Oomycetes associated with soybean disease and improved diagnostics

Martin Chilvers & OSCAP Extension Network

chilvers@msu.edu

@MartinChilvers1

517-353-9967

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Michigan Soybean Promotion Committee www.michigansoybean.org

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NCSRP NORTH CENTRAL SOYBEAN RESEARCH PROGRAM

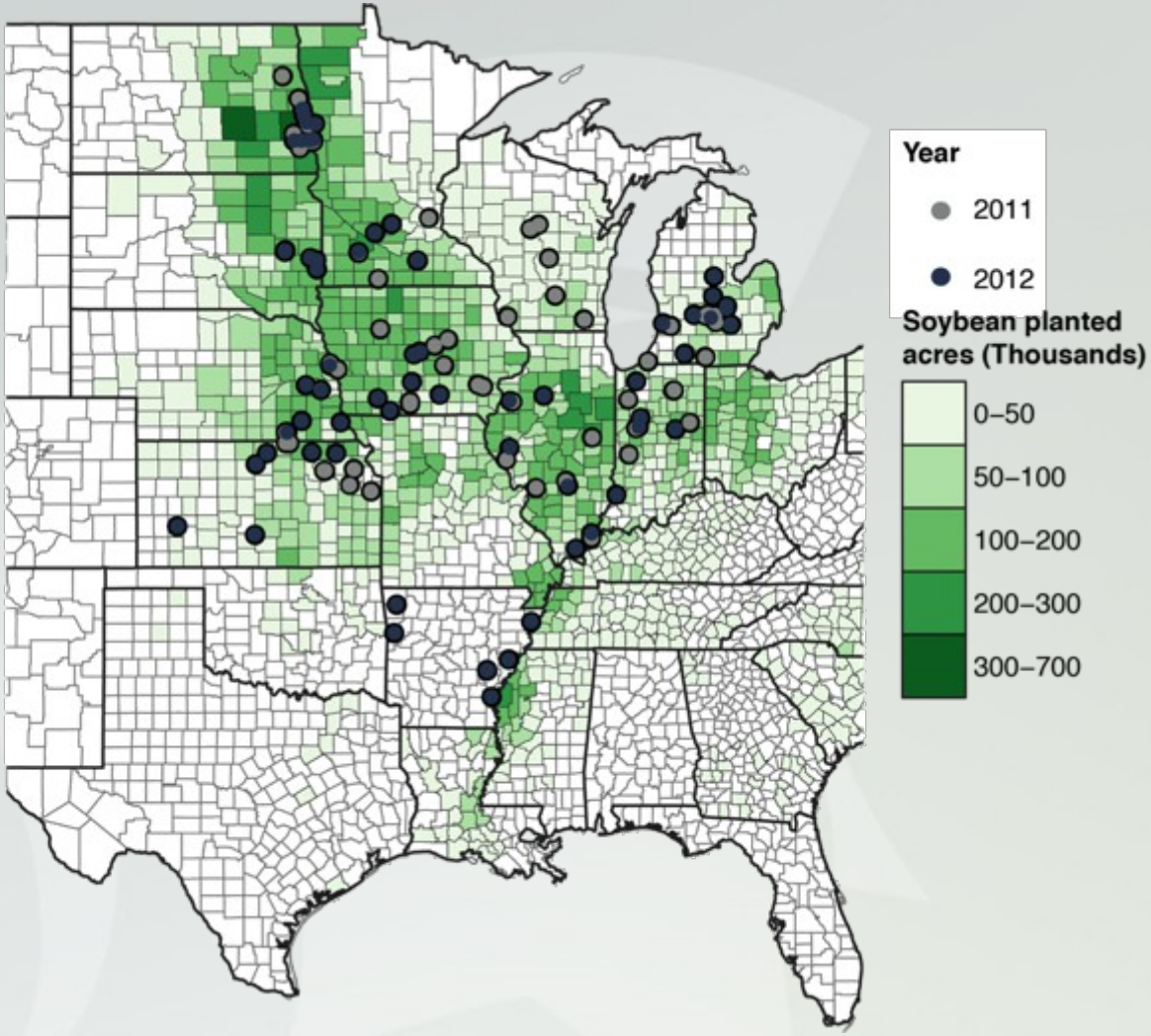
USDA NIFA

MICHIGAN STATE UNIVERSITY | Department of Plant, Soil and Microbial Sciences

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

USDA NIFA

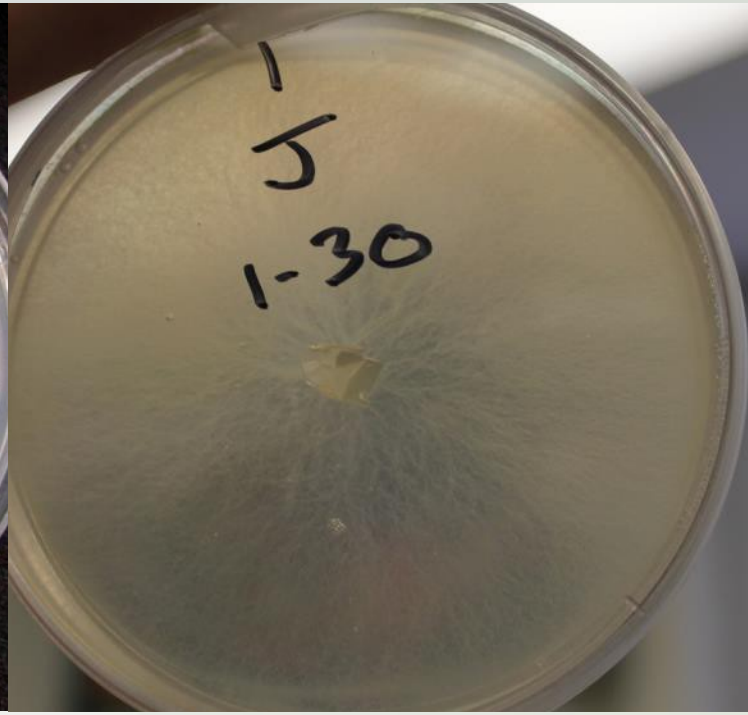


Two year survey conducted in conjunction with OSCAP network

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

