Biology and management of *Pantoea* sp. in Georgia: Sharing our experiences

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COASTAL PLAIN EXPERIMENT STATION
UNIVERSITY OF GEORGIA, TIFTON
Importance of Vidalia onion in GA

- Georgia ranks number one in spring-grown onion in US
- Vidalia sweet onion is number one vegetable commodity in GA
- Farm gate value: **$150 million**; area: **11,000 acres**
- Vidalia onion is grown in 21 counties is SE GA (Toombs and Tattnall Co. being major contributors)
Center rot of onion

- First described in GA in 1997
- White streaks and water soaked lesions in leaves
- Wilting of plants and bulb infection
- 100% losses in some fields
- Severe in late maturing genotypes
Center rot: *Pantoea ananatis*, *P. agglomerans* and *P. alli*

Belongs to *Enterobacteraceae* (family of gut bacteria)

- *P. ananatis* (*Erwinia ananas*): Large host range (pineapple, tomato, cantaloupe, honeydew melons, onion, rice, corn, Sudangrass, Eucalyptus)

- *P. agglomerans* (*Erwinia herbicola*): epiphytic resident of many hosts also a pathogen of onion, corn, cotton

- *P. alli*: Onion

- Any other causal agents: ???

**Sources of *P. ananatis* inoculum**: Infested seeds, weeds and thrips (tobacco thrips and onion thrips)
**MLSA:** *atpD, gyB, infB, rpoB*

Another partner in crime identified: *Pantoea stewartii* subsp. *indologenes*

Known center rot causing pathogens

- *P. stewartii* subsp. *indologenes*  
- *P. stewartii* subsp. *indologenes*  
- *P. ananatis* 97-1

*P. stewartii* subsp. *indologenes*  

*P. ananatis* 97-1

*PNA 03-3*  

*PNA 14-12*

Stumpf et al. 2018 Plant Disease
Management of center rot requires an integrated approach

• How do weeds and vectors are involved in *P. ananatis* infection?

• How do weeds and vector management affect center rot management?

• Are there any physiological growth stage/stages of onion where it is susceptible to bulb infection?

• Cultivar susceptibility?

• How diverse are *P. ananatis* populations in GA?

• Reliable diagnostic methods to detect pathogenic *P. ananatis*
\textit{P. ananatis} can survive as an epiphyte on Florida pusley (\textit{Richardia scabra}) under conditions that prevail in Vidalia onion region.

- \textit{P. ananatis} survived significantly better with a 12h wet/12h dry cycle or a continuous wet period for 96 hpi at both 15.5° and 21.1°C.

Dutta et al., 2016 Plant Disease
Thrips can acquire *P. ananatis* from weeds and can transmit to onion seedlings

<table>
<thead>
<tr>
<th>Weed species</th>
<th>Number of weed samples assayed</th>
<th>Weed samples harbored epiphytic populations of <em>P. ananatis</em> (%)</th>
<th>Weed samples harbored pathogenic strains of <em>P. ananatis</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida pusley</td>
<td>40</td>
<td>95</td>
<td>60</td>
</tr>
<tr>
<td>Verbena</td>
<td>40</td>
<td>55</td>
<td>35</td>
</tr>
<tr>
<td>Carolina geranium</td>
<td>40</td>
<td>70</td>
<td>45</td>
</tr>
<tr>
<td>Yellow nutsedge</td>
<td>40</td>
<td>55</td>
<td>25</td>
</tr>
</tbody>
</table>

- Pathogenic strains of *P. ananatis* was isolated from weeds
- Thrips can acquire and transmit natural populations of *P. ananatis* to onion seedlings
- Thrips and weeds are important component in *P. ananatis* spread and infection
No data on how diverse *P. ananatis* is population in Georgia: Important information required for resistance breeding
TABLE 1. Isolates of *Pantoea ananatis* and their associated phenotypic characteristics.

