td>5.9 a</td>
<td>1.43 bc</td>
<td>0.59 a</td>
<td>0.31 a</td>
<td>29.9 a</td>
<td>35.8 d</td>
<td>38.3 d</td>
<td>9.2 a</td>
</tr>
<tr>
<td>3.5</td>
<td>5.7 a</td>
<td>1.68 a</td>
<td>0.59 a</td>
<td>0.31 a</td>
<td>30.9 a</td>
<td>36.1 cd</td>
<td>39.7 cd</td>
<td>9.3 a</td>
</tr>
<tr>
<td>4.2</td>
<td>5.9 a</td>
<td>1.53 b</td>
<td>0.57 a</td>
<td>0.32 a</td>
<td>31.0 a</td>
<td>34.4 d</td>
<td>36.5 d</td>
<td>9.3 a</td>
</tr>
</tbody>
</table>

Plant nutrients not affected by rates of limestone: N, P, K, Mg, S, B, Cu, Fe
Results: 2006
Seed germination & health assays

<table>
<thead>
<tr>
<th>Main factor</th>
<th>Seed germination (%)</th>
<th>Freeze-blotter seed health assay (% of seed)</th>
<th>Fusarium spp.</th>
<th>Verticillium spp.</th>
<th>Stemphylium botryosum</th>
<th>Cladosporium variabile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinach parent line</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod. Female</td>
<td>62.0 a</td>
<td>0.6 a</td>
<td>38.7 a</td>
<td>13.0 a</td>
<td>0.6 a</td>
<td></td>
</tr>
<tr>
<td>Susc. Female</td>
<td>21.3 b</td>
<td>0.1 a</td>
<td>8.6 b</td>
<td>17.5 a</td>
<td>1.3 a</td>
<td></td>
</tr>
<tr>
<td>Limestone rate (tons/acre)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>43.2 a</td>
<td>0.3 a</td>
<td>11.2 c</td>
<td>15.8 a</td>
<td>0.8 a</td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>36.1 a</td>
<td>0.1 a</td>
<td>19.6 bc</td>
<td>14.6 a</td>
<td>0.7 a</td>
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<tr>
<td>2.1</td>
<td>39.2 a</td>
<td>0.5 a</td>
<td>20.0 bc</td>
<td>17.6 a</td>
<td>1.5 a</td>
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<tr>
<td>2.8</td>
<td>47.1 a</td>
<td>0.4 a</td>
<td>29.1 ab</td>
<td>17.7 a</td>
<td>0.6 a</td>
<td></td>
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<tr>
<td>3.5</td>
<td>42.2 a</td>
<td>0.6 a</td>
<td>35.7 a</td>
<td>12.9 a</td>
<td>1.6 a</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>44.3 a</td>
<td>0.2 a</td>
<td>26.4 ab</td>
<td>12.7 a</td>
<td>0.6 a</td>
<td></td>
</tr>
</tbody>
</table>
Results: 2006

Colonies of *Fusarium oxysporum* at harvest

![Graph showing the comparison of CFUs of *Fusarium oxysporum* in moderate and susceptible female samples.]

- **Moderate female**
- **Susceptible female**

Legend:
- Grey bar: Moderate female
- Blue bar: Susceptible female
Limestone trials: Conclusions


- Limestone is a potentially valuable tool for reducing losses to Fusarium wilt in spinach seed crops in the PNW
- Longer rotations may be needed following spinach seed crops with Fusarium wilt-susceptible inbred(s)
- Research needs:
  - Optimize limestone rates to reduce rotation intervals without increasing Fusarium wilt OR Verticillium wilt
  - Foliar applications of micronutrients to counter reduced availability at higher soil pH (Zn, Mn?)
  - Impact of fertilizers high in Zn (e.g., 11-52-0) on suppression of Fusarium wilt
  - Assess mechanisms of suppression for optimization
Cucumber mosaic virus (CMV) in spinach
Seed transmission of CMV in spinach:

Influence of parent line on rate of seed transmission

du Toit et al., 2007. Phytopathology 97:S30
Integrated disease management in spinach seed crops in Washington State

- Rotation - long intervals necessary:
  - driven by Fusarium wilt, need assess Verticillium wilt
  - resistance of previous spinach lines?
  - avoid Verticillium wilt-susceptible crops?
  - biofumigant cover crops or amendments?
- Clean stock seed:
  - assay for fungi & CMV, use seed treatments
- Know susceptibility of parent lines to various diseases
- Orientation of rows for lines susceptible to leaf spots
- Limestone: 1-2 tons/acre (soil pH 6.5+)
- Fertilizers: Nitrate vs. ammonium? Low Zn? Foliar feeds?
- Fungicides: timing (anthesis & rains), modes of action
- Diligent scouting & roguing
- Accurate diagnosis of problems
- Timely, regional management of crop residues & volunteers
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