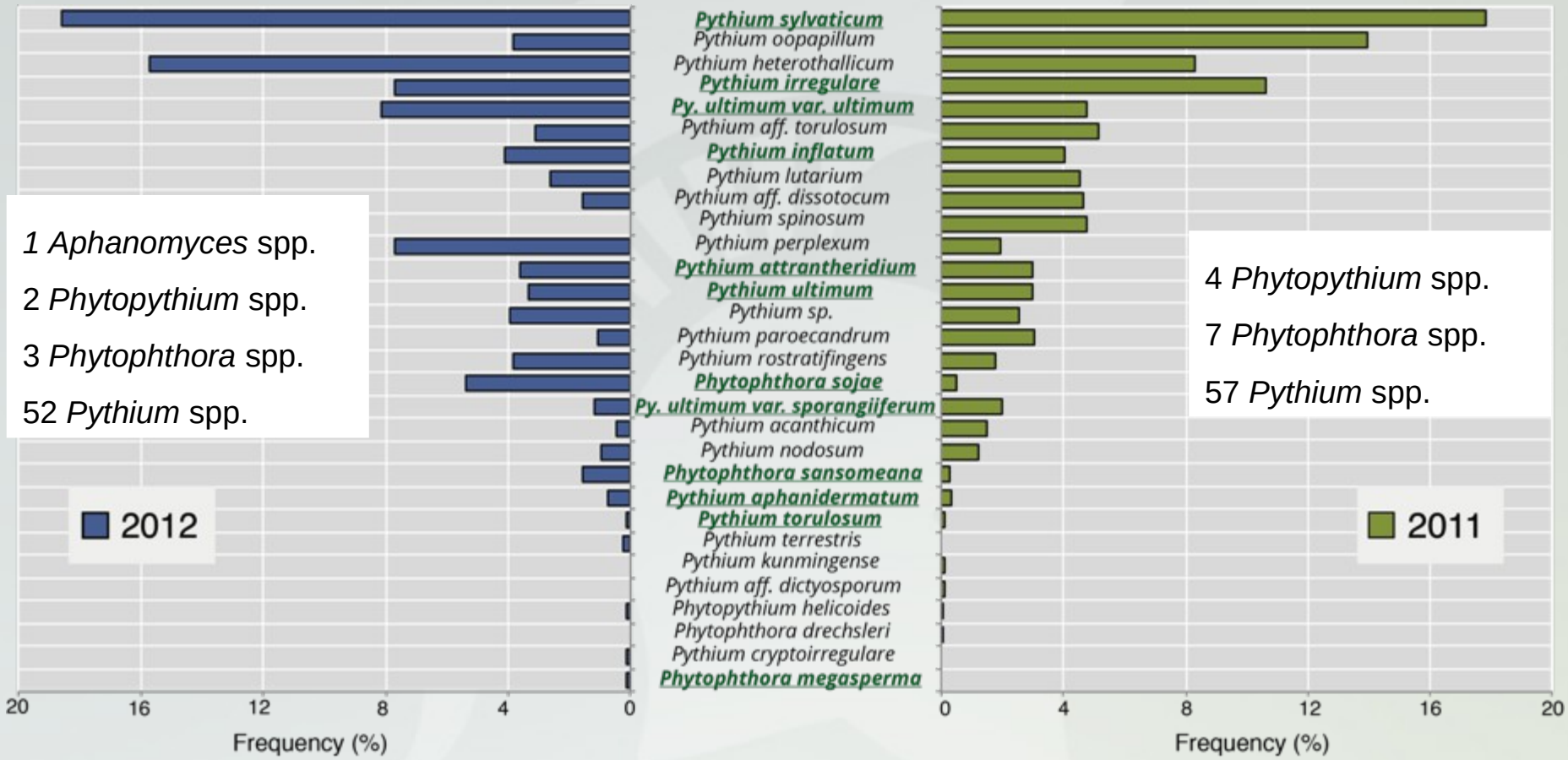
Soybean seedling disease survey

- Isolation from diseased seedlings



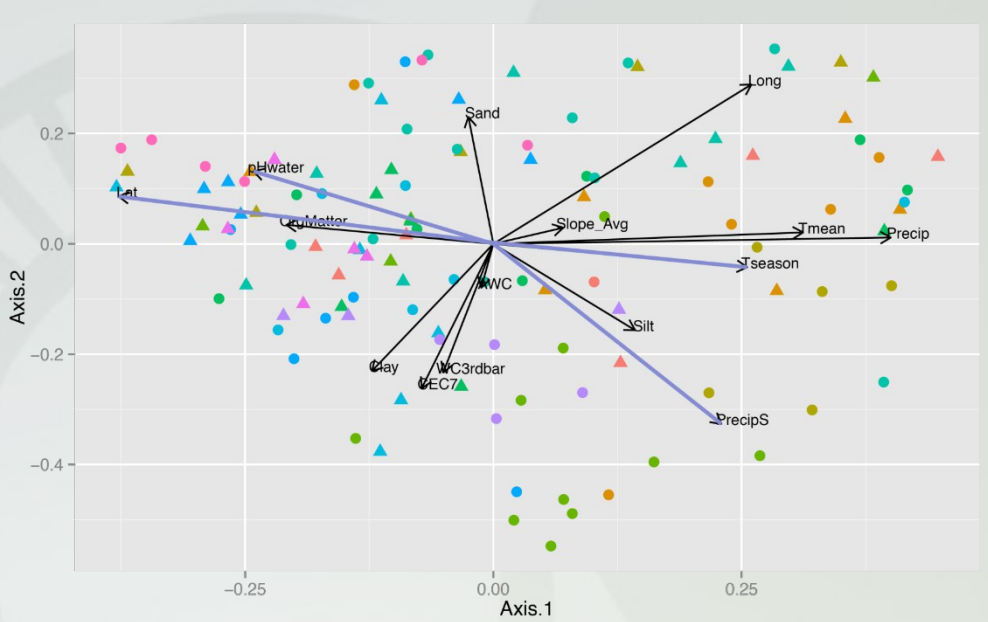
Survey of oomycete species

Results



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

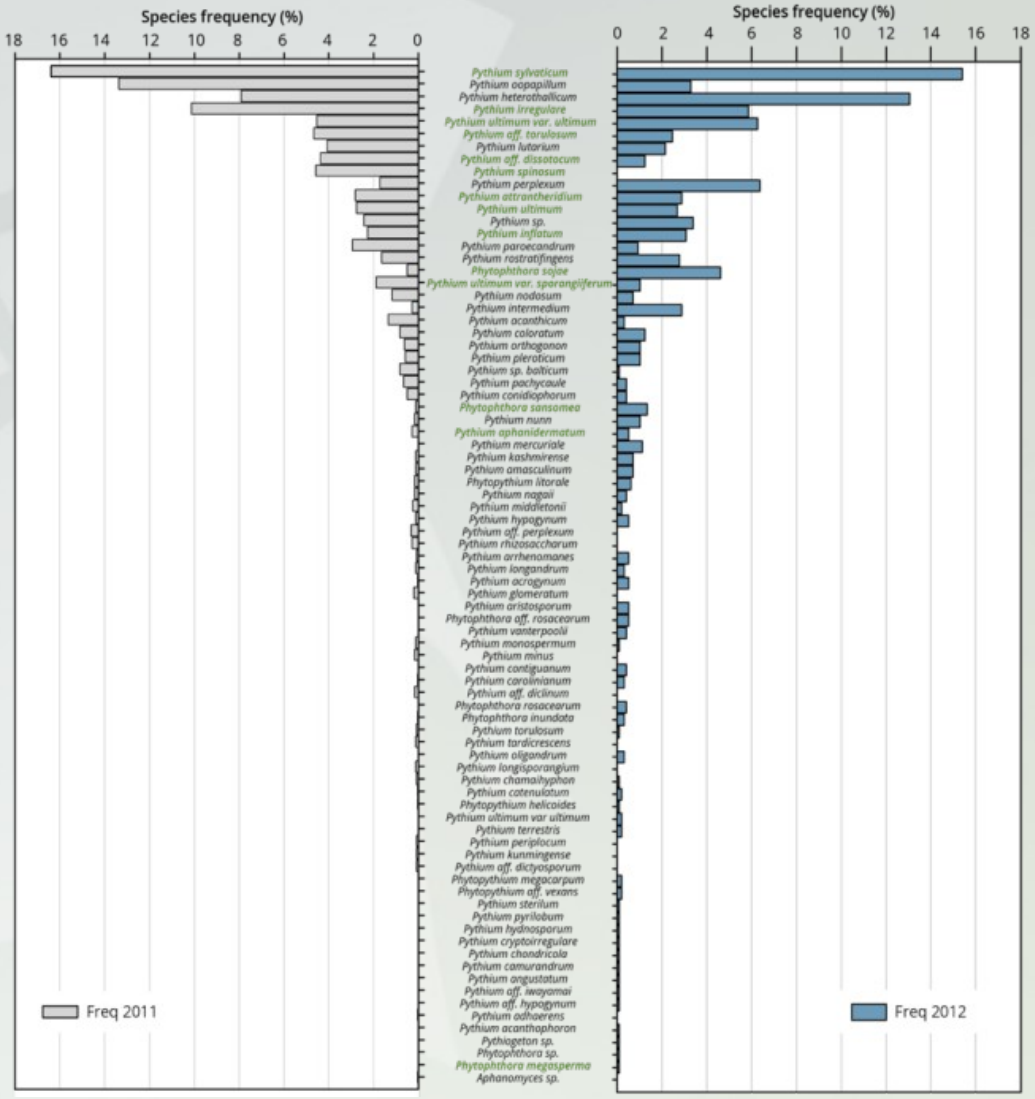
Oomycetes community structure



State ANOSIM R: 0.2343
Significance: 0.001

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

- 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

7days in dark

13C and 20C

Disease Index = (DSI)

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

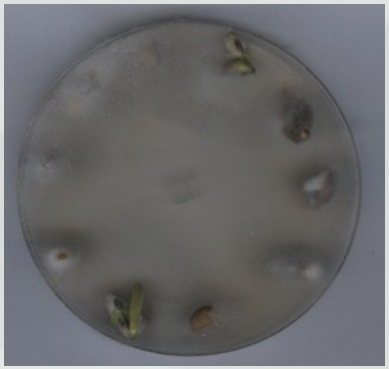
$$DSI = \frac{\Sigma(\text{severity} \times n)}{N}$$