<table>
<thead>
<tr>
<th>Strain Name&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Host</th>
<th>Place of isolation in Georgia (county)</th>
<th>Ice nucleation&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Copper tolerance&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANS 99-1</td>
<td><em>Richardia scabra</em> L.</td>
<td>Tift</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>PANS 99-3</strong></td>
<td><em>Richardia scabra</em> L.</td>
<td>Tift</td>
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<tr>
<td>PANS 99-11</td>
<td><em>Digitaria sanguinalis</em></td>
<td>Tift</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PANS 99-27</td>
<td><em>Desmodium tortuosum</em></td>
<td>Toombs</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PANS 99-29</td>
<td><em>Digitaria sanguinalis</em></td>
<td>Tift</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>PANS 01-2</strong></td>
<td><em>Thrips tabaci</em>&lt;sup&gt;z&lt;/sup&gt;</td>
<td>Tift</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PNA 97-1</td>
<td><em>Allium cepa</em> L.</td>
<td>Toombs</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PNA 98-8</td>
<td><em>Allium cepa</em> L.</td>
<td>Toombs</td>
<td>+</td>
<td>-</td>
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<tr>
<td>PNA 99-3</td>
<td><em>Allium cepa</em> L.</td>
<td>Tift</td>
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<tr>
<td>PNA 200-11</td>
<td><em>Allium cepa</em> L. seed</td>
<td>Tift</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PNA 02-18</td>
<td><em>Allium cepa</em> L.</td>
<td>Tattnall</td>
<td>+</td>
<td>-</td>
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<tr>
<td><strong>PNA 06-1</strong></td>
<td><em>Allium cepa</em> L.</td>
<td>Wayne</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PNA 07-1</td>
<td><em>Allium cepa</em> L.</td>
<td>Tattnall</td>
<td>+</td>
<td>-</td>
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<td>PNA 08-1</td>
<td><em>Allium cepa</em> L.</td>
<td>Tattnall</td>
<td>+</td>
<td>-</td>
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<tr>
<td><strong>PNA 15-1</strong></td>
<td><em>Allium cepa</em> L.</td>
<td>Tattnall</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PANS 99-22</td>
<td><em>Digitaria sanguinalis</em></td>
<td>Tift</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PANS 01-5</td>
<td><em>Thrips tabaci</em>&lt;sup&gt;z&lt;/sup&gt;</td>
<td>Tift</td>
<td>+</td>
<td>-</td>
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<tr>
<td>PANS 01-6</td>
<td><em>Thrips tabaci</em>&lt;sup&gt;z&lt;/sup&gt;</td>
<td>Tift</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PNA 07-5</td>
<td><em>Allium cepa</em> L.</td>
<td>Wayne</td>
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<td>-</td>
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<tr>
<td>PNA 13-1</td>
<td><em>Allium cepa</em> L.</td>
<td>Toombs</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PNA 14-1</td>
<td><em>Allium cepa</em> L.</td>
<td>Toombs</td>
<td>-</td>
<td>-</td>
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<tr>
<td>PNA 99-14</td>
<td><em>Allium cepa</em> L.</td>
<td>Toombs</td>
<td>+</td>
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<tr>
<td>PNA 14-4</td>
<td><em>Allium cepa</em> L.</td>
<td>Toombs</td>
<td>-</td>
<td>-</td>
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<td>PANS 99-24</td>
<td><em>Vigna unguiculata</em></td>
<td>Toombs</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>PNA 200-3</strong></td>
<td><em>Allium cepa</em> L. seed</td>
<td>Tift</td>
<td>-</td>
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<tr>
<td>PANS 99-33</td>
<td><em>Richardia scabra</em> L.</td>
<td>Coffee</td>
<td>-</td>
<td>-</td>
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<tr>
<td>PANS 99-33</td>
<td><em>Richardia scabra</em> L.</td>
<td>Coffee</td>
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<tr>
<td>PANS 02-5</td>
<td><em>Frankliniella fusca</em>&lt;sup&gt;z&lt;/sup&gt;</td>
<td>Tift</td>
<td>+</td>
<td>-</td>
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<tr>
<td>PNA 97-11</td>
<td><em>Allium cepa</em> L.</td>
<td>Toombs</td>
<td>+</td>
<td>-</td>
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<tr>
<td>PNA 98-2</td>
<td><em>Allium cepa</em> L.</td>
<td>Tift</td>
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<td>Tattnall</td>
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</tbody>
</table>

<sup>a</sup> Strains collected from Vidalia onion counties (1997-2015)

<sup>b</sup> Ice nucleation

<sup>c</sup> Copper tolerance
Strains collected from Vidalia onion counties (1997-2015)

- Fifty strains isolated from onion ($n=23$), onion seed ($n=5$), weeds ($n=16$), and thrips ($n=6$) were used in this study
  - 1997-2015 (isolation period)
  - Various counties in southern Georgia
  - Pathogenic on onion ($n=33$)
  - Ice nucleation positive ($n=31$)

- Strains ($n=50$) were assessed for ice nucleation phenotype

- Strains ($n=50$) were diluted to $1 \times 10^6$ CFU/ml and plated on media amended with 50 ppm of CuSO$_4$
  - No strains grew on copper amended media
### Phenotypic diversity (aggressiveness) observed on different hosts (onion, chives, leek, shallot)

