Update on Verticillium wilt, New Species and their Disease Potential

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Prior to 1995, lettuce was not considered a host of *V. dahliae*; even when grown in rotation with other diseased crops.
VERTICILLIUM WILT

Foliar Symptoms:
• Angular Chlorosis
• Necrosis
• Wilting
• Darkening of Leaf Veins
• Acropetal Progression
• Collapse of Head
VERTICILLIUM WILT

Root Symptoms:
- Vascular Discoloration
- Occur ~2 wk before foliar symptoms
144 Fields identified
≈ 1085 hectares infested
(2,680 acres)
Verticillium wilt of lettuce

- First report in 1995
  - Extensive damage in Watsonville, CA
- Detected in 175 fields from 1995 to 2010
- ~75 fields in 2010 alone
- Incidence 70-100%
- Random distribution of fields
- 2-3 crops following fumigation
- Often first observed by field edge
- Complete plant death
- Pathogen is seedborne
Verticillium Wilt of Lettuce

- Assess seed lots of lettuce types for *V. dahliae* infestation.

- Determine the potential of host-directed evolution of *V. dahliae* genotypes of differential virulence from a single genotype.

- Continue characterization of the spinach and lettuce pathogen populations.
• *Verticillium dahliae* is seedborne in lettuce and other crops and weeds.
• It is both externally seedborne and internally seedborne.
• Infested seed germinates, plants grow and develop wilt.
• Seed harvested from infected plants are infested with *V. dahliae*. 
## Lettuce types (2008-10)

<table>
<thead>
<tr>
<th>Type</th>
<th>Seed lots</th>
<th>Infested</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby Leaf</td>
<td>34</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Crisphead</td>
<td>84</td>
<td>13</td>
<td>0.1-4</td>
</tr>
<tr>
<td>Romaine</td>
<td>38</td>
<td>8</td>
<td>0.5-2</td>
</tr>
<tr>
<td>Red Romaine</td>
<td>7</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Green Leaf</td>
<td>31</td>
<td>2</td>
<td>0.5-2</td>
</tr>
<tr>
<td>Red Leaf</td>
<td>22</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Butterhead</td>
<td>5</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Other/unknown</td>
<td>44</td>
<td>22</td>
<td>0.5-5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>265</strong></td>
<td><strong>48</strong></td>
<td></td>
</tr>
<tr>
<td>Country</td>
<td>Seed lots</td>
<td>Infested</td>
<td>Range</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------</td>
<td>----------</td>
<td>--------</td>
</tr>
<tr>
<td>USA</td>
<td>195</td>
<td>33</td>
<td>0.1-5</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>3</td>
<td>2</td>
<td>0.0-4</td>
</tr>
<tr>
<td>China</td>
<td>22</td>
<td>8</td>
<td>0.5-3</td>
</tr>
<tr>
<td>Chile</td>
<td>33</td>
<td>5</td>
<td>0.5-2</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>5</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Australia</td>
<td>7</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>265</strong></td>
<td><strong>48</strong></td>
<td></td>
</tr>
</tbody>
</table>
Verticillium wilt of lettuce

- Exceedingly high production of microsclerotia
  - Calculated ~3 million microsclerotia/plant
- Two races
  - Race 1 currently ~75% of population
  - Resistance for race 1 available
Why did lettuce succumb to *Verticillium dahliae*?

- What is the source of these “apparently” novel isolates?
  - Endemic; Parasexual cycle (mitotic recombination)?
  - No teleomorph ever observed.
  - Exotic; Just another invasive in CA?
  - How diverse is the *V. dahliae* population in the Salinas Valley?
  - Did these isolates wash in from elsewhere during the 1995 floods?

- Big implications for disease management & resistance durability.
Verticillium Wilt of Lettuce

• Determine whether or not nitrate fertilizers used in strawberries following fumigation render soil Verticillium wilt-conducive relative to ammonium fertilizers.
## Effect of different form of Nitrogen on Verticillium wilt of lettuce

### Treatments
- Ammonium sulfate
- Ammonium nitrate
- Calcium nitrate
  (60 lb N per acre)

### Other essential nutrients
- Hoagland solution without nitrogen was prepared and applied three times per week

### Pathogen
- *Verticillium dahliae*

### Time of sampling for N analysis
- 0 days after fertigation
- 3 days after fertigation
- 7 days after fertigation

### Data
- Disease incidence
- Disease severity
Levels of Ammonium and Nitrate in Sand

**NH₄**

**NO₃**

<table>
<thead>
<tr>
<th>Level of NH₄ (mg/kg of soil)</th>
<th>Level of NO₃ (mg/kg of soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium sulfate</td>
<td>Ammonium nitrate</td>
</tr>
<tr>
<td>Ammonium nitrate</td>
<td>Calcium nitrate</td>
</tr>
</tbody>
</table>
Effects of Nitrogen form on Verticillium wilt

![Graph showing effects of nitrogen form on Verticillium wilt]