Broders et al., 2007

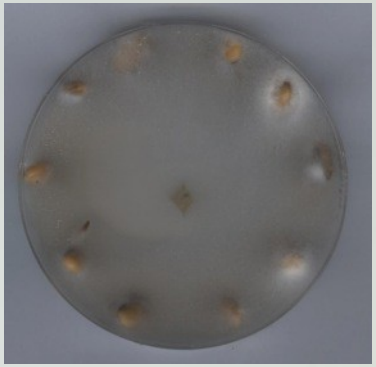
Seed Rot Assays

Control

Pythium oopapillum



Pythium irregulare

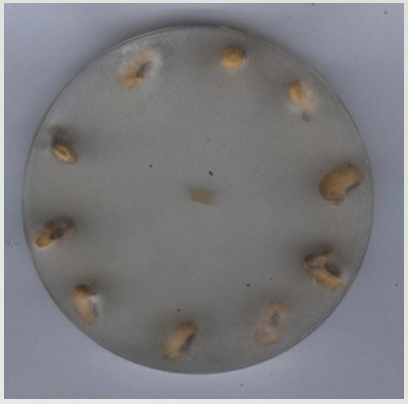


Pythium ultimum var. ultimum

Phytophthora sojae

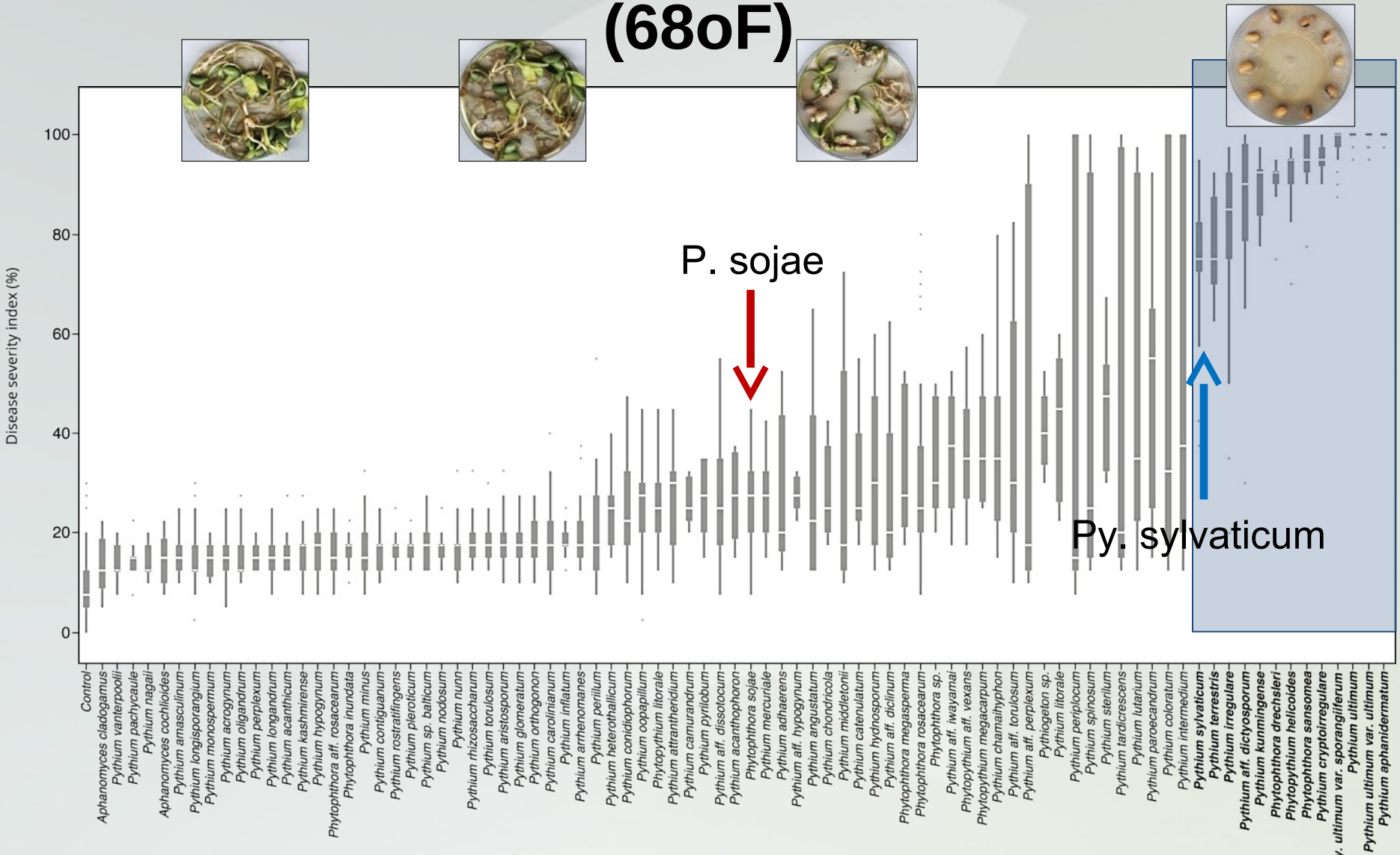
Pythium sylvaticum

Pythium attrantheridium

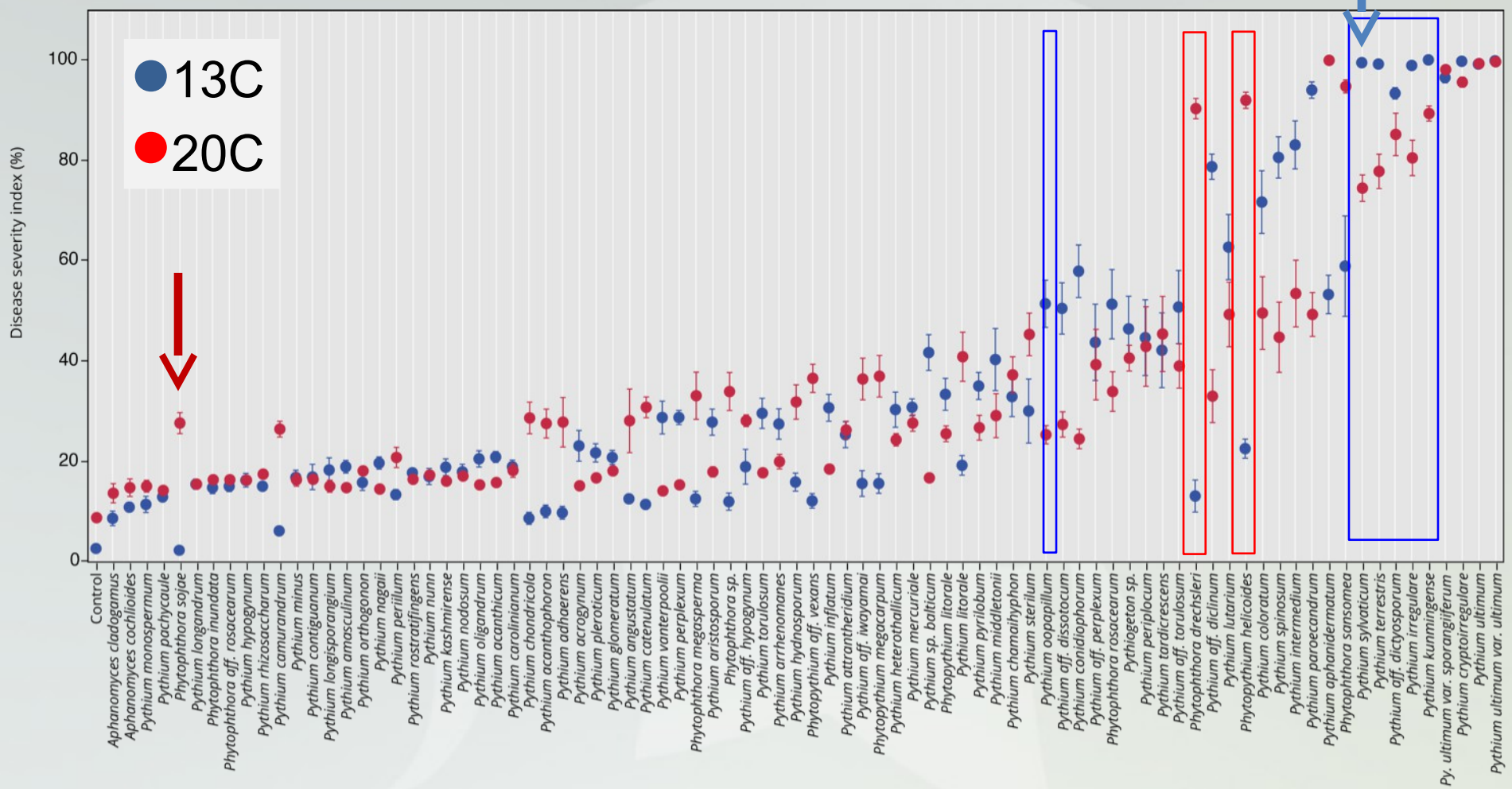


Pythium ultimum var. sporangiiferum

Seed rot severity index DSI at 20oC (68oF)



Seed rot DSI – 13oC (55oF) vs 20oC (68oF)



- *P. oopapillum* **prevalent in cooler 2011 – more pathogenic at 55oF than 68oF**
- *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

Pythium oopapillum

Pythium irregulare

Pythium ultimum var. ultimum

Phytophthora sojae

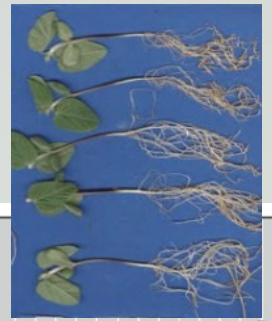
Pythium sylvaticum

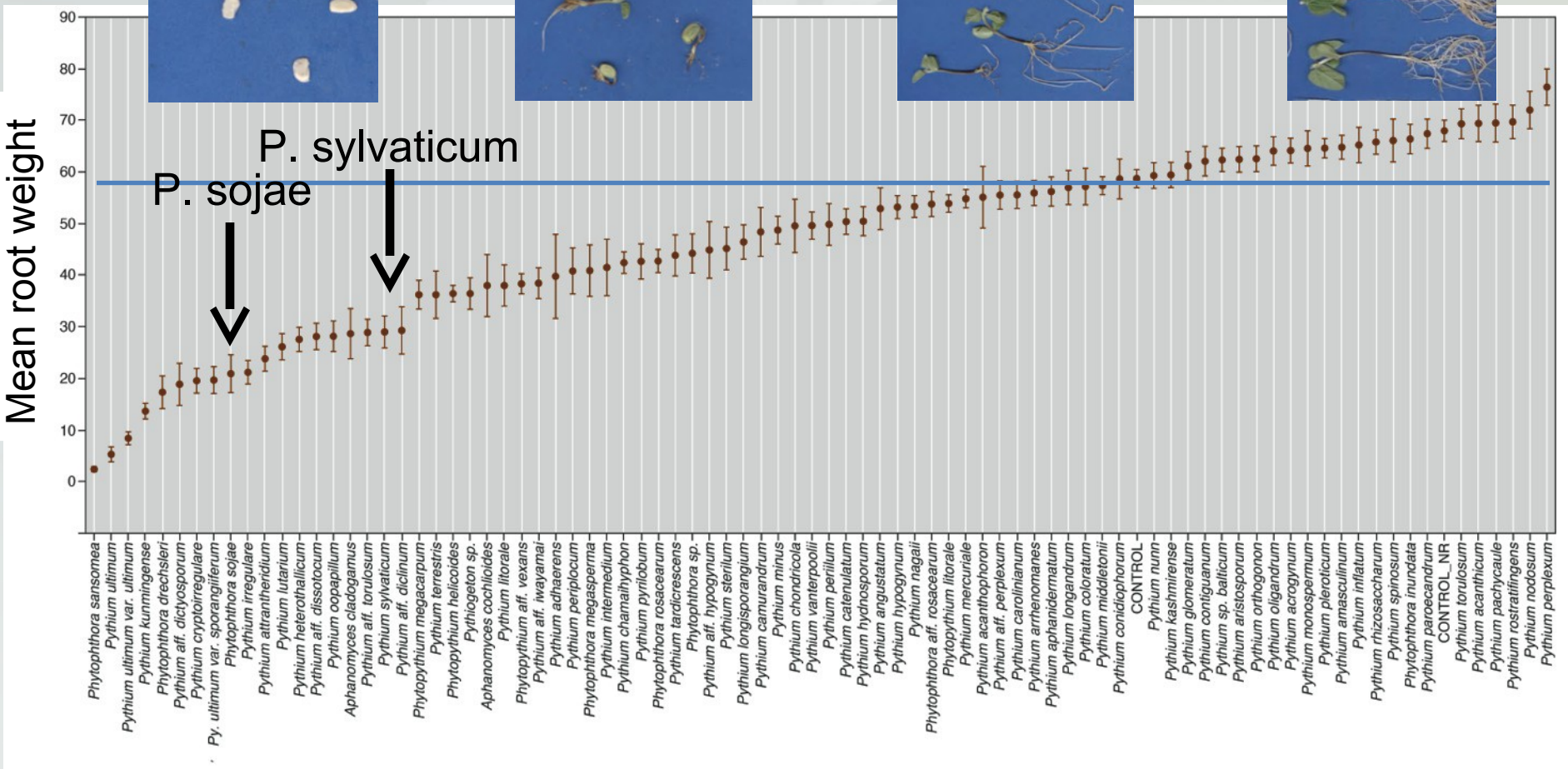
Pythium attrantheridium

Pythium ultimum var. sporangiiferum

Variability in seedling root rot at 20oC





Molecular diagnostics

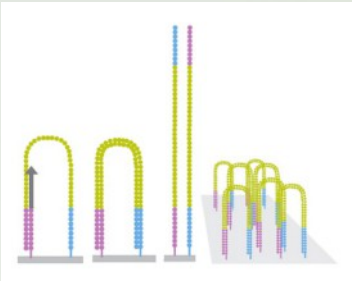
- **Different end users**
  - **Different requirements**

Quantitative PCR
Hydrolysis probe

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

Isothermal PCR

Amplicon-based community analysis



- **Testing and validation**

Chilvers 2012. Fung. Genom. & Biol.