<table>
<thead>
<tr>
<th>Code</th>
<th>Species</th>
<th>Geographical Distribution</th>
<th>Onion</th>
<th>Chives</th>
<th>Leek</th>
<th>Shallot</th>
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<tr>
<td>PANS 99-1</td>
<td><em>Richardia scabra</em> L.</td>
<td>Tift</td>
<td>-</td>
<td>-</td>
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<td>PANS 99-3</td>
<td><em>Richardia scabra</em> L.</td>
<td>Tift</td>
<td>-</td>
<td>-</td>
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<td><em>Thrips tabaci</em></td>
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<tr>
<td>PNA 200-11</td>
<td><em>Allium cepa</em> seed</td>
<td>Tift</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>PNA 02-18</td>
<td><em>Allium cepa</em> L.</td>
<td>Tattnall</td>
<td>+</td>
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<td>Tattnal</td>
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<td><em>Digitaria sanguinalis</em></td>
<td>Tift</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>PANS 01-5</td>
<td><em>Thrips tabaci</em></td>
<td>Tift</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PANS 01-6</td>
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<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>PNA 07-5</td>
<td><em>Allium cepa</em> L.</td>
<td>Wayne</td>
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<td>PNA 13-1</td>
<td><em>Allium cepa</em> L.</td>
<td>Toombs</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PNA 14-1</td>
<td><em>Allium cepa</em> L.</td>
<td>Toombs</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PNA 99-14</td>
<td><em>Allium cepa</em> L.</td>
<td>Toombs</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PNA 14-4</td>
<td><em>Allium cepa</em> L.</td>
<td>Toombs</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
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<td>PANS 99-24</td>
<td><em>Vigna unguiculata</em></td>
<td>Toombs</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>PNA 200-3</td>
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<td>ND</td>
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<tr>
<td>PANS 99-33</td>
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<td>Coffee</td>
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<td>ND</td>
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<td>PANS 02-5</td>
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<td>ND</td>
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<td>PNA 97-11</td>
<td><em>Allium cepa</em> L.</td>
<td>Toombs</td>
<td>+</td>
<td>-</td>
<td>Y</td>
<td>ND</td>
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<td>+</td>
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</tr>
</tbody>
</table>

**Less aggressive (+)**

**Moderately aggressive (++)**

**Highly aggressive (+++)**
Multi-Locus Sequence Analysis (MLSA): fusA, gyrB, leuS, pyrG, rplB, and rpoB

- Limited genotypic diversity using MLSA and REP-PCR
- Even pathogenic and non-pathogenic onion strains were not separated out
- MLSA may not be a suitable method for determining intra-species strain diversity in *P. ananatis*
Whole genome sequencing

1. Identify 10 diverse strains
2. WGS 10 strains
3. Pacbio of proto-type strain PNA 97-1
4. Comparative Pipelines (ROARY & PGAP)
5. Identify potential genes of interest
Red Onion Scale Assay: Is it an alternative to seedling assay for determining pathogenicity of *Pantoea* sp. strains?

- The pathogenic and non-pathogenic strains showed a high correlation ($r= 0.98; P=0.002$) and ($r=0.91; P=0.037$) respectively between the foliar assay and the onion scale assay.
Twenty strains were selected based on their pathogenicity on Alliaceae species, isolation source, and location.
Can Whole Genome Sequences unravel underlying diversity in *P. ananatis* strains?

A

**ROARY**

Pathogenic

- PANS 06-01
  - PNA 15-1
  - PNA 200-3
  - PANS 99-3
  - PANS 01-02
  - PNA 97-1
- PANS 99-23
  - PANS 04-02
  - PNA 99-7
  - PANS 99-36

B

**PGAP**

Non-Pathogenic

- PNA 97-1
  - PANS 01-02
  - PANS 99-3
  - PNA 15-1
  - PANS 06-1
  - PNA 200-3
- PNA 99-7
  - PANS 04-02
  - PANS 99-23
  - PANS 99-36
Can Whole Genome Sequences unravel underlying diversity in *P. ananatis* strains?
**Can Whole Genome Sequences unravel pathogenicity and/or virulence factors in P. ananatis?**

- Two groups of genes in *P. ananatis* correlate with onion pathogenicity

  ✓ Onion Virulence Region (OVR A-D): (Stice & Stumpf et al. 2018)
    - Four gene clusters found on **plasmids** of *P. ananatis*

  ✓ HiVir Cluster: (Asselin et al. 2018)
    ✓ Gene cluster found in **genome** of pathogenic *P. ananatis* strains
    ✓ **Required** for pathogenicity (foliar and bulb)
Pathogenic strains

HiVir and OVR present = pathogenic strains (72%)

HiVir present only = pathogenic strains (28%)

Non-Pathogenic strains

HiVir and OVR absent = non-pathogenic strains (78%)

Only HiVir = non-pathogenic strains (15%)

Only HiVir = non-pathogenic strains (7%)

Genome screening of *P. ananatis* strains (n = 26)
Can we use these genes to detect pathogenic *P. ananatis* strains?