- **Percent disease incidence**
  - Ammonium sulfate: 80%
  - Ammonium nitrate: 50%
  - Calcium nitrate: 30%
  - Control: 10%

- **Disease severity**
  - Ammonium sulfate: 3.5
  - Ammonium nitrate: 1.5
  - Calcium nitrate: 1.0
  - Control: 0.5
• Sample spinach seedlings in Monterey, San Benito, and Santa Barbara counties and assay for *Verticillium dahliae*. 
Number of fields Surveyed: Monterey = 23; Santa Barbara = 25; Santa Cruz = 6; and San Benito = 15.
Distribution of *Verticillium* spp. on spinach in four California counties

<table>
<thead>
<tr>
<th>County</th>
<th>V. t (petiole)</th>
<th>V. t (root)</th>
<th>V. d (petiole)</th>
<th>V. d (root)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monterey</td>
<td>104</td>
<td>99</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Santa Barbara</td>
<td>108</td>
<td>129</td>
<td>34</td>
<td>35</td>
</tr>
<tr>
<td>Santa Cruz</td>
<td>15</td>
<td>19</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>San Benito</td>
<td>7</td>
<td>15</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
Verticillium spp. recovered per field

<table>
<thead>
<tr>
<th>Location</th>
<th>V. t (petiole)</th>
<th>V. t (root)</th>
<th>V. d (petiole)</th>
<th>V. d (root)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monterey</td>
<td>6.93</td>
<td>6.6</td>
<td>0.2</td>
<td>1.36</td>
</tr>
<tr>
<td>Santa Barbara</td>
<td>4.32</td>
<td>5.16</td>
<td>0.87</td>
<td>1.4</td>
</tr>
<tr>
<td>Santa Cruz</td>
<td>1.36</td>
<td>1.4</td>
<td>2.5</td>
<td>3.17</td>
</tr>
<tr>
<td>San Benito</td>
<td>2.5</td>
<td>3.17</td>
<td>0.33</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Number of Verticillium spp. recovered per field.
Acres of Spinach Grown in Monterey and Santa Barbara Counties from 1993
Figure. Phylogenetic relationships of the ten *Verticillium* species based on the combined ACT, EF, GPD and TS dataset of 2658 characters and 77 taxa, with *Gibellulopsis nigrescens* as outgroup. One of the most parsimonious trees is shown. Species in bold are newly described in this study, strain identifiers in bold represent ex-type strains. Numbers by the branches are parsimony and Bayesian and likelihood support values above 70.

Verticillium tricorpus: Is now three species, *V. tricorpus*, *V. isaacii* and *V. klebahnii*, that are morphologically indistinguishable. And they may differ in pathogenicity and virulence. Which of these are present in CA?

Also of interest, *V. zaregamsianum*, a lettuce pathogen in Japan. It looks similar to *V. dahliae*, forms mostly microsclerotia, only a few brown-pigmented hyphae, and has yellow-pigmented hyphae. However, the pigmented hyphae may not be formed in culture, and thus *V. zaregamsianum* may be confused with *V. dahliae*. Or if judged solely by the yellow pigmentation, it could be confused with species in the *V. tricorpus* group. Is *V. zaregamsianum* present in CA?

All these species can be differentiated by DNA sequencing, or more easily, by PCR.
Verticillium tricorpus

Verticillium zaregamsianum
Verticillium klebahnii

Verticillium isaacii
V. dahliae  V. isaacii  V. tricorpus
V. dahliae  V. klebahnii  V. zaregamsianum

Actin  ITS  EF-1α  EF-1α  Actin  GPD
**Lettuce - Verticillium dahliae Disease Cycle**

**Initial 48 hours PI**
- Conidiophores
- Germinating conidium with germtube and appressorium
- Systemic colonization of lateral root
- Colonization of root elongation zone
- Colonization of root tip

**By 2 weeks PI**
- or

**6 to 8 weeks PI**
- Close-up of taproot exhibiting vascular discoloration
- Leading edge of mycelia advancing into the taproot via a colonized lateral root
- Advance of mycelia into taproot via xylem vessels
- Discoloration of vascular tissues in the taproot in advance of mycelia

**8 to 10 weeks PI**
- Eruption of mycelia from xylem vessels into surrounding cortical tissues of taproot
- Advance of mycelia into taproot via xylem vessels
- Discoloration of vascular tissues in the taproot in advance of mycelia

**10+ weeks PI**
- Mature microsclerotia embedded in taproot tissues
- Colonization of taproot and microsclerotia development
- Taproot colonization coincides with foliar symptom development

**14+ weeks PI**
- Anthropogenic dispersal of microsclerotia throughout soil
- Colonization of taproot and microsclerotia development
- Taproot colonization coincides with foliar symptom development

**Determinative Phase**
- Systemic spread via hyphal budding in stem tissues
- Colonization with sporulation on achenes in a dehiscent capitulum
- Internal colonization of achenes limited to maternal tissues; seedborne phase