Penn State Microbiome Center Presents

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Mighty microbes: The tri-trophic interactions of endophytic *Metarhizium* in maize.
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Project team

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Plants, microbes and beyond!
Microbes are mighty!

In 1 teaspoon of soil there are...

- **Bacteria**: 100 million to 1 billion
- **Fungi**: 6-9 ft fungal strands put end to end
- **Protozoa**: Several thousand flagellates & amoeba
  One to several hundred ciliates
- **Nematodes**: 10 to 20 bacterial feeders and a few fungal feeders
- **Arthropods**: Up to 100
- **Earthworms**: 5 or more
Metarhizium

- Hypocreales: Clavicipitaceae
- Insect pathogens
- Plant colonizer
- Broad host range

Life cycle of *Metarhizium*

Metarhizium: A multifunctional fungus

**Phytohormone-mediated plant defense**

**JA:** chewing insects, necrotrophic and symbiotic fungi. Growth-defense switch.

**SA:** Biotrophic phytopathogens and phloem-feeding insects.

Fine-tuning of plant growth and defense

Objectives

- Ability of *M. robertsii* to form endophytic relationship with maize.
- Effects of endophytic *M. robertsii* on maize growth.
- Effects of endophytic *M. robertsii* on growth of Black Cutworm (BCW).
- Effects of endophytic *M. robertsii* on expression of key plant defense genes.
• Detection greatest in maize phase of feed grain rotation.

• Effects on maize growth and defense gene expression, and growth of BCW?

Plant species affect prevalence of *M. robertsii*

Randhawa et al. 2018. Biological Control
Maize colonization by *M. robertsii*

\[ F_{2,15} = 17.7 \]
\[ P = 0.0013 \]

\[ F_{1,171} = 19.7 \]
\[ P = 0.0001 \]
Height is correlated with tissue colonization

**Leaf colonization**

- Average height (cm)
  - P = 0.02
  - Est. = 1.87
  - $R^2_{adj} = 0.02$

**Root colonization**

- Average height (cm)
  - P = 0.01
  - Est. = 1.64
  - $r^2_{adj} = 0.02$

**Statistical Analysis**

- **Inoculated and Detected**
  - Average height: 92.5 cm
  - P = 0.03
  - $F_{2,227} = 3.73$

- **Inoculated and Not Detected**
  - Average height: 91.5 cm

- **Not Inoculated**
  - Average height: 90.5 cm

**Legend:**
- a
- ab
- b
Biomass is correlated with root colonization

Above-ground biomass (g) vs. Leaf colonization

Above-ground biomass (g) vs. Root colonization

$r^2_{Adj} = 0.0007$

$P = 0.16$

Est. = 0.41

$r^2_{Adj} = 0.03$

$P = 0.006$

Est. = 0.67

$F_{2,211} = 3.78$

$P = 0.02$
Jasmonic acid biosynthesis pathway

$P = 0.0001$
$F_{1,22} = 29.5$

$P = 0.02$
$F_{1,22} = 6.56$

**Relative expression**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>lox1</strong></td>
<td>1.0</td>
<td>5.0</td>
</tr>
<tr>
<td><strong>opr7</strong></td>
<td>1.0</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Significance levels:
- a: $P < 0.05$
- b: $P < 0.01$
JA and SA response pathways

**JA response**

Maize protease inhibitor (mpi)

- **Control**
- **Treated**

**Relative expression**

$P = 0.002$

$F_{1,22} = 13.23$

**SA response**

Pathogenesis-related protein 5 (pr5)

- **Control**
- **Treated**

**Relative expression**

$P = 0.0001$

$F_{1,22} = 24$
Plant-derived chitinases

Endochitinase A

- Control: 0
- Treated: 16

Relative expression: $P = 0.0036$  
$F_{1,22} = 10.63$

Pathogenesis-related protein 4 ($pr4$)

- Control: 0.2
- Treated: 0.4

Relative expression: $P = 0.006$  
$F_{1,22} = 16.11$
RGR of BCW is correlated with colonization

Inoculated and Detected
Inoculated and Not Detected
Not Inoculated

$r^2_{Adj} = 0.02$
$P = 0.03$
$Est. = -0.01$

$P = 0.01$
$F_{2,211} = 4.66$
• *M. robertsii* recovered from 91% of treated maize plants. Systemic recovery.

• Endophytic had greater plant height, above-ground biomass and modulated defense gene expression.

• Relative growth rate of black cutworm was lower on leaves from endophytic plants.

• Results support model of integrated response vs. trade-off between plant growth and defense.
Thanks for your attention!