- A multiplex PCR with HiVir and OVR regions can be developed
- Combined with red-onion scale bioassay
- Studies are underway to decipher pathogenicity/virulence factors in *P. ananatis*
- Information will be utilized in developing a robust diagnostic assay
Host factor: Which growth stage of onion is highly susceptible to bulb infection?

- Seedling stage
- True leaf stage (4-6)
- First leaf senescence
- Bulb initiation
- Bulb swelling
- Pseudostem hardening
- Flowering

Vegetative phase

Reproductive phase
Significant higher incidence of center rot was observed for bulbs whose foliage were inoculated during first leaf senescence stage (64%) compared to bulb initiation (55%) and bulb swelling (52%) stages ($P=0.048$).
Conclusions

• Onion growth stage can influence incidence of *P. ananatis* in bulbs

• Can we protect these growth stages using antimicrobials (Cu-bactericide) and growth regulators (Actigard)?
Investigate if growth stage targeted foliar treatments can reduce *P. ananatis* bulb infection

- Growth stage (bulb initiation and bulb swelling) targeted chemical treatments: Kocide 3000 (copper hydroxide), Actigard 50WG (acibenzolar-s-methyl), and Actigard 50WG + Kocide 3000

- Additionally, we assessed if thrips infestation could reduce the efficacy of foliar treatments
Onion growth stage directed chemical application

24 h

Exposed to thrips

Actigard

24 h

Inoculation

Induction of PR genes upon Actigard application

Mean relative expression

Time (hour post application)
Thrips feeding can reduce the efficacy of protective chemical treatments against *P. ananatis*.
Conclusions

• Chemical protection with Kocide 3000 during bulb initiation and bulb swelling stages were effective in reducing center rot incidence in onion bulbs

• Actigard displayed limited efficacy in reducing center rot symptoms in bulb

• Thrips feeding can reduce the efficacy of protective chemical treatments against *P. ananatis*

• How relevant is this finding under field conditions?
At First Leaf Senescence
Treatment 1: Kocide 3000 @ 1.5 lb/A for 3 consecutive weeks
Treatment 2: Actigard @ 0.5 oz/A for 3 consecutive weeks
Treatment 3: Kocide+Actigard at previously mentioned rate. Actigard were applied 24 hours prior to Kocide application.
Treatment 4: No treatment

At Bulb Initiation
Treatment 5: Kocide 3000 @ 1.5 lb/A for 3 consecutive weeks
Treatment 6: Actigard @ 0.5 oz/A for 3 consecutive weeks
Treatment 7: Kocide+Actigard at previously mentioned rate. Actigard were applied 24 hours prior to Kocide application.
Treatment 8: No treatment

At Bulb Swelling
Treatment 9: Kocide 3000 @ 1.5 lb/A for 3 consecutive weeks
Treatment 10: Actigard @ 0.5 oz/A for 3 consecutive weeks
Treatment 11: Kocide+Actigard at previously mentioned rate. Actigard were applied 24 hours prior to Kocide application.
Treatment 12: No treatment

All Growth Stages
Treatment 13: Kocide 3000 @ 1.5 lb/A throughout growing season
Treatment 14: Actigard @ 0.5 oz/A throughout growing season
Treatment 15: Kocide+Actigard at previously mentioned rate. Actigard were applied 24 hours prior to Kocide application.
Treatment 16: No treatment
Treatment 17: No treatment

Growth stage targeted foliar protection:
Field trials in 2017 and 2018
Intensive insecticide program was followed for thrips control
Field incidence + post curing incidence (7 days) = Total bulb incidence

>9000 onion bulbs were cut open and individually rated for center rot symptoms
Significant effect of treatments were not observed for onions that were treated during first leaf senescence stage

Dutta et al., 2018
Bulb incidence of center rot of onion

Bulb incidence of center was significantly lower for Kocide or Kocide+Actigard treatments

Dutta et al., 2018
Bulb incidence of center rot of onion

Center rot bulb incidence (%)

UTC Kocide Actigard Kocide+Actigard

P=0.021

Chemical protection throughout onion growing period

Marketable yield (lb)

Bulb initiation $P<0.001$

Kocide Actigard Kocide+Actigard UTC

Bulb swelling $P<0.001$

Kocide Actigard Kocide+Actigard UTC

• Significantly higher marketable yields for Kocide treatment when onions were applied at either bulb initiation or bulb swelling stages

Dutta et al., 2018
Impact of center rot research

• Importance of weed and vector management were extended to our onion growers

• Threshold based insecticide spray for thrips may not be effective in center rot management

• Growth stage based targeted spray can reduce frequency/number of Cu spray (at least 3), which may account for $80-100/acre of saving

• No added benefit from Actigard; removed from onion spray guide

• Overall, added information to the knowledge base in *P. ananatis* biology, critical step in devising sustainable management options for center rot.
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