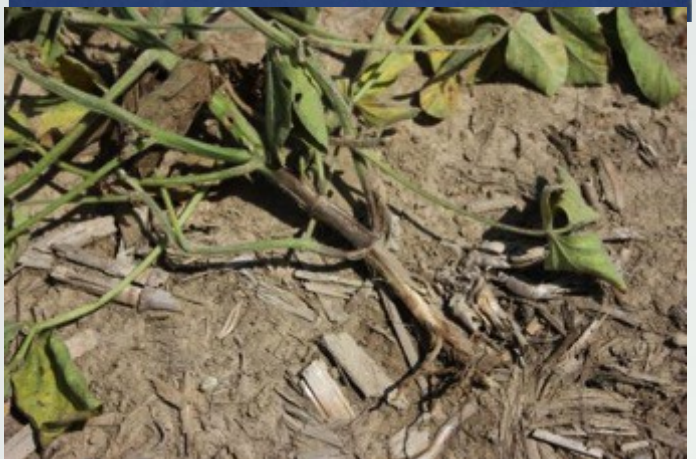
Phytophthora sojae

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

Phytophthora sansomeana **(described 2009)**

- **Not as well recognized**
- **Wide host range - soybean, corn, Douglas-fir**

Phytophthora sojae

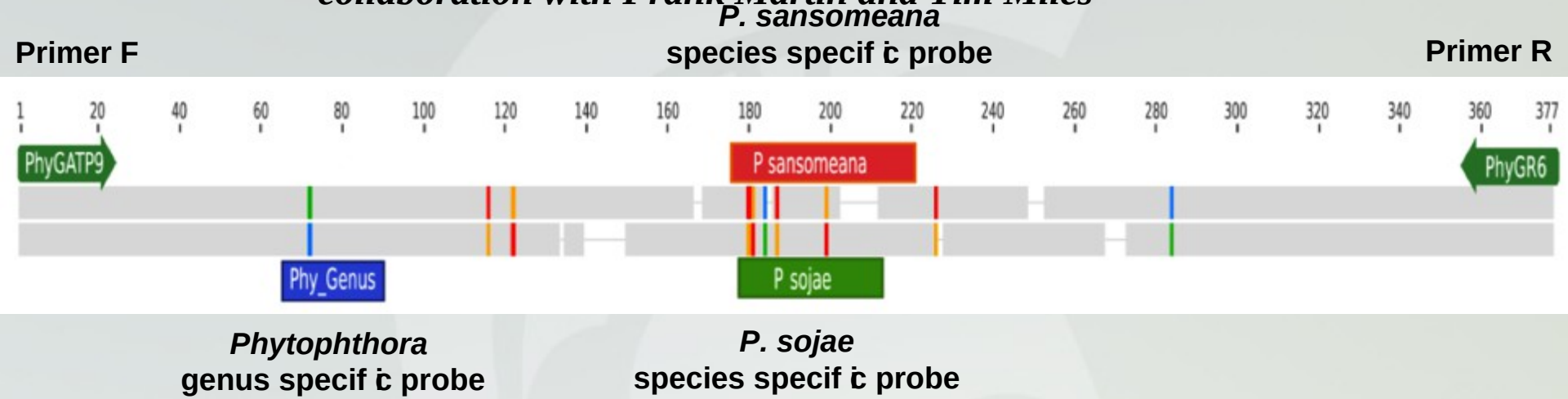


Phytophthora sansomeana

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)

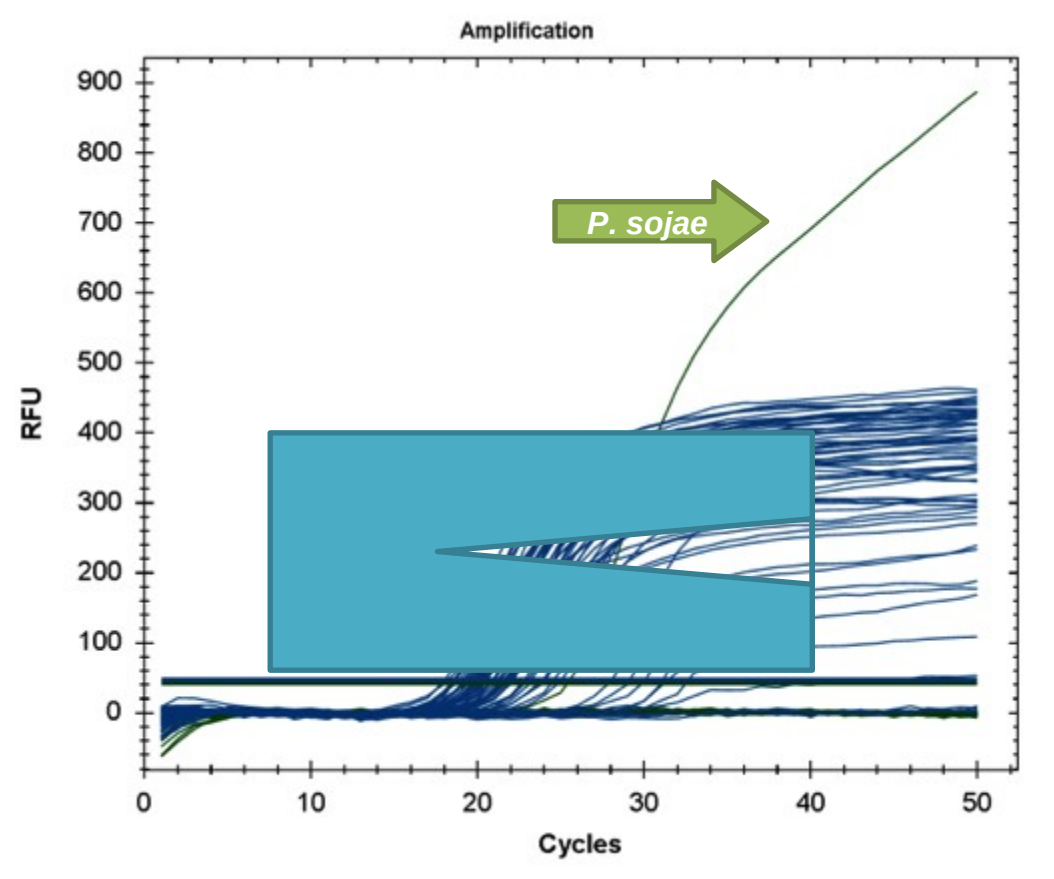
Bilodeau et al. Phytopath. 104:733

Validation of qPCR assay

Specificity test panel

- 96 different *Phytophthora* spp.
- 14 provisional *Phytophthora* species
- 10 *Pythium* spp.

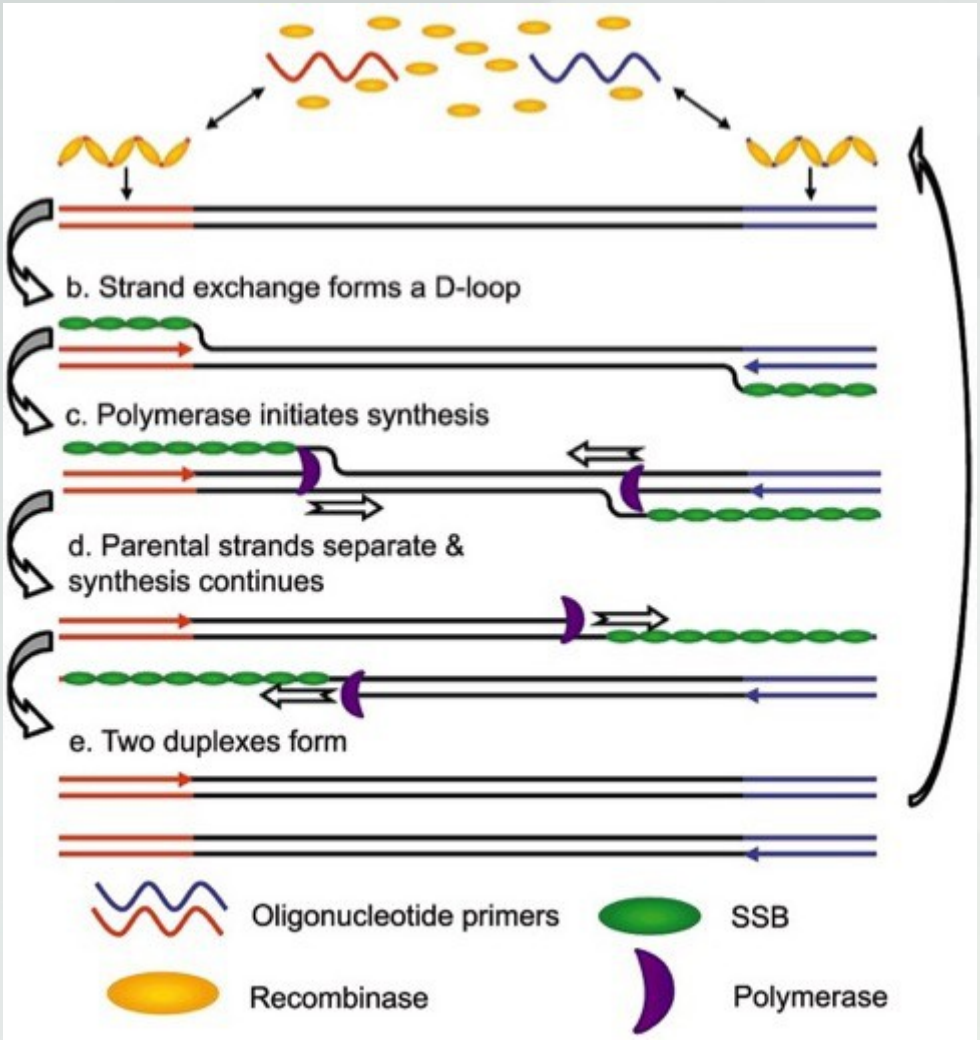
Genus



Isothermal amplification Recombinase Polymerase Amplification (RPA)

Smart Dart (BioRanger)

The RPA Cycle



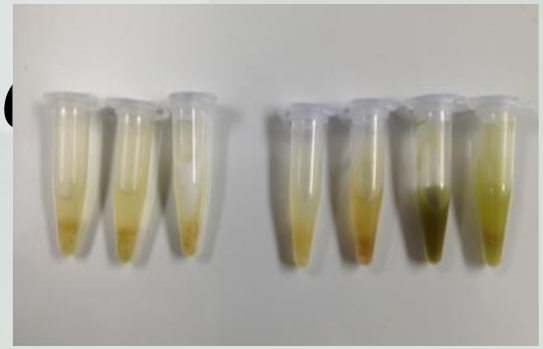
RPA advantages

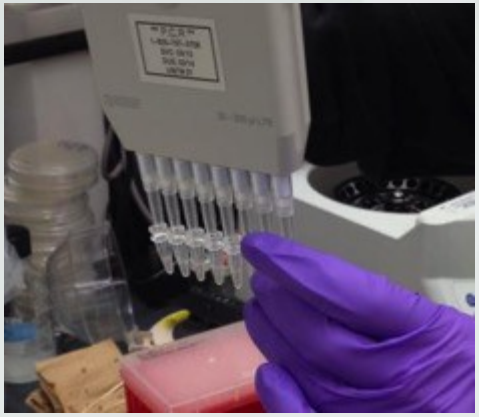
- All steps at 37ºC
- Recombinase opens dsDNA
- End point or real-time

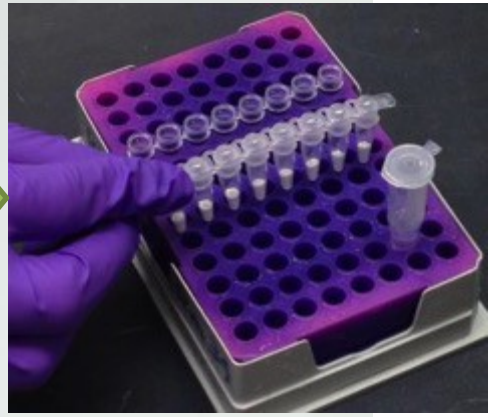
Detection in ~ 20 minutes!

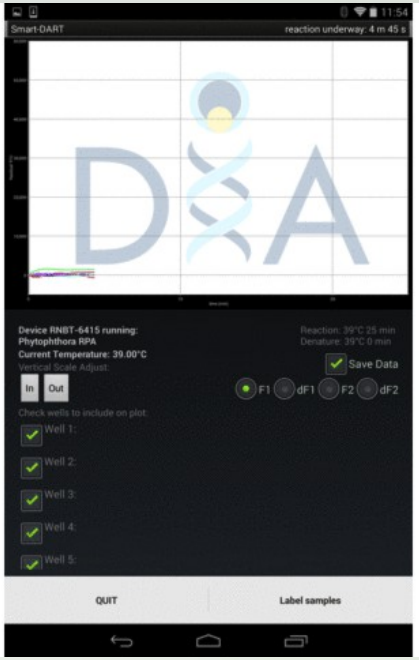
http://www.twistdx.co.uk/our_technology/

