Oomycetes associated with soybean disease and improved diagnostics

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Integrated management of oomycete diseases of soybean and other crop plants

- Survey oomycetes associated with diseased soybean seedlings
- Characterize pathogenicity and aggressiveness
- Determine fungicide sensitivity – high throughput
- Develop diagnostics for improved management
Two year survey conducted in conjunction with OSCAP network

- Which oomycetes are associated with soybean seedling disease?
- Are oomycete communities similar across locations?
Soybean seedling disease survey

- Isolation from diseased seedlings
84 unique species found ~3200 cultures
Pathogenicity/Virulence assessed for a subset of isolates across all species
- Seed rot assay
- Seedling root rot assay

Survey of oomycete species

Results

1 Aphanomyces spp.
2 Phytophthora spp.
3 Phytophthora spp.
52 Pythium spp.
4 Phytophthorum spp.
7 Phytophthora spp.
57 Pythium spp.
Oomycetes community structure

- States were clustered based on relative abundance of species
- Latitude is correlated with species richness
- Temperature and precipitation were main drivers of community structure

States
- ANOSIM R: 0.2343
  Significance: 0.001
- Many beneficial species
- Which species cause disease?
- Does plant growth stage affect susceptibility?
- What effect does temperature have on disease?
- Two different methods:
  - Seed rot
  - Seedling root rot
Seed Rot Assays

Pathogen + Seed

Severity
0    Germinated
1    Delayed germination
2    Germination and some lesions
3    Germination with coalesced lesions
4    Seed colonized

Disease Index = (DSI)

DSI = \( \sum(\text{severity} \times n) \) / \( N \)

Broders et al., 2007
Seed Rot Assays

Control

Pythium oopapillum

Pythium irregulare

Pythium ultimum var. ultimum

Phytophthora sojae

Pythium sylvaticum

Pythium attrantheridium

Pythium ultimum var. sporangiiferum
Seed rot severity index DSI at 20°C (68°F)

P. sojae

Py. sylvaticum
**Seed rot DSI – 13°C (55°F) vs 20°C (68°F)**

- *P. oopapillum* prevalent in cooler 2011 – more pathogenic at 55°F than 68°F
- *P. sojae* not very aggressive on seed, slightly more at warmer temp.
Seedling root rot assays

- Dry weight of roots and shoots
- Root area and root length
Seedling root rot assays

Control

Phytophthora sojae

Pythium oopapillum

Pythium ultimum var. sporangiiferum

Pythium sylvaticum

Pythium irregulare

Pythium ultimum var. ultimum

Pythium atrantheridium
Variability in seedling root rot at 20°C

P. sojae

P. sylvaticum
Molecular diagnostics

- Different end users
  - Different requirements

- Hierarchical approach
  - Genus
    - Phytophthora
  - Species
    - P. sojae
    - P. sansomeana

- Testing and validation
  - Quantitative PCR
  - Hydrolysis probe
  - Isothermal PCR
  - Amplicon-based community analysis

**Phytophthora sojae**
- Well recognized
- Narrow host range – soybean, lupin

**Phytophthora sansomeana** *(described 2009)*
- Not as well recognized
- Wide host range - soybean, corn, Douglas-fir

*Photo Credit: Anette Phibbs, DATCP.*
Multiplex qPCR for *P. sojae* and *P. sansomeana*

*collaboration with Frank Martin and Tim Miles*

- Multiplex assay for detection of two species
  - Plant samples (Plant internal control)
  - Soil samples (Internal control)

Bilodeau et al. Phytopath. 104:733
Validation of qPCR assay

Specificity test panel

- 96 different *Phytophthora* spp.
- 14 provisional *Phytophthora* species
- 10 *Pythium* spp.
Isothermal amplification
Recombinase Polymerase Amplification (RPA)

Smart Dart (BioRanger)
The RPA Cycle

RPA advantages
• All steps at 37°C
• Recombinase opens dsDNA
• End point or real-time

Detection in ~ 20 minutes!

http://www.twistdx.co.uk/our_technology/
RPA process: *P. sojae* and *P. sansomeana*
Isothermal RPA - Sample Results

Real-time data collection

End-point diagnostic data

Control Band
Positive Rxn
Positive
Negative
Amplicon based community analysis

- Oomycetes poorly represented in metagenomes
- But play important role in microbial ecology
- Pair w/ phenotype data
Oomycetes - Summary and future work

• Oomycete species vary by region.

