Polygalacturonase gene expression in wild collected *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae)

D.E. Fleming, N. Krishnan, and F.R. Musser
Lygus lineolaris

- Tarnished plant bug
- Highly polyphagous
- Similar to *L. hesperus* in western US
- Major cotton pest in SE US
  - #1 cotton pest in MS
  - Cost MS growers $28 million per year for insecticides and still caused an average of $8 million damage per year (2009-2011)
  - $194/ha in Delta vs. $46/ha in Hills
- Resistant to many insecticides
Damage

- Cause damage by inserting stylet into plant tissue and inject salivary enzymes to breakdown plant pectins
Polygalacturonase

- Salivary enzymes used by plant bugs to breakdown plant pectins
- Shown to degrade plant tissue, associated with *Lygus* type damage (Strong and Kruitwagen 1968, Shackel 2005)
- Three different PG genes in *L. lineolaris* (Allen and Mertens, 2008)
- Genes may be up and down regulated based on current host (Walker and Allen 2010)
- PG may be used in oviposition site preparation (Cooper and Spurgeon 2011)
- Anti-PG genes have been discovered
PG expression in wild *L. lineolaris* populations collected from cotton, pigweed, and horseweed in the Delta and Hills regions of MS
Materials and Methods

- **Field**
  - Collected 10 adult TPBs per location per host
  - 5 locations
  - Stored in plastic bags in -80°C freezer

- **Preparation for molecular analysis**
  - Surface sterilized in 70% EtOH
  - Salivary glands removed (head removed from body)
  - Stored in DNAse and RNAse free microcentrifuge tubes in -80°C freezer
Materials and Methods

- Molecular analysis
  - Homogenized as 10 insect samples in Trizol (Sigma)
  - RNA isolated
  - Genomic DNA removed (rDNAse I-Takara)
  - RNA purified and quantity estimated by Take 3 nonodrop in BioTek H1M reader
  - cDNA synthesized using Iscript cDNA synthesis kit (Biorad)
  - Gene expression measured by qRT-PCR in Eppendorf realplex² Master Cycler
Materials and Methods

- **Statistical analysis**
  - Raw data converted to fold change
    - \( \text{Ct mean - housekeeper} = \Delta \text{Ct} \)
      - Housekeeper rpl6
    - \( \Delta \text{Ct} - \text{normalizer} = \Delta \Delta \text{Ct} \)
      - Normalizer \( \Delta \text{Ct} \) values are from a colony reared on diet
    - Fold change = \( 2^{-\Delta \Delta \text{Ct}} \)
    - Log transformed
  - Analyzed using Statistica Statsoft software GLM
  - Comparisons made of
    - Delta vs. Hills
    - PG expression by host (ex. PG1 in cotton vs. horseweed vs. pigweed)
Results (Overall)

- Region by Host interactions
  - PG1 (df=2, 17, F=5.70, p=0.01)
  - PG2 (df=2, 17, F=2.60, p=0.10)
  - PG3 (df=2, 17, F=3.20, p=0.07)

- Main effects
  - Region
    - PG2 (df=1, 19, F= 2.16, p=0.16)
    - PG3 (df=1, 19, F=0.12, p=0.73)
  - Host
    - PG2 (df=1, 19, F=8.67, p<0.01)
    - PG3 (df=2, 19, F=3.83, p=0.04)
Results (Delta vs. Hills)

- **Cotton**
  - PG₁
    - More expression in Hills TPBs
    - \((df=1, 6, F=9.55, p=0.02)\)

- **Horseweed**
  - No significant differences for any PG

- **Pigweed**
  - PG₂
    - More expression in Hills TPBs
    - \((df=1, 6, F=9.00, p=0.02)\)
Delta vs. Hills Cotton

<table>
<thead>
<tr>
<th>Region*Target</th>
<th>Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta*PG1</td>
<td>-3.5</td>
</tr>
<tr>
<td>Hills*PG1</td>
<td>-3.0</td>
</tr>
<tr>
<td>Delta*PG2</td>
<td>-2.5</td>
</tr>
<tr>
<td>Hills*PG2</td>
<td>-2.0</td>
</tr>
<tr>
<td>Delta*PG3</td>
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<tr>
<td>Hills*PG3</td>
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</table>

log fold change

- a
- b
Delta vs. Hills Horseweed

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<td>Hills*PG1</td>
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</tr>
<tr>
<td>Delta*PG2</td>
<td>-2.0</td>
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<tr>
<td>Hills*PG2</td>
<td>-1.5</td>
</tr>
<tr>
<td>Delta*PG3</td>
<td>-1.0</td>
</tr>
<tr>
<td>Hills*PG3</td>
<td>-0.5</td>
</tr>
</tbody>
</table>
PG expression by host

- **Delta**
  - **PG1**
    - Less expression in cotton than horseweed or pigweed
    - $(df=2, 8, F=7.80, p=0.01)$
  - **PG2**
    - More expression in horseweed than in cotton or pigweed
    - $(df=2, 8, F=14.16, p<0.01)$
  - **PG3**
    - Less expression in cotton than in horseweed or pigweed
    - $(df=2, 9, F=4.72, p=0.04)$
- **Hills**
  - No significant differences
PG2 expression by host

| Region*Host          | Mean±SE
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Delta*Cotton</td>
<td>-3.0</td>
</tr>
<tr>
<td>Delta*Horseweed</td>
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<tr>
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<tr>
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<td>Hills*Horseweed</td>
<td>-1.0</td>
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<tr>
<td>Hills*Pigweed</td>
<td>-0.5</td>
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</tbody>
</table>

log fold change

-3.0 -2.5 -2.0 -1.5 -1.0 -0.5 0.0
PG3 expression by host

![Bar chart showing log fold change for different region-host combinations.](chart)

<table>
<thead>
<tr>
<th>Region*Host</th>
<th>Delta*Cotton</th>
<th>Delta*Horseweed</th>
<th>Delta*Pigweed</th>
<th>Hills*Cotton</th>
<th>Hills*Horseweed</th>
<th>Hills*Pigweed</th>
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</thead>
<tbody>
<tr>
<td>log fold change</td>
<td>-4.0</td>
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<td>-3.0</td>
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<tr>
<td></td>
<td>-2.0</td>
<td>-1.5</td>
<td>-1.0</td>
<td>-0.5</td>
<td>0.0</td>
<td>-0.5</td>
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- **a** indicates statistically significant difference.
- **b** indicates another statistically significant difference.
Summary

- All samples show a general down regulation of PG expression compared to the normalizer (artificial diet).
- Few differences between Delta vs. Hills and Hosts.
- At this point it is difficult to determine what may be a real effect vs. random effect
  - Movement between hosts
  - Time on current host
  - Age and health of the plants
  - Age and health of the insects
Future Work

- Male vs. Female
- Age
- Nymphs vs. Adults
- Changes from artificial diet to host tissue
Questions and suggestions???

Have any PG antibodies been developed?