RPA process: *P. sojae* and *P. omeana*



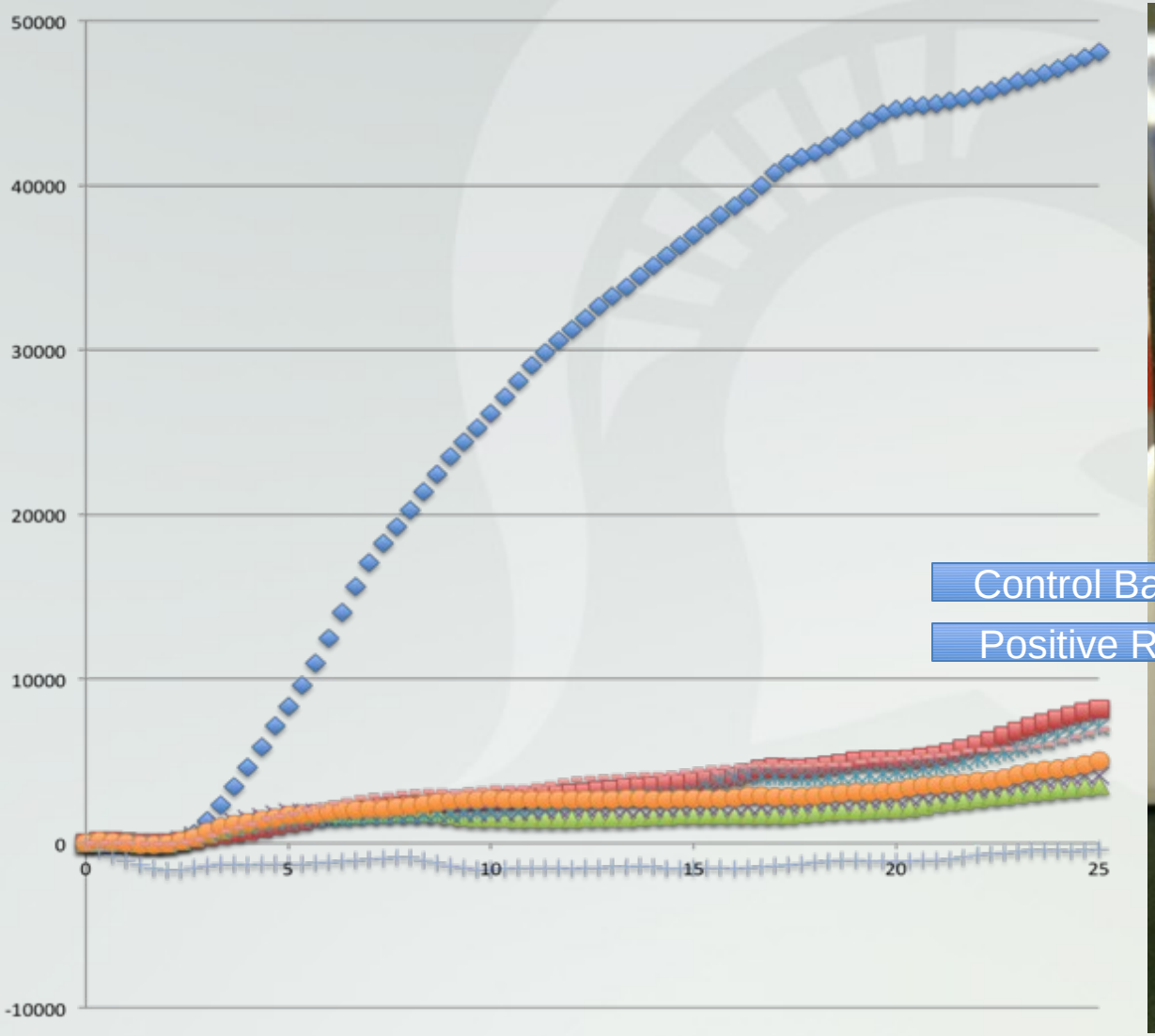




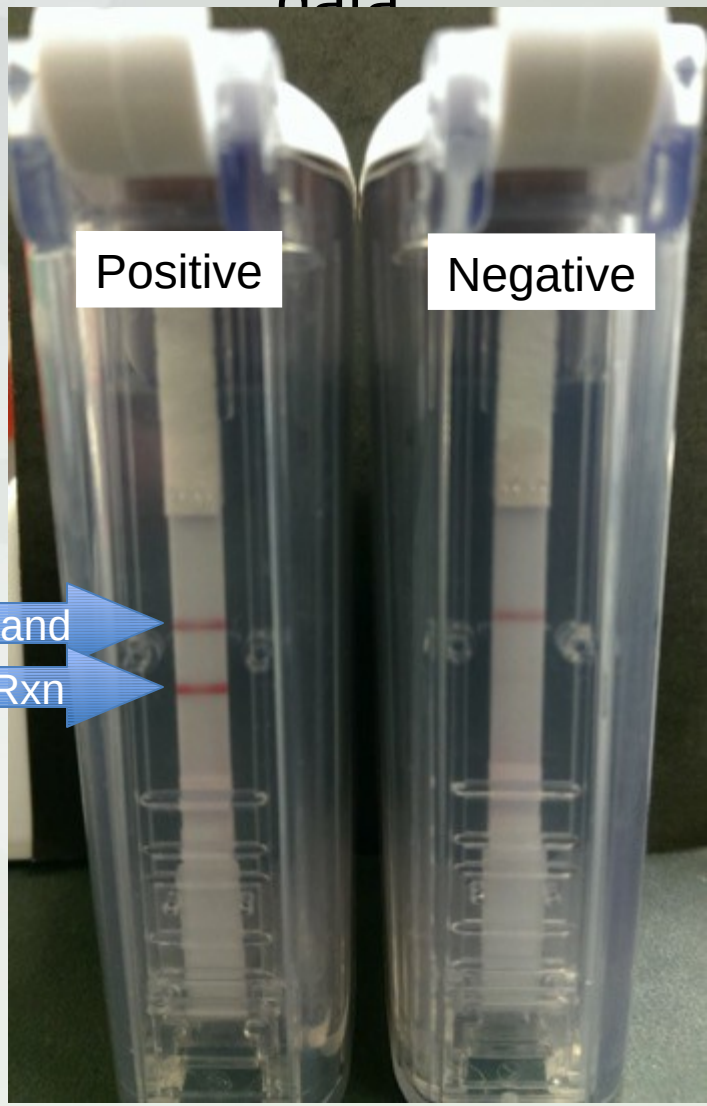


Isothermal RPA - Sample Results

Real-time data collection

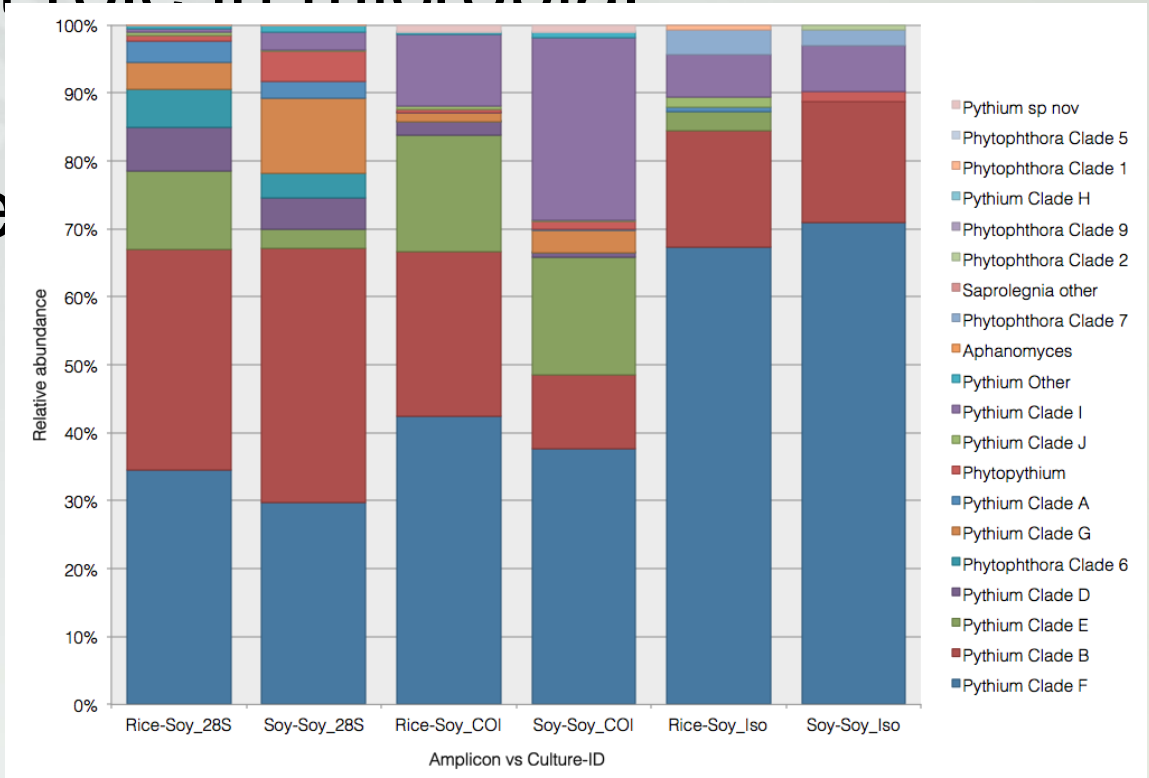


End-point diagnostic data



Amplicon based community analysis

- Oomycetes rarely represented in metagenomes
- But play important role in microbial ecology
- Pair w/ phenotype



Amplicon vs Culture

Oomycetes - Summary and future work

- Oomycete species vary by regio
- 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

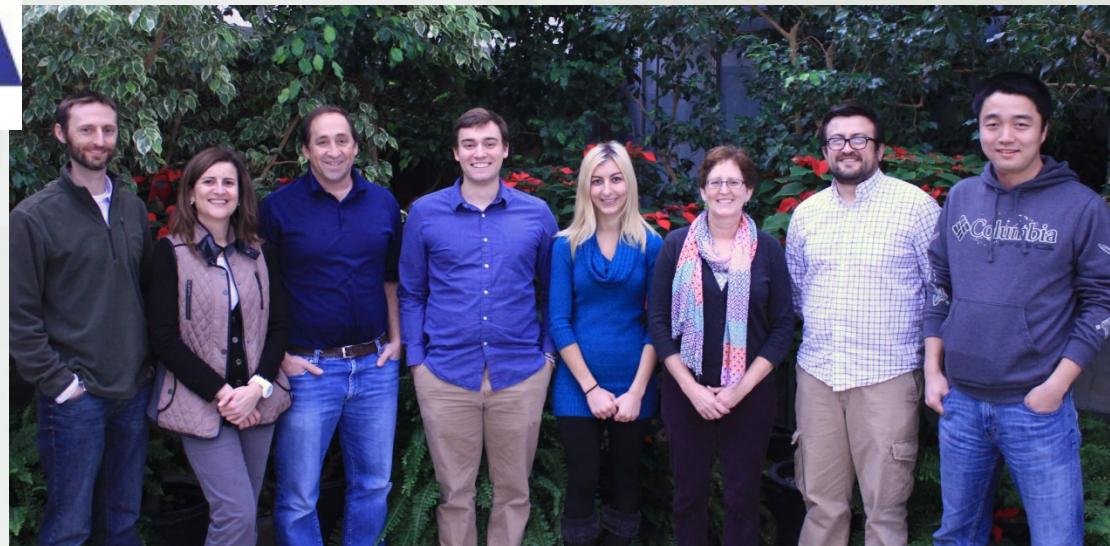
Acknowledgements

- **OSCAP Extension Network:**

Carl Bradley, Tom Chase, Paul Esker, Loren Giesler, Doug Jardine, Dean Malvick, Sam Markell, Berlin Nelson, Alison Robertson, John Rupe, Damon Smith, Laura Sweets, Albert Tenuta, Kiersten Wise

- **MSU diagnostic lab**
- **John Boyse and Randy Laurenz**

Project No. 2011-68004-30104 ("Integrated management of oomycete diseases of soybean and other crop plant



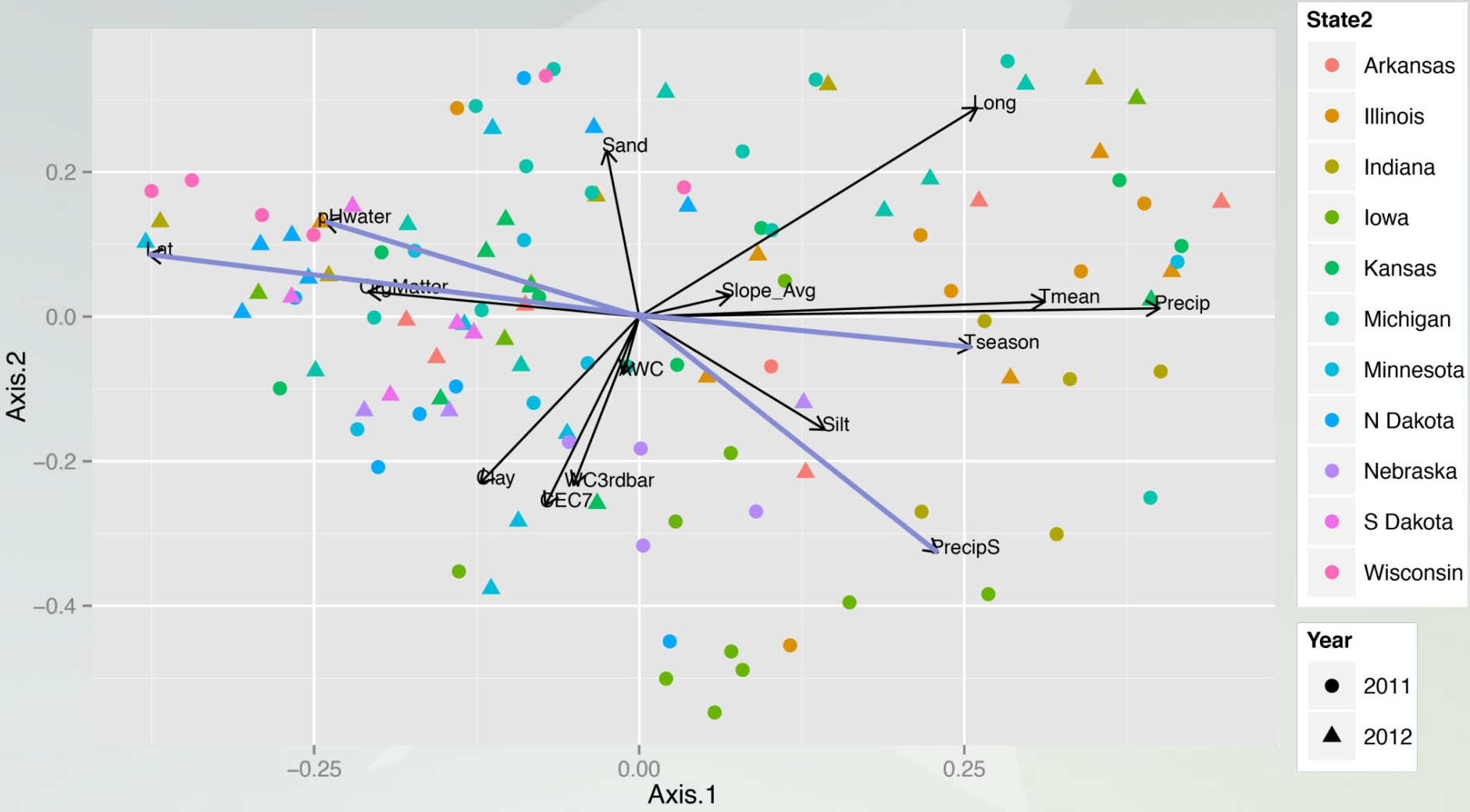
Oomycete Survey Summary

- 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

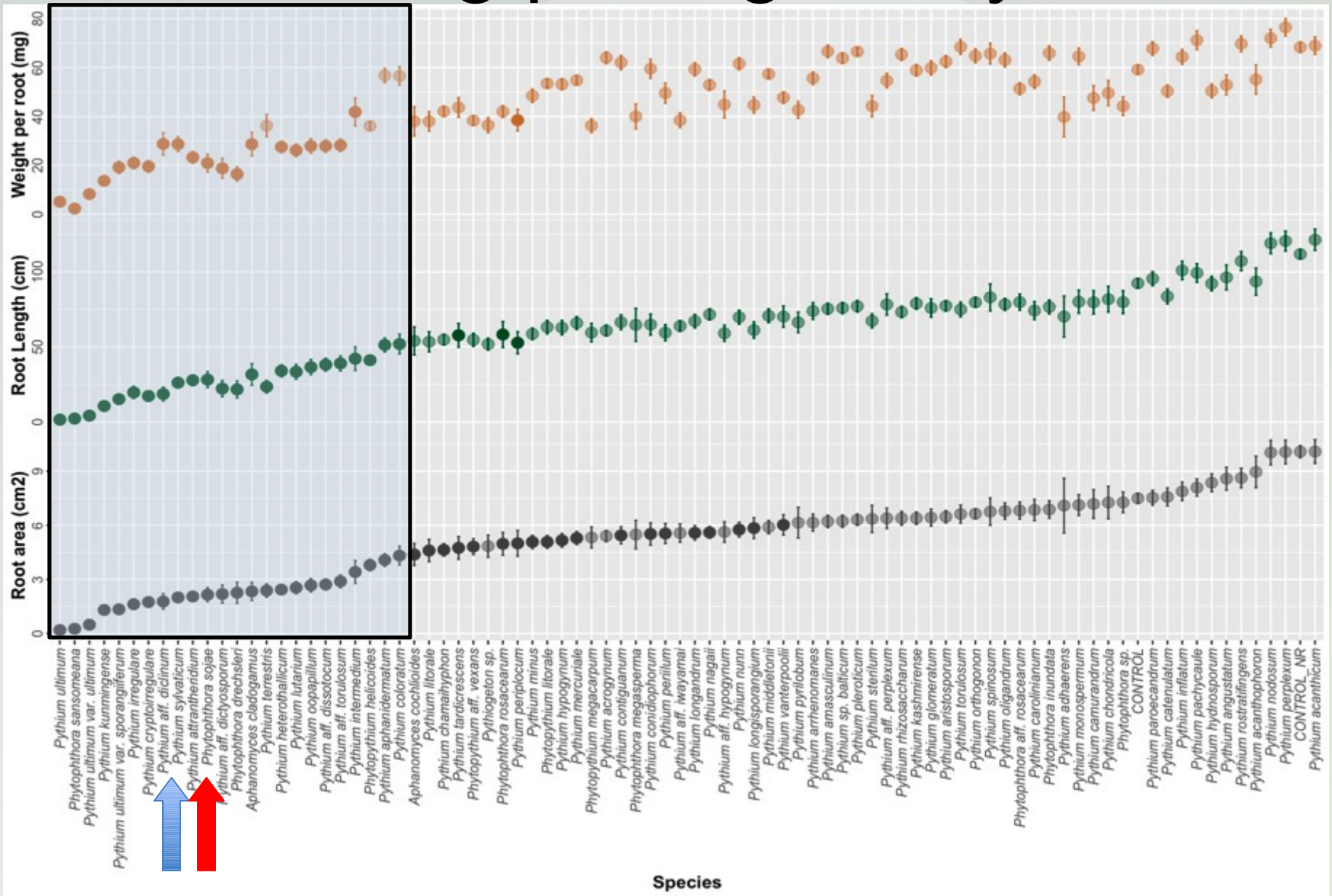
- Multiplex hierarchical qPCR assay for *Phytophthora* genus, *P. sojae*, *P. sansomeana*
- Implemented by diagnosticians
- Hierarchical RPA assay for *Phytophthora*, *P. sojae*, *P. sansomeana*
- qPCR and RPA validated with field

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

NCSRP NORTH CENTRAL SOYBEAN RESEARCH PROGRAM

USB UNITED SOYBEAN BOARD Progress Powered by U.S. Farmers

Courtesy Ahmad Fakoury, Univ. of Southern Illinois

Fusarium species

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

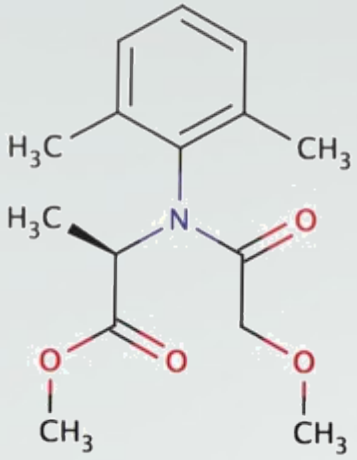
RNA Polymerase 1

Ethaboxam

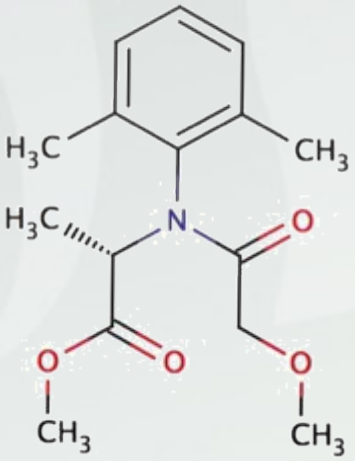
FRAC group B3

β-tubulin assembly – Uchida et al. 2005

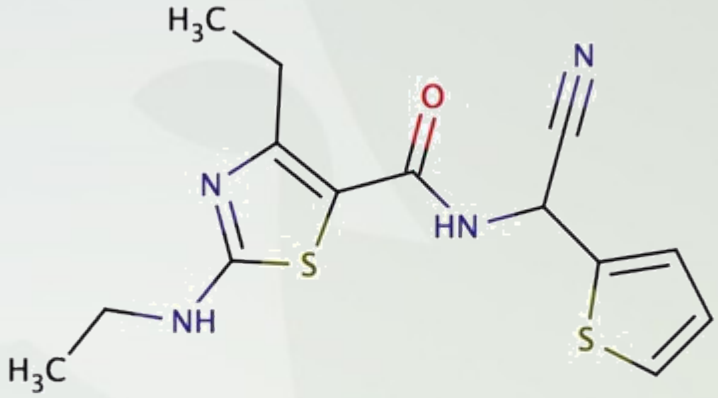
Mefenoxam



S-metalaxyl



R-metalaxyl



Ethaboxam

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

10 ml syringe

20G needle

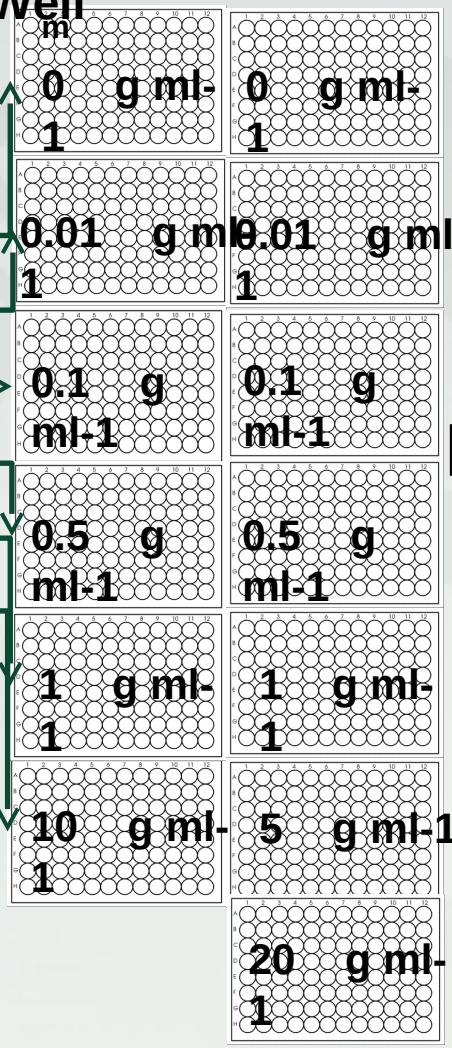
Vortex for 10 seconds

2. Distribute to 96 Well

50 L

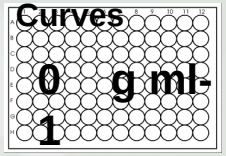
mefenoxam

ethaboxam



½ strength V8 broth + Fungicide

Growth Curves



3. Measure

Controls

Inoculated control

Non-inoculated control

½ strength V8 broth

24 - 48 hours growth

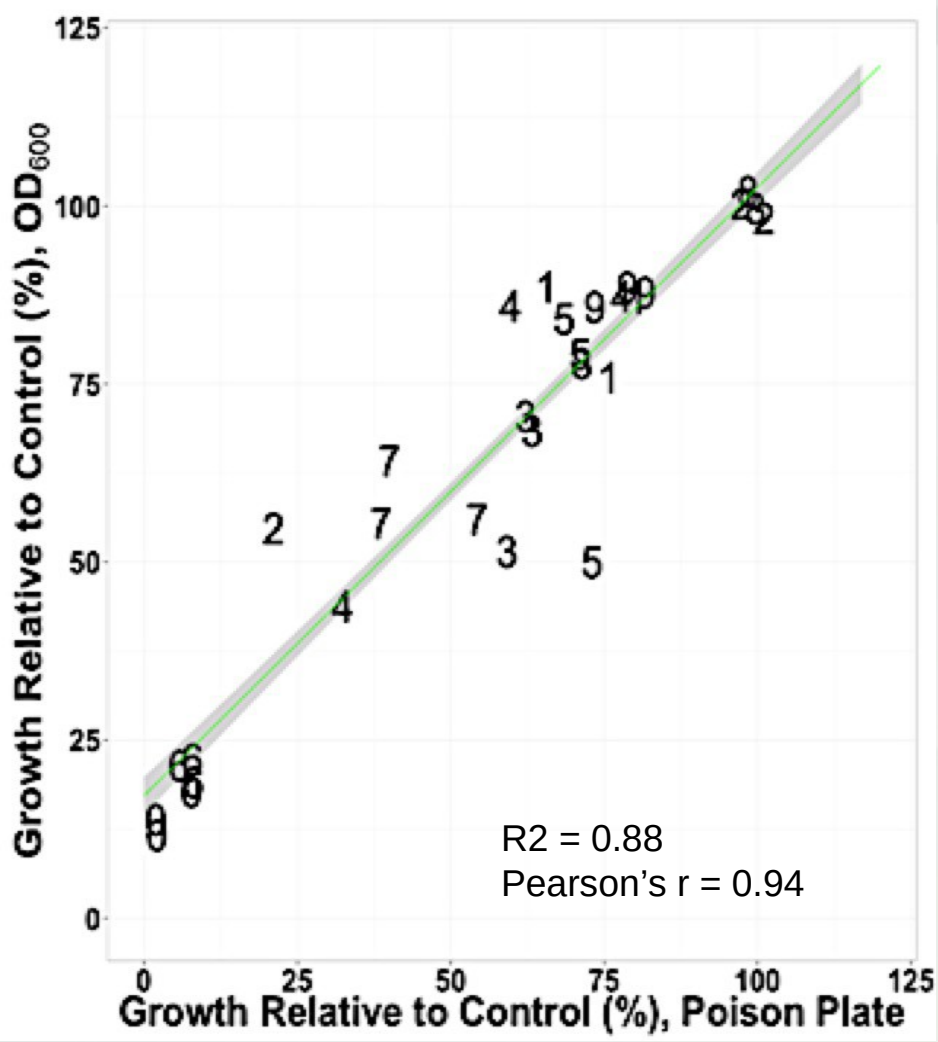
Optical density 600nm

Tecan Plate reader

High-throughput fungicide sensitivity

Assay validation

Ethaboxam – *P* 0.05

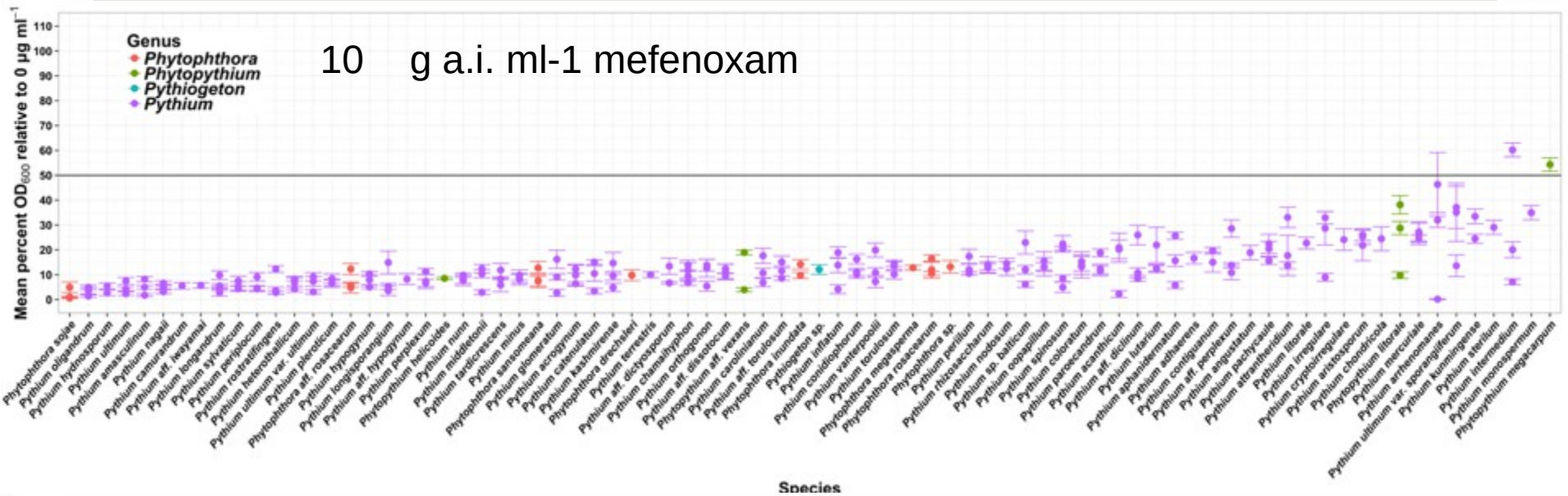


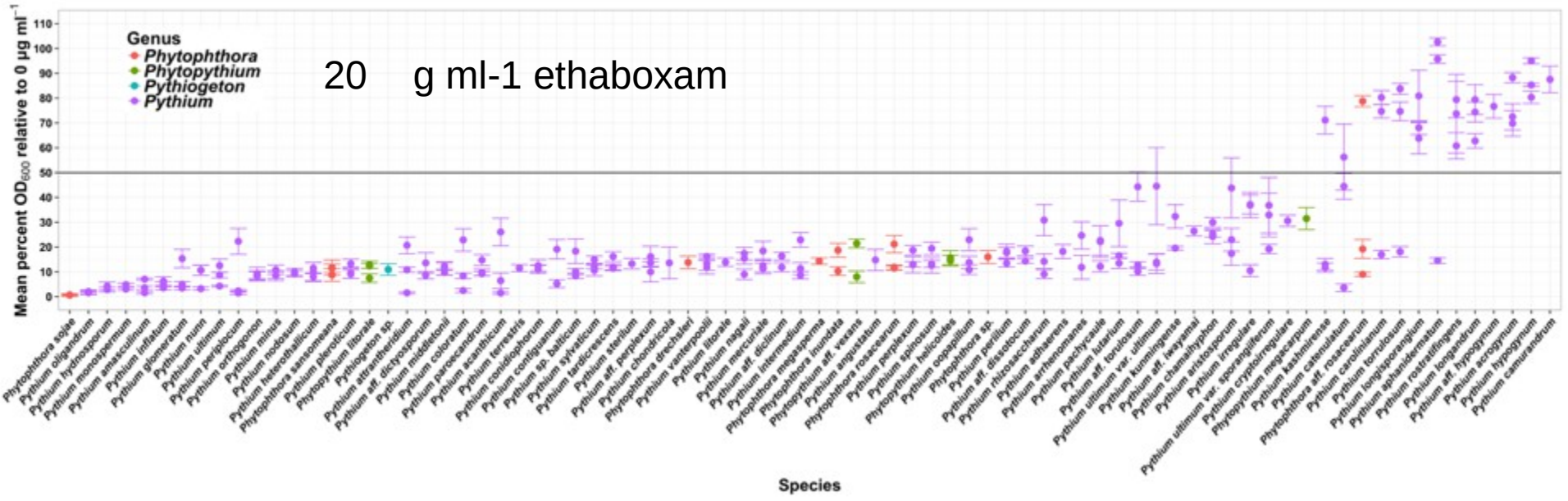
Species

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*

High-throughput fungicide sensitivity

Community inference





Fungicide Sensitivity Summary

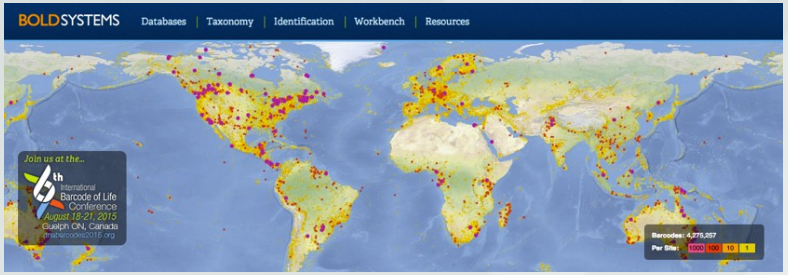
- High throughput ~4 hours set up 30 isolates, multiple chemistries
- Poison plate ~1 day set up 12 isolates, one chemistry
- Most species appear sensitive to mefenoxam
- Ethaboxam insensitivity conserved amongst monophyletic groups

Oomycete resources

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

MOLECULAR ECOLOGY RESOURCES
Molecular Ecology Resources (2011) 11, 1002–1011 doi: 10.1111/j.1755-0998.2011.03041.x

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

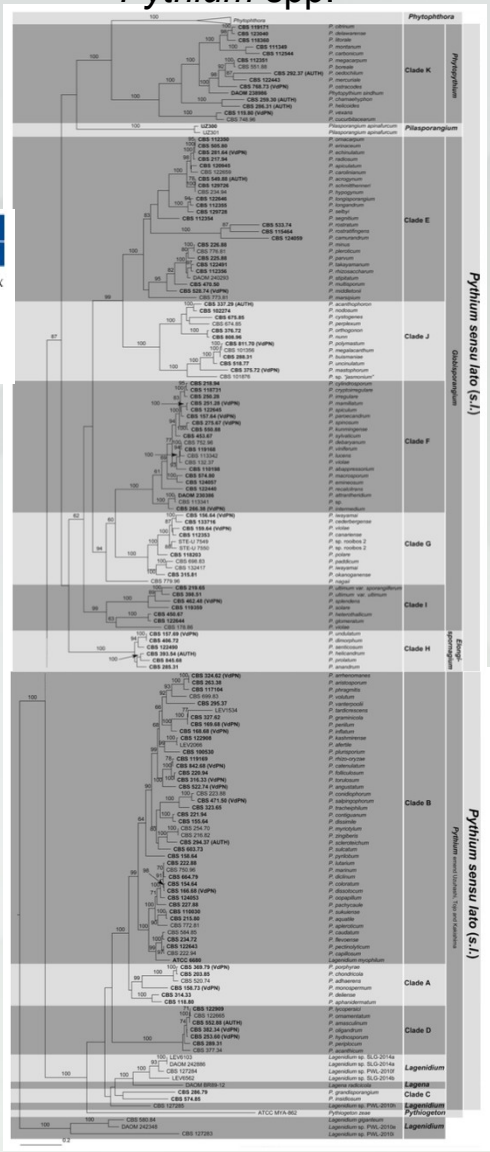


- Taxonomy
  - Species?
  - Consolidated taxonomy

- A. Levesque and AW de Cock
- F. Martin and J. Blair (2014)

Hyde et al. 2014

Pythium spp.



Phytophthora spp.