• 84 unique oomycete species associated with soybean seedlings
  – 17 pathogenic in seed rot assay
  – 43 pathogenic in seedling assay
  – 15 species pathogenic in both assays

• Hierarchical qPCR and RPA diagnostic assays

• Which chemistries are most effective?

• Breeding for resistance – Dechun Wang and Yingdong Bi
Acknowledgements

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- MSU diagnostic lab

- John Boyse and Randy Laurenz

Project No. 2011-68004-30104 (“Integrated management of oomycete diseases of soybean and other crop plant...”)
Oomycete Survey

Summary

- 84 unique oomycete species associated with soybean seedlings
- Oomycete community structure correlated with geographical proximity
- Oomycete species abundance varied by latitude
- 17 species were pathogenic in the seed rot assay
- 43 species pathogenic on seedlings for at least one parameter
Diagnostic Assay Summary

- Multiplex hierarchical qPCR assay for *Phytophthora* genus, *P. sojae*, *P. sansomeana*

- Implemented by diagnosticians

- Hierarchical RPA assay for *Phytophthora*, *P. sojae*, *P. sansomeana*

- qPCR and RPA validated with field samples from 8 U.S. states and Ontario
Oomycete community structure

- Samples analyzed based on relative abundance of the species
- Temperature and precipitation were main drivers of community structure
Seedling pathogenicity
Fungi associated with diseased soybean seedlings

3000 isolates

Courtesy Ahmad Fakoury, Univ. of Southern Illinois
Fusarium species

Courtesy Ahmad Fakoury, Univ. of Southern Illinois
Fungicide sensitivity

Introduction

- Roughly 70% of soybeans are treated (Munkvold 2009)
- Critical to understand fungicide sensitivity for best management
- Sensitivity of species recovered in survey not well understood
Fungicides used in seed treatments

*What is mefenoxam and ethaboxam?*

**Metalaxyl**
- FRAC group A1
- RNA Polymerase 1

**Ethaboxam**
- FRAC group B3
- β-tubulin assembly – Uchida et al. 2005

*Well documented resistance in oomycetes*  
*Registered for soybean seed treatments 2014*
High-throughput fungicide sensitivity
Assay development

1. Macerate Agar

- 5 or 6, 16.5 mm plugs
- 2 - 5 day old cultures
- ¼ strength PCA 0.5% agar

Vortex for 10 seconds

10 ml syringe 20G needle

2. Distribute to 96 Well

- mefenoxa
- ethaboxam

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<th>mefenoxa</th>
<th>ethaboxam</th>
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3. Measure

- Controls
  - Inoculated control
  - Non-inoculated control
- ½ strength V8 broth

Growth Curves

- 24 - 48 hours growth
- Optical density 600 nm

Tecan Plate reader
High-throughput fungicide sensitivity
Assay validation

Ethaboxam – $P$ 0.05

Species
0. *Phytophthora sansomeana*
1. *Pythium aff. dissotocum*
2. *Pythium aphanidermatum*
3. *Pythium irregulare*
4. *Pythium lutarium*
5. *Pythium oopapillum*
6. *Pythium perplexum*
7. *Pythium sylvaticum*
8. *Pythium torulosum*
9. *Pythium ultimum var. ultimum*

$R^2 = 0.88$
Pearson’s $r = 0.94$
High-throughput fungicide sensitivity

Community inference

10 g a.i. ml-1 mefenoxam

20 g ml-1 ethaboxam
Fungicide Sensitivity Summary

- High throughput ~4 hours set up 30 isolates, multiple chemistries
- Poison plate ~1 day set up 12 isolates, one chemistry
- Most species appear sensitive to mefenoxam
- Ethaboxam insensitivity conserved amongst monophyletic groups
Oomycete resources

- Databases!
  - Curated and consolidated database
  - Joint effort among the community

- Taxonomy
  - Species?
  - Consolidated taxonomy
    - A. Levesque and AW de Cock
    - F. Martin and J. Blair (2014)
    - Hyde et al. 2014

Phytophthora spp.

DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer