

# Characterizing the significance of *Pratylenchus penetrans* on soybean

Kanan Saikai

Exit seminar – 12/03/2019



# Soybean

**Soybean is the leading U.S. agricultural export, valued at more than \$23 billion**

**In Wisconsin:**

**There are 11,000 soybean growers.**  
**2.2 million acres** planted with soybean  
**produced 106 million bushels/acre**  
**in 2018**

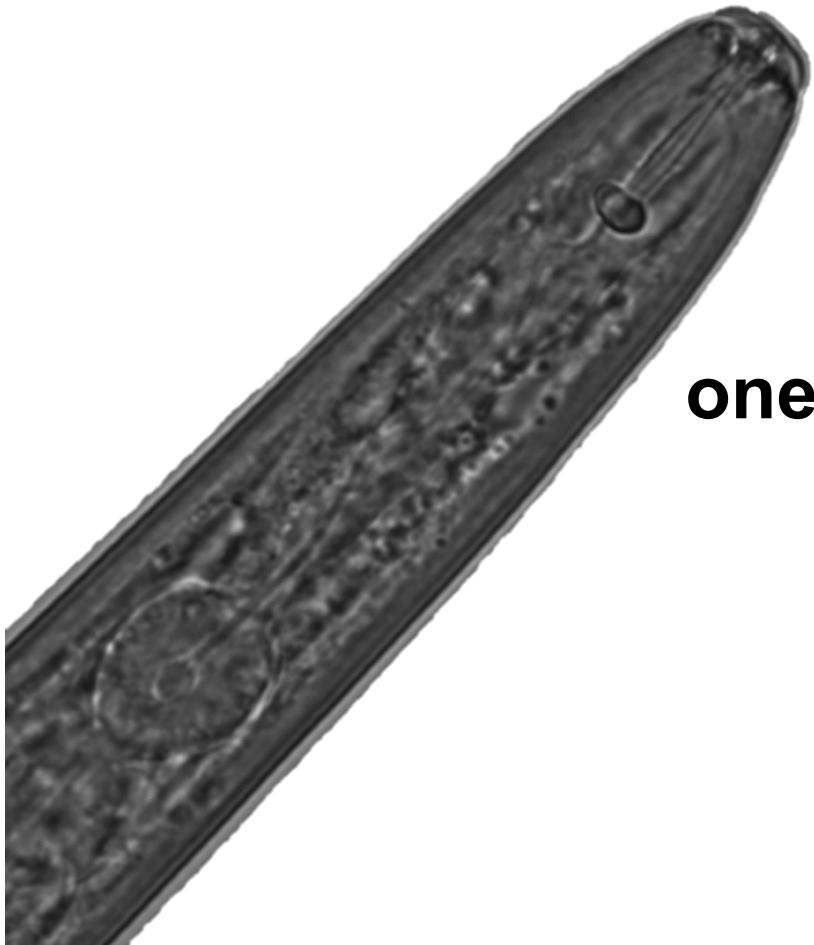
USDA-NASS (2019)



# *Pratylenchus penetrans* (RLN)

*Pratylenchus* is the **third most economically important genus** with very wide host range and worldwide distribution.

*P. penetrans* has been recognized as **one of the most damaging species of *Pratylenchus*** and it is frequently detected on soybean fields in Wisconsin.

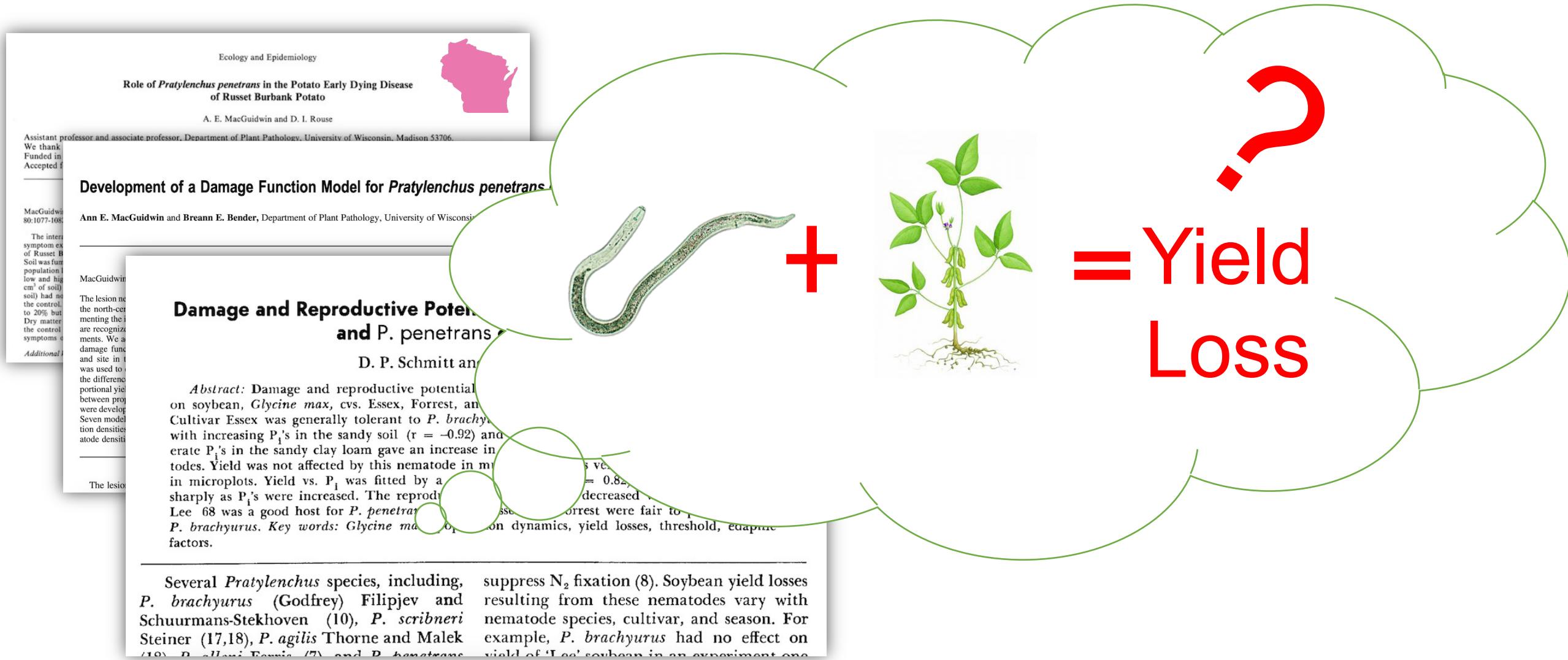


# Life cycle and symptoms of *P. penetrans*



Source: Jones, M. G. K. and Fosu-Nyarko, J. 2014. Molecular biology of root lesion nematodes (*Pratylenchus* spp.) and their interaction with host plants. Annals of Applied Biology 164:163-181.  
Photo credit (pea): Ann MacGuidwin

# *P. penetrans* on soybean



# Outline

**Chapter 1.** Soybean response to *P. penetrans* in field and greenhouse environments.

**Chapter 2.** Gonochoristic species of *Pratylenchus* in soybean cropping systems in Wisconsin.

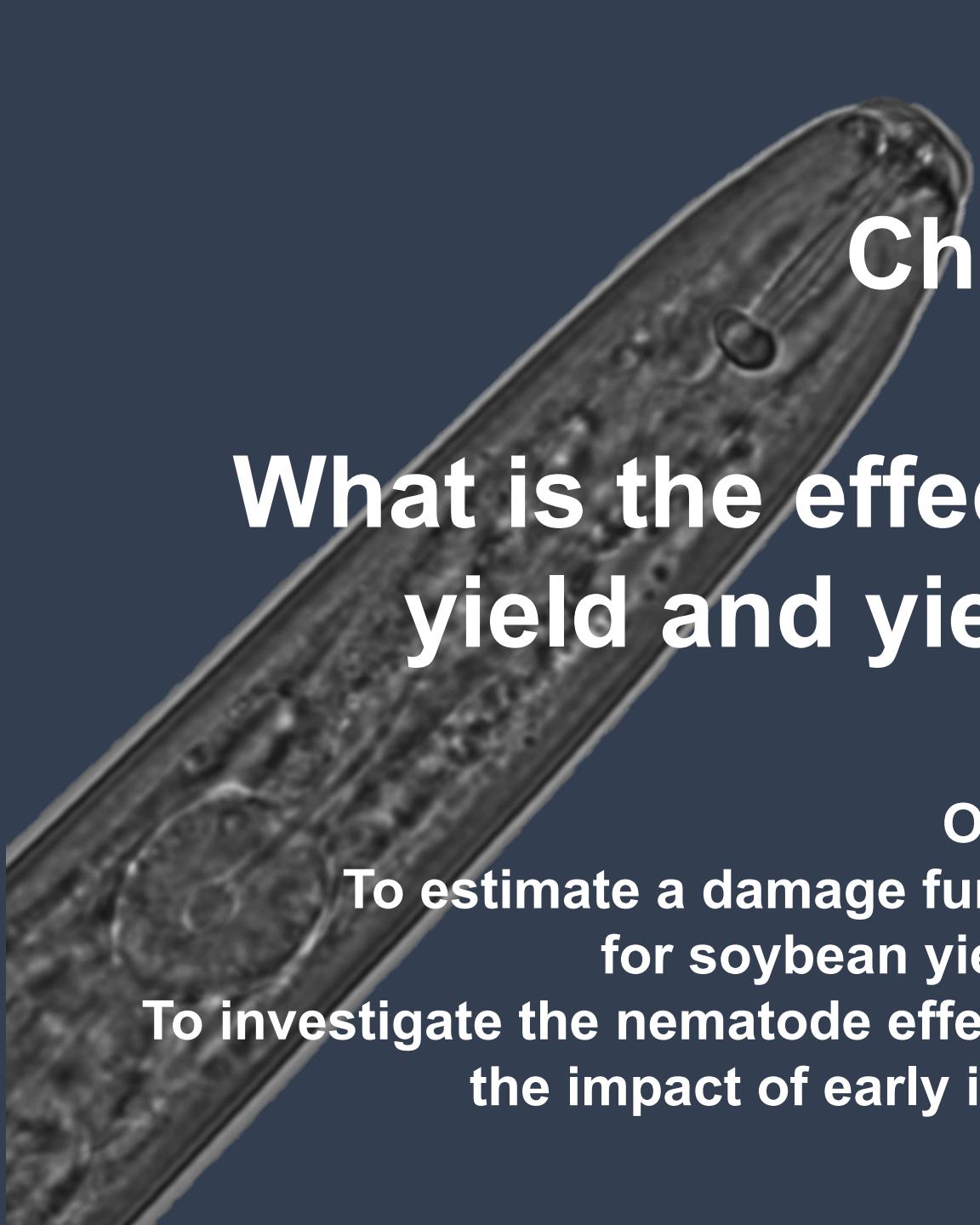
**Chapter 3.** Gender difference of *P. penetrans* for symptom development and severity.

# Outline

**Chapter 1.** Soybean response to *P. penetrans* in field and greenhouse environments.

**Chapter 2.** Gonochoristic species of *Pratylenchus* in soybean cropping systems in Wisconsin.

**Chapter 3.** Gender difference of *P. penetrans* for symptom development and severity.



# Chapter 1.

## What is the effect of *P. penetrans* on yield and yield components?

### Objectives:

To estimate a damage function of *Pratylenchus penetrans* for soybean yield and its components

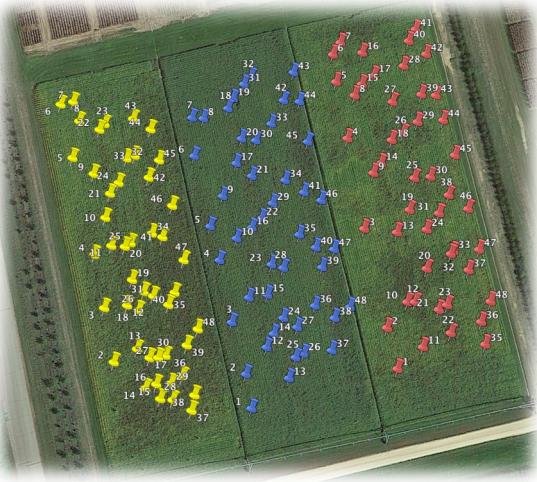
To investigate the nematode effect on soybean plants at the V2 stage and the impact of early infection on final seed yield.

# Field study

Conducted at Hancock Agricultural Research Station in 2017 & 2018.

## Emergence stage

- Two-meter plots were established.
- Soil samples (Pi)



## Second Trifoliolate Stage (V2)

- 2 plant samples
- Root biomass
- Shoot biomass
- Shoot : total biomass



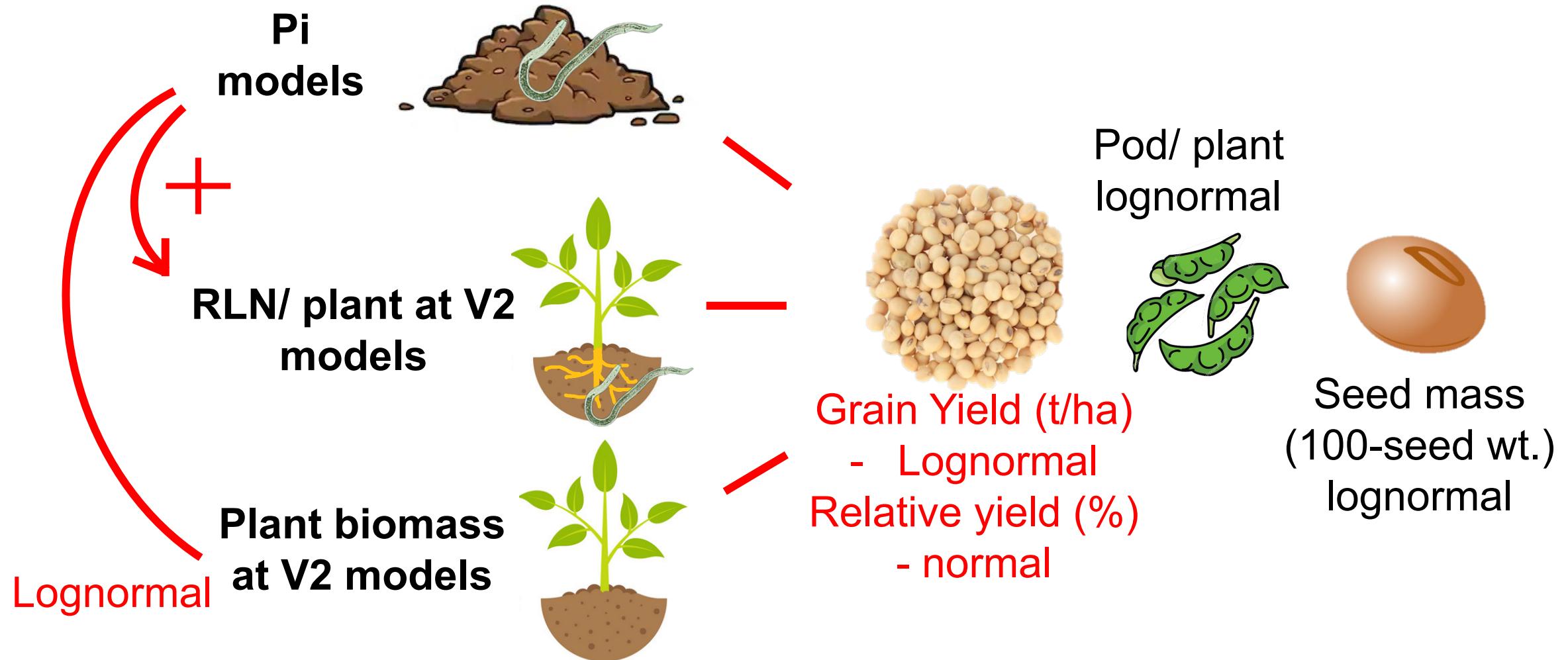
## Final maturity

- Harvest plots
- Grain yield
- Pod number/ plant
- Seed mass
- (100 seed wt.)

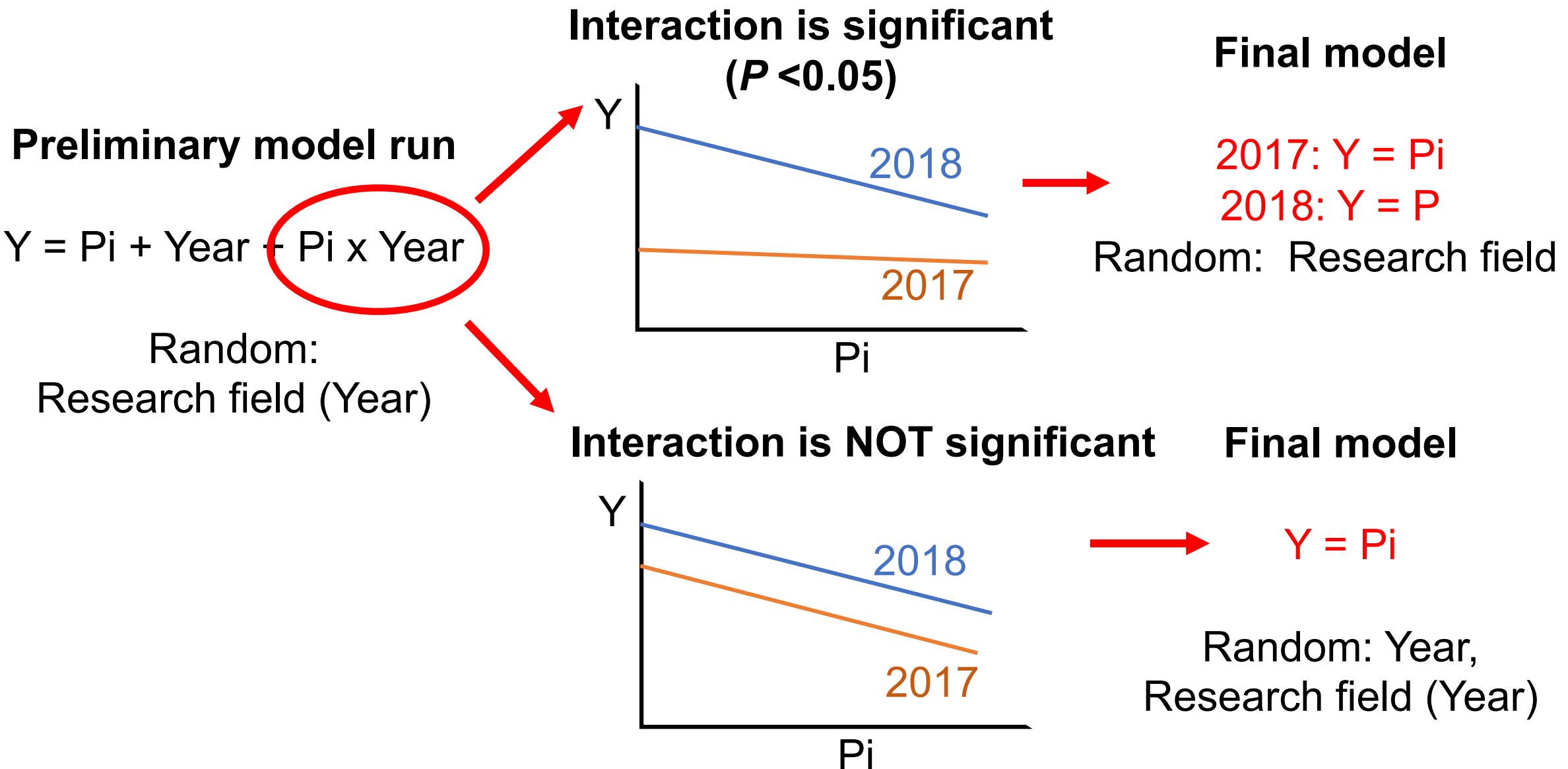


# Field study models

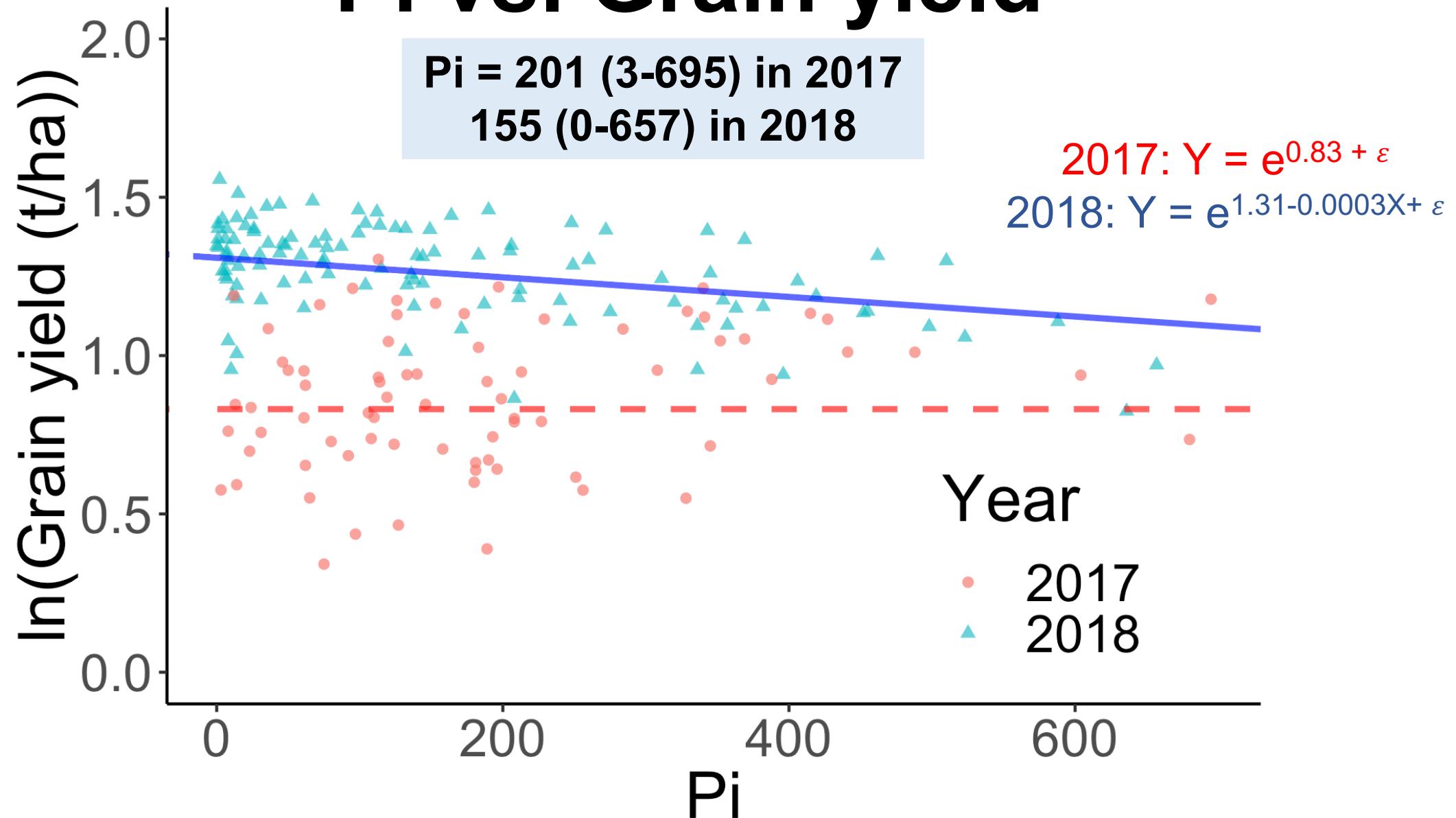
Generalized Linear Mixed Model (GLMM) using PROC GLIMMIX in SAS.



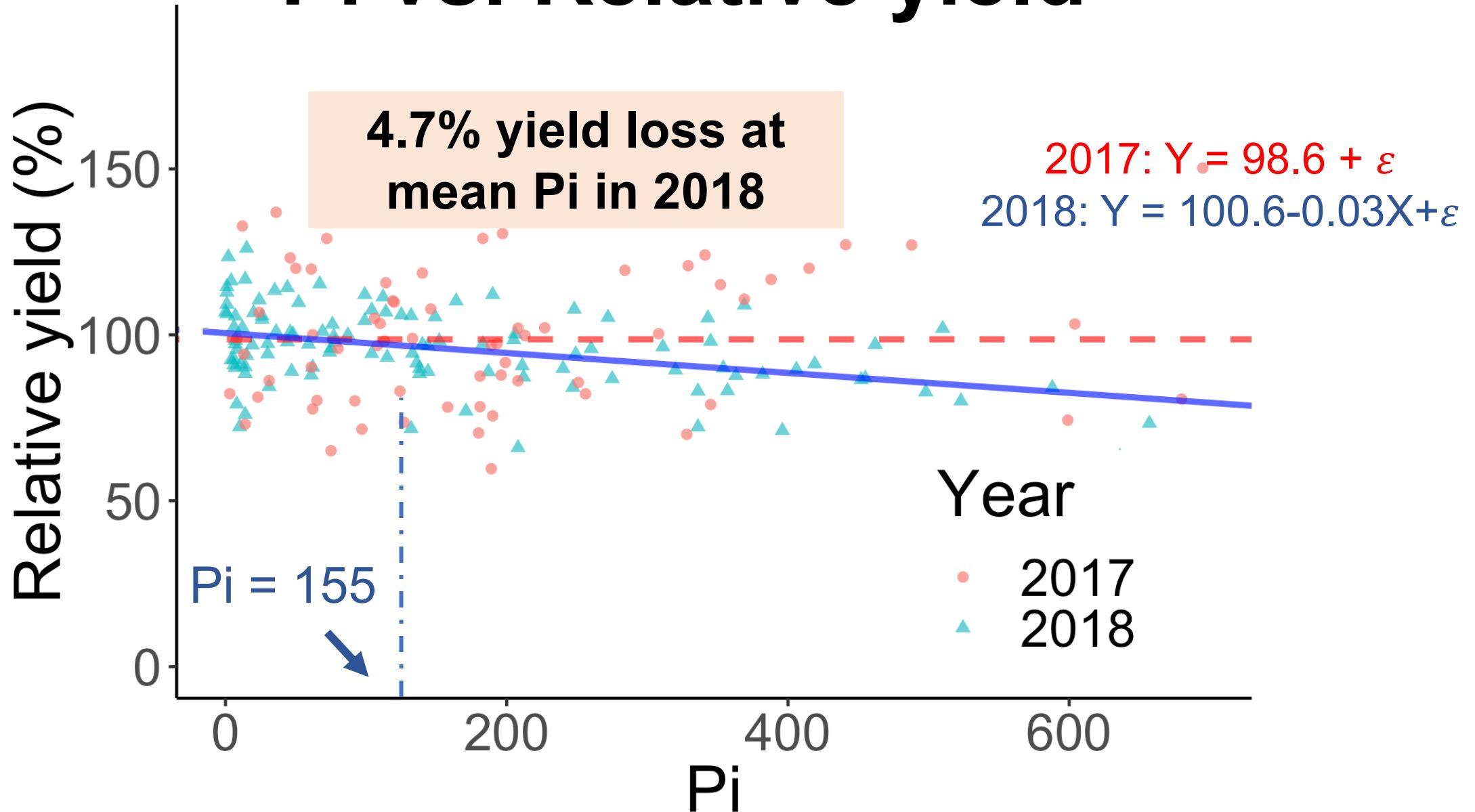
# Field study models



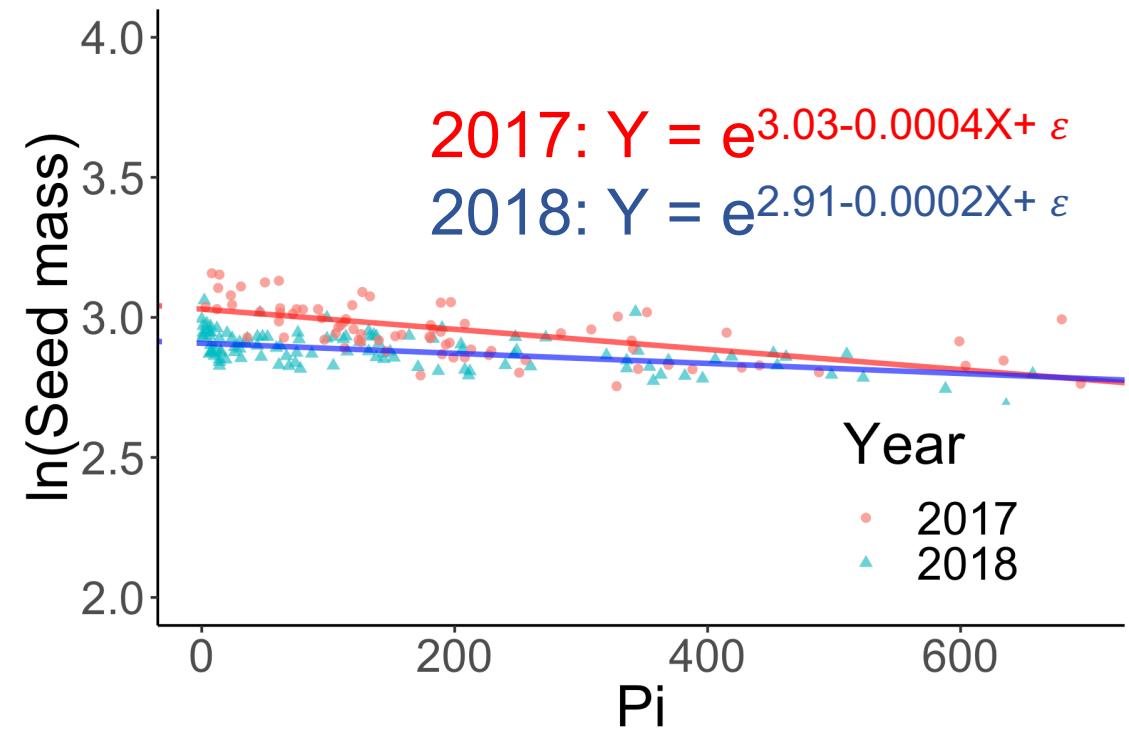
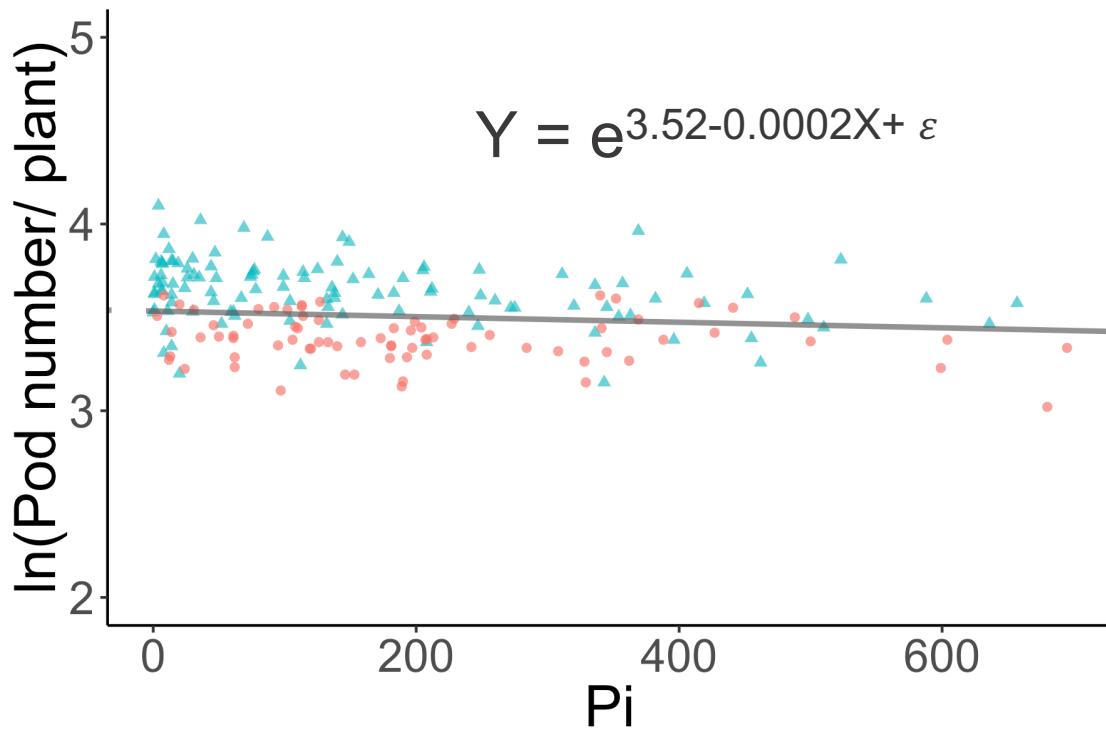
# Pi vs. Grain yield



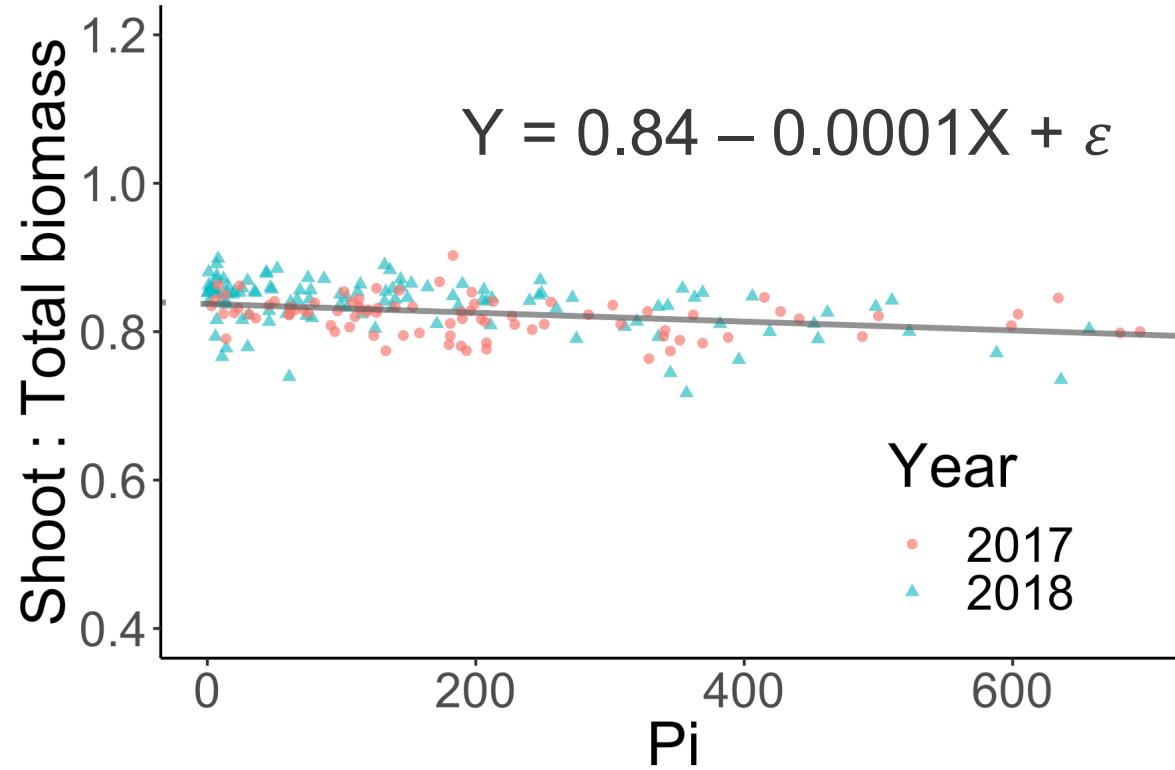
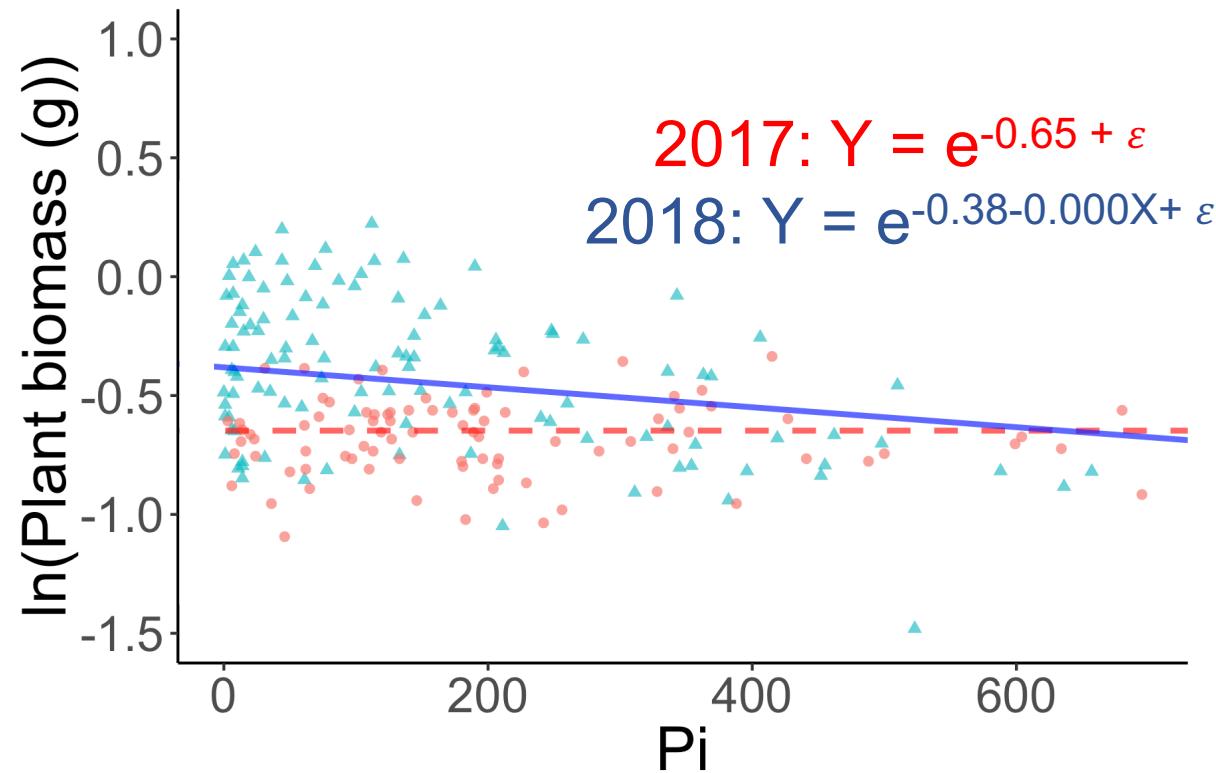
# Pi vs. Relative yield



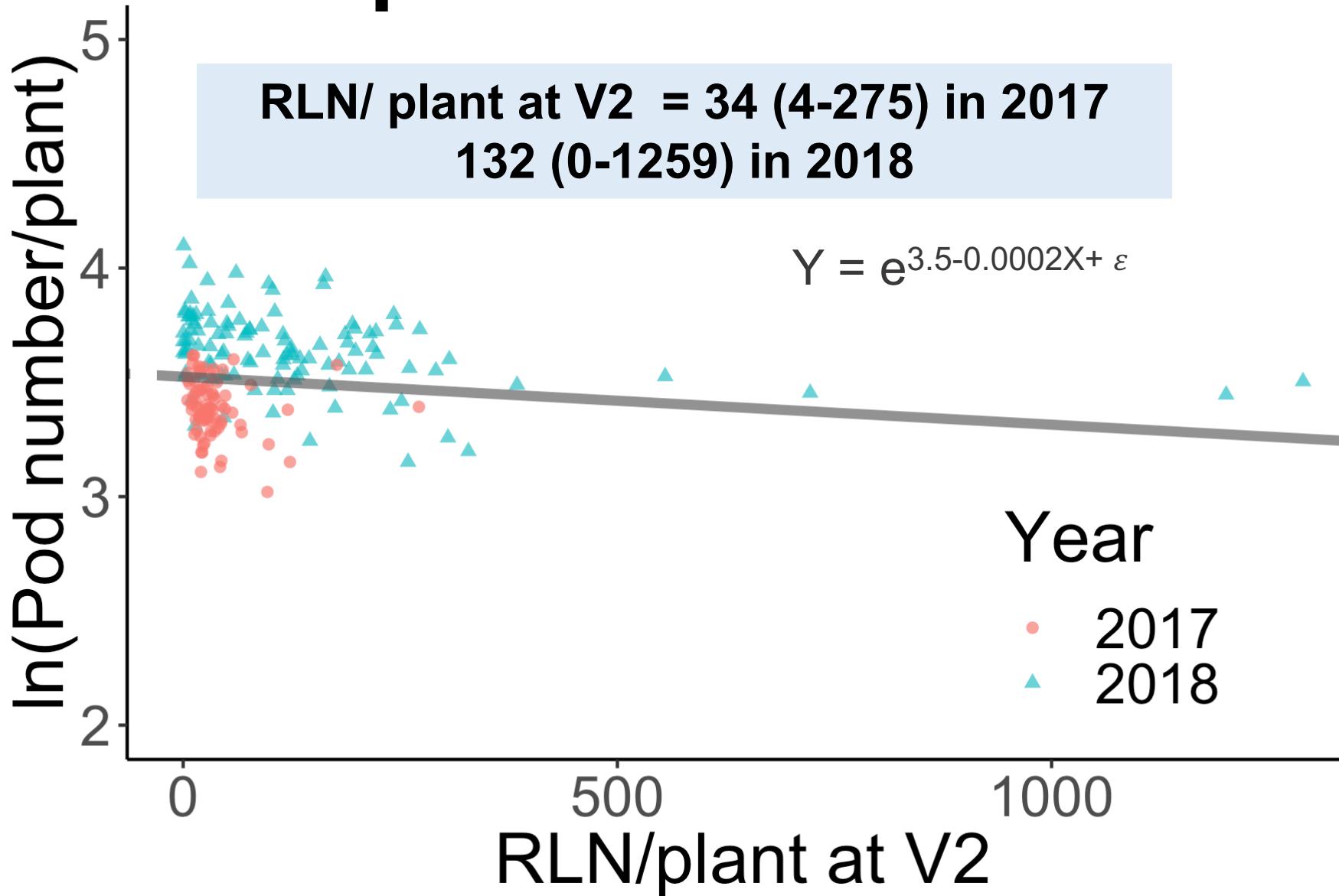
# Pi vs. yield components



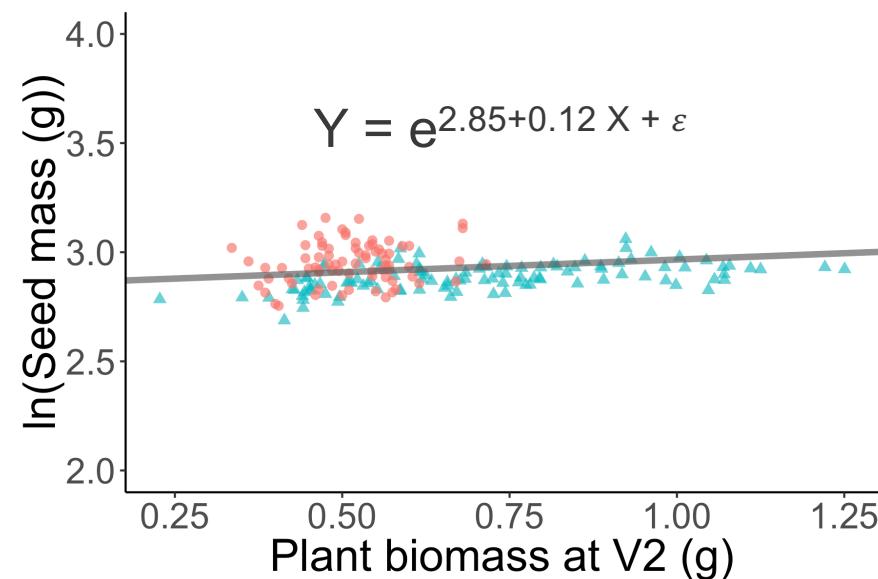
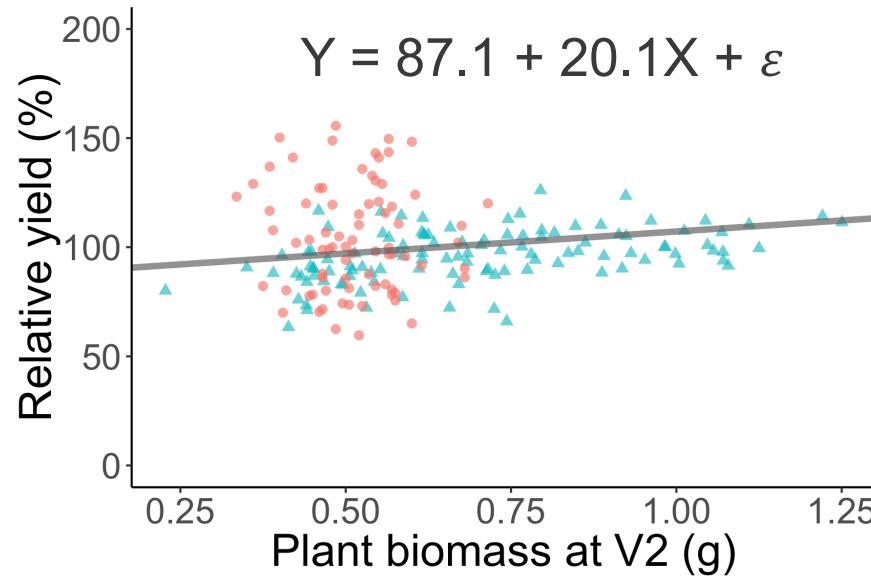
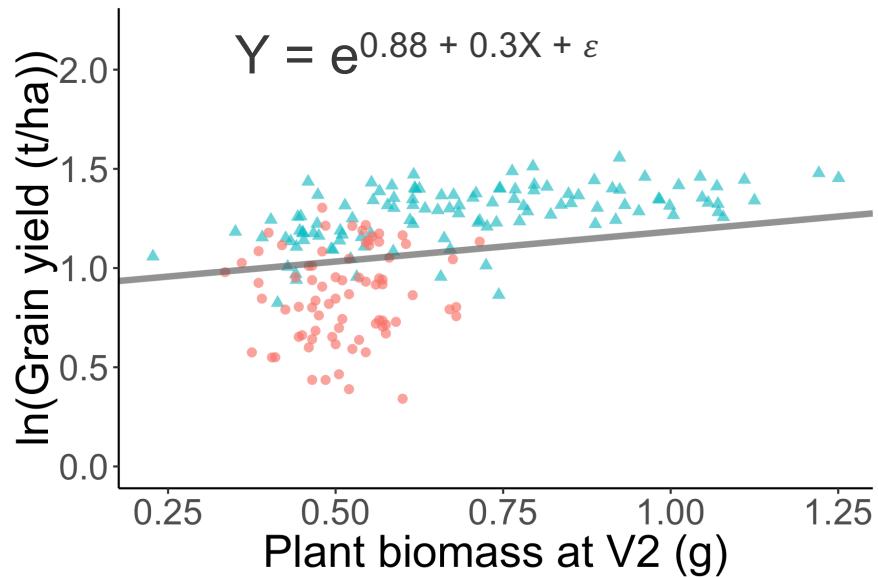
# Pi vs. Plant biomass at V2



# RLN/plant at V2 vs. Yield



# Plant biomass at V2 vs. Yield



Year

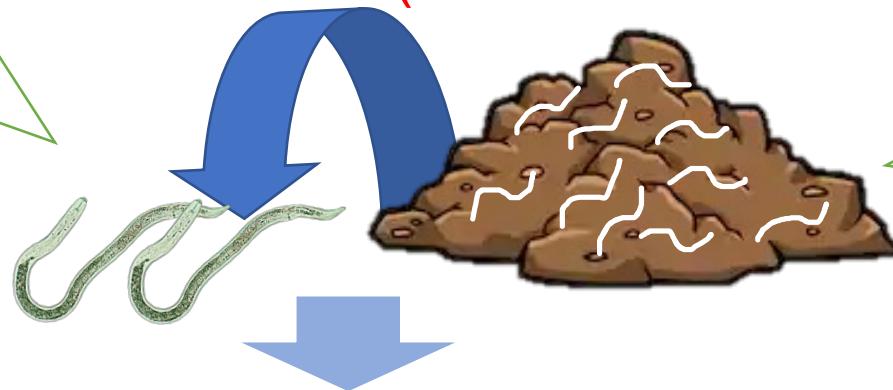
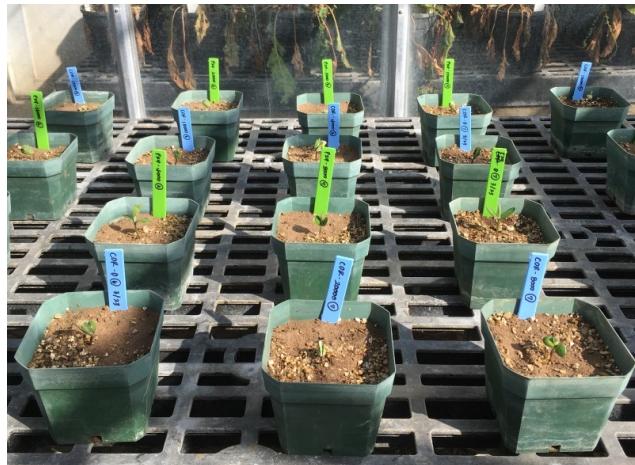
- 2017
- ▲ 2018

# Greenhouse study

20% extraction efficiency  
(MacGuidwin 1989)

Field Pi =  
201 (3-695) in 2017  
155 (0-657) in 2018

Planted in  
pasteurized 500 cm<sup>3</sup> soil



Total nematodes  
in 100cm<sup>3</sup> soil:  
0 – 3475

Dose Level	Trial 1 - Pi	Trial 2 - Pi
Control	0	0
1	1927±168	2066±191
2	3853±534	3995± 225
3	8330±510	7233±285
4	11889±208	11764±300
5	16161±367	18403±297
6	20819±1017	20323±313

# Greenhouse study

## 1. Nematode inoculation



2 soybean genotypes  
8 replications/ dose level

## 2. First harvest at V2 (Rep. 1-3)



Data collection on  
plant biomass

## 3. Second harvest at R8 (Rep. 4-8)



Data collection on  
accumulative plant biomass  
Yield and yield components

# Pi vs. Plant biomass

At V2

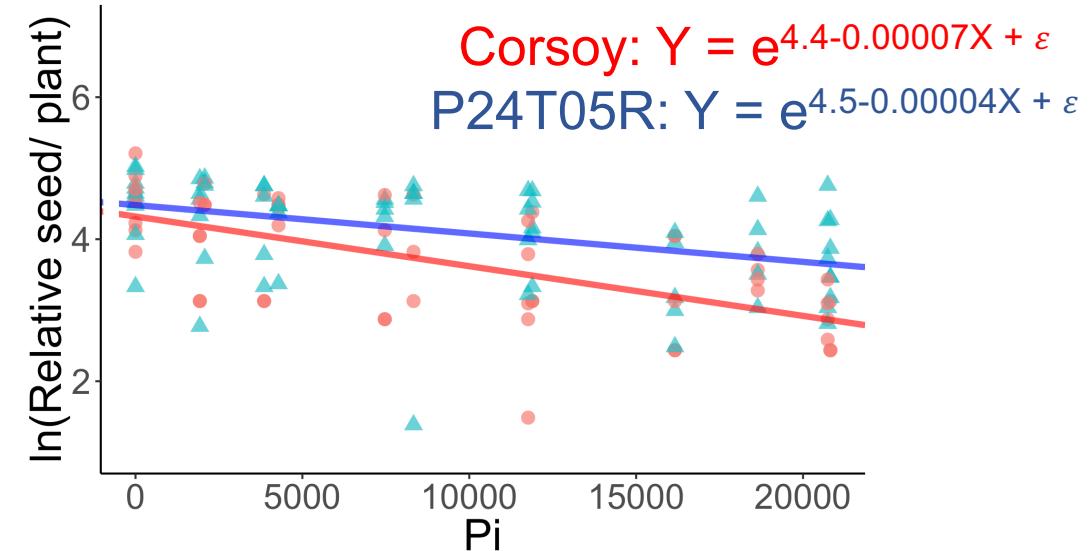
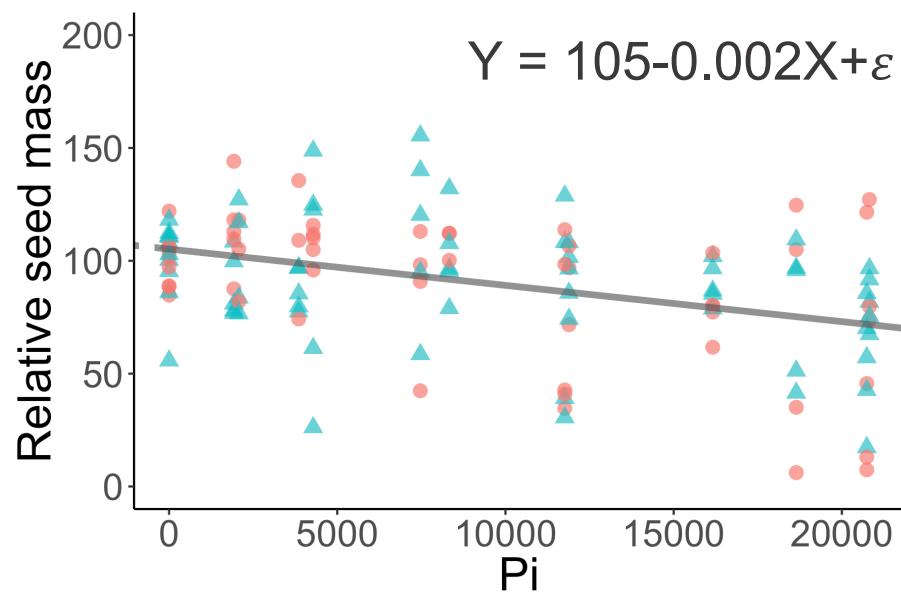
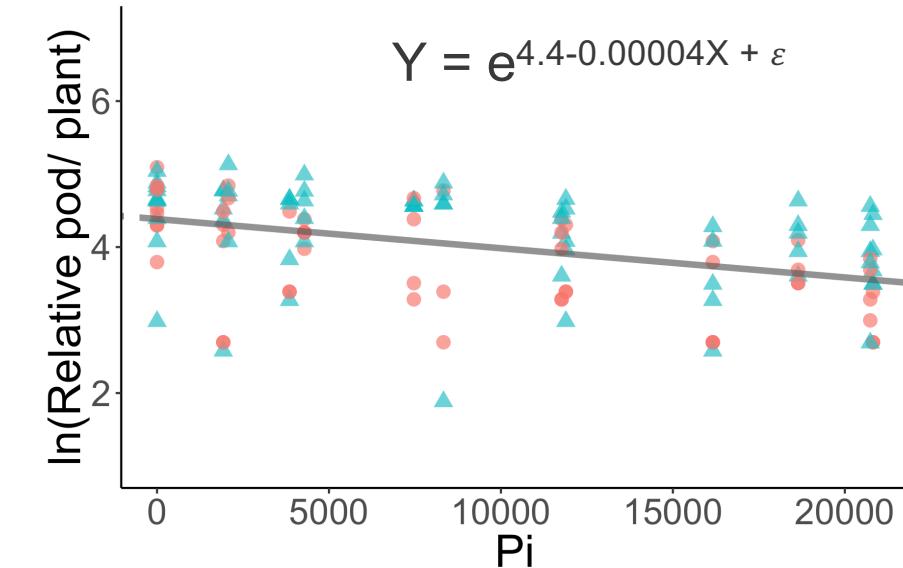
Response variable	Distribution	MS estimate	p
Relative total biomass	lognormal	-0.00001	0.007
Relative shoot biomass	lognormal	-0.00001	0.002
Relative root biomass	normal	-0.00030	NS

*P. penetrans* reduce shoot biomass  
but has no effect on root biomass.

At R8

Response variable	Distribution	Genotype	MS estimate	p
Relative total biomass	lognormal	Random	-0.00003	< 0.0001
Relative shoot biomass	lognormal	Random	-0.00001	< 0.0001
Relative root biomass	normal	Corsoy	-0.00200	0.0003
		P24T05R	-0.00080	NS

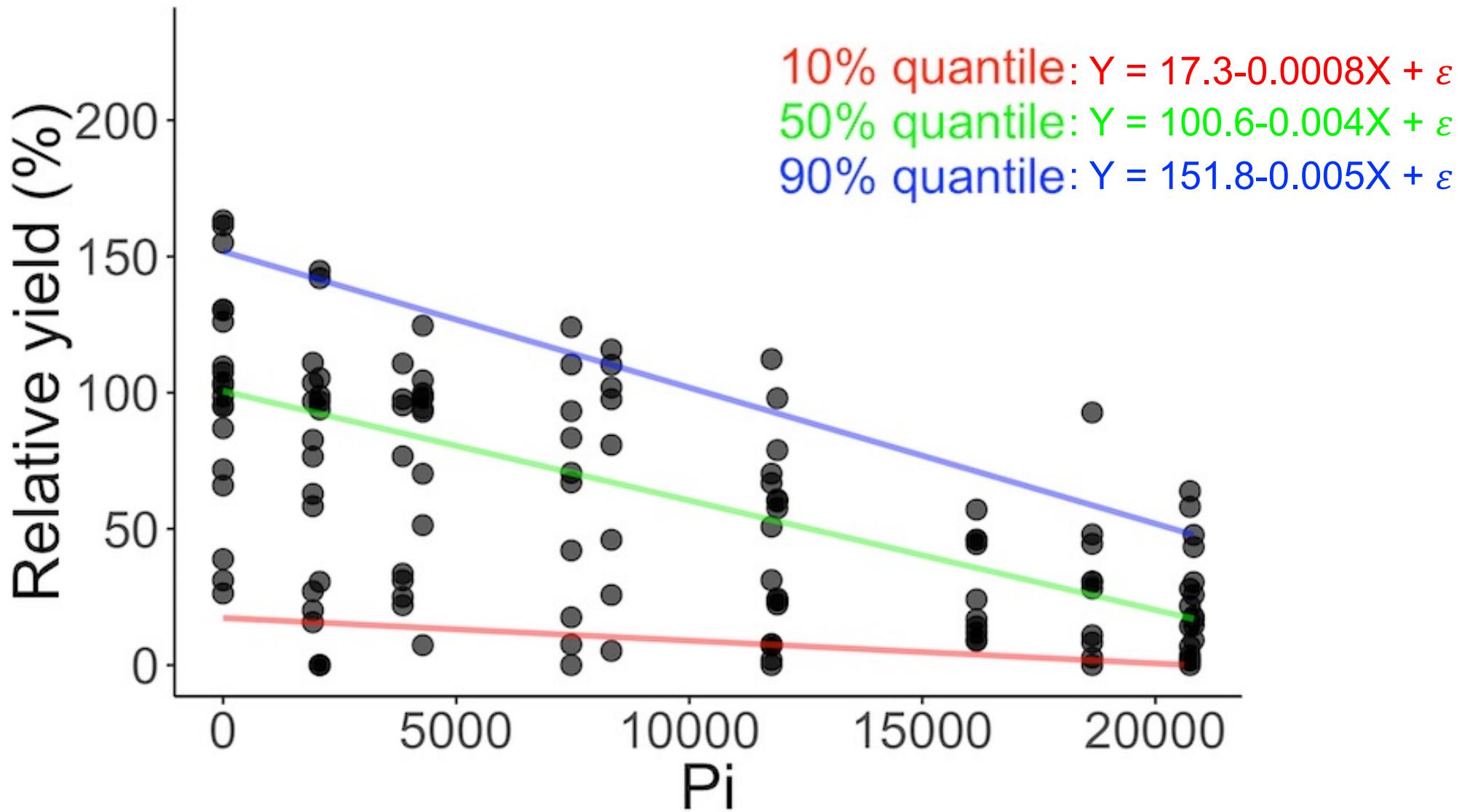
# Pi vs. Yield components



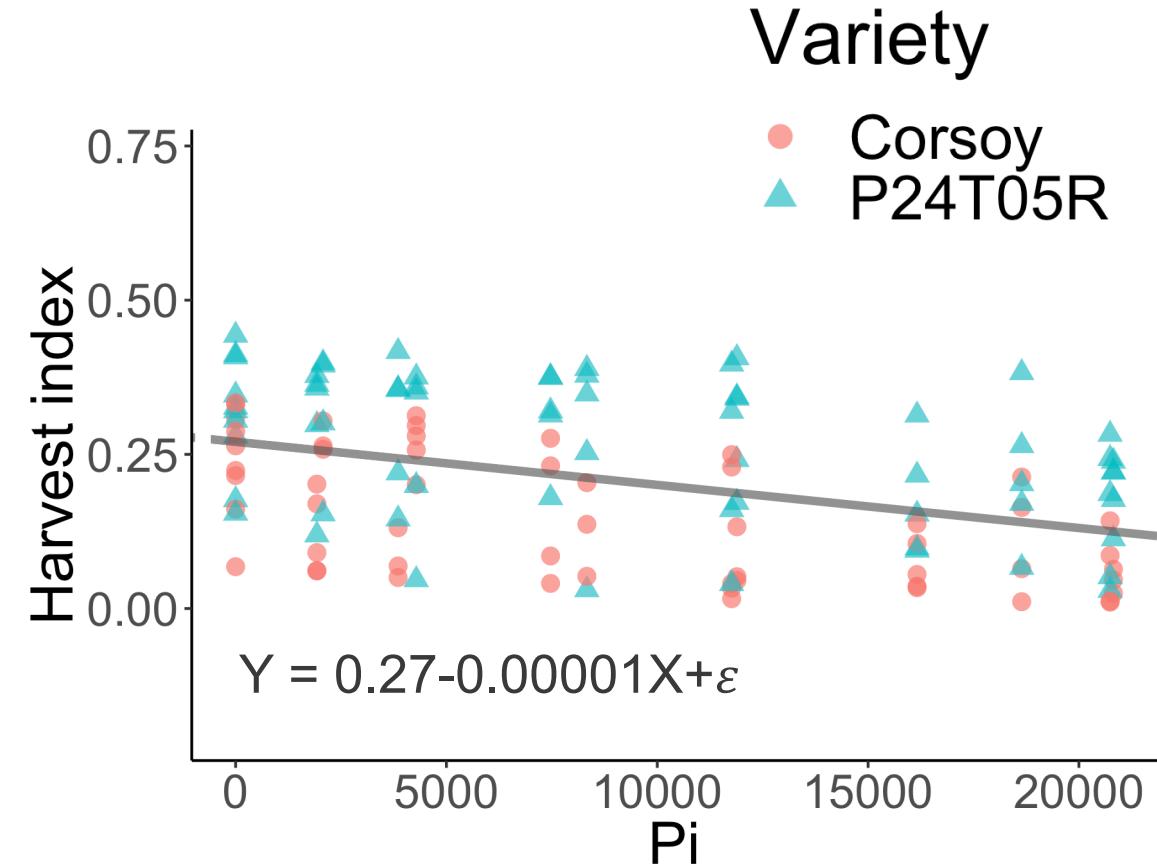
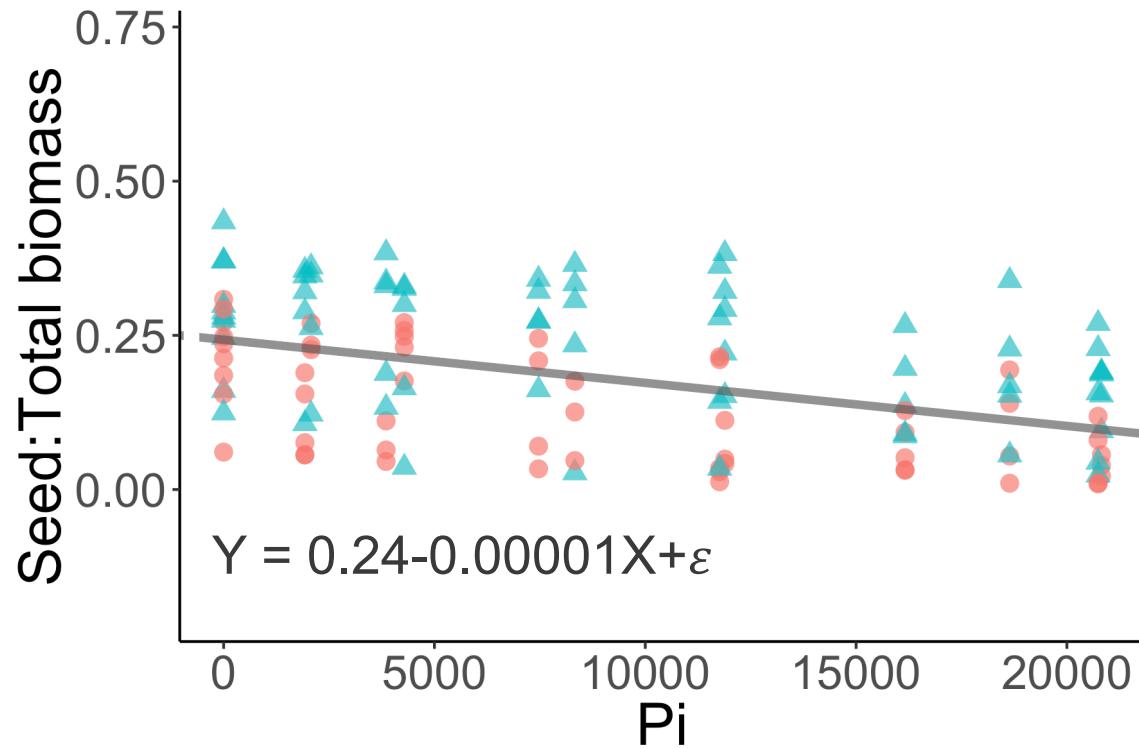
## Variety

- Corsoy
- ▲ P24T05R

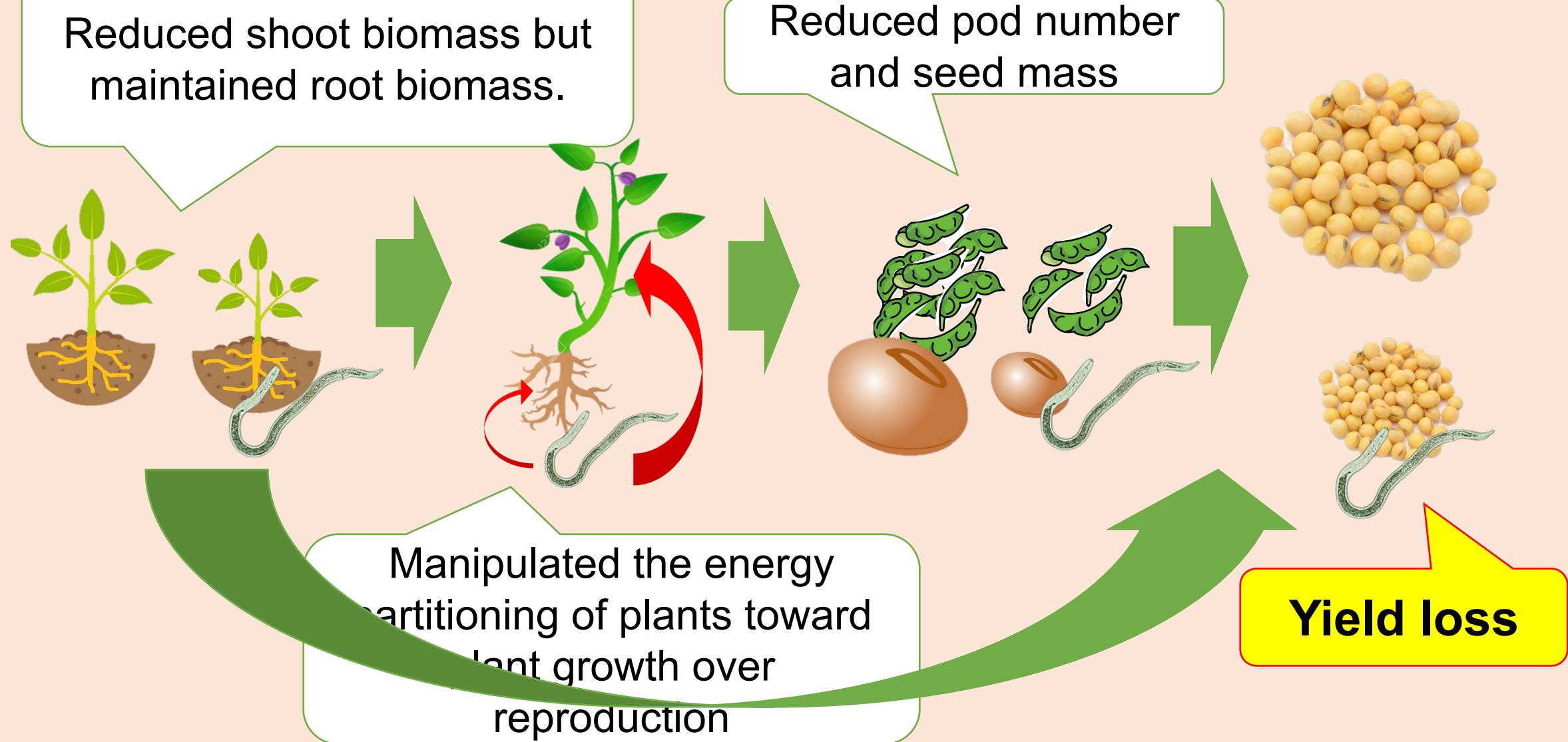
# Pi vs. Relative yield



# Effect of Pi on resource partitioning



# Chapter 1 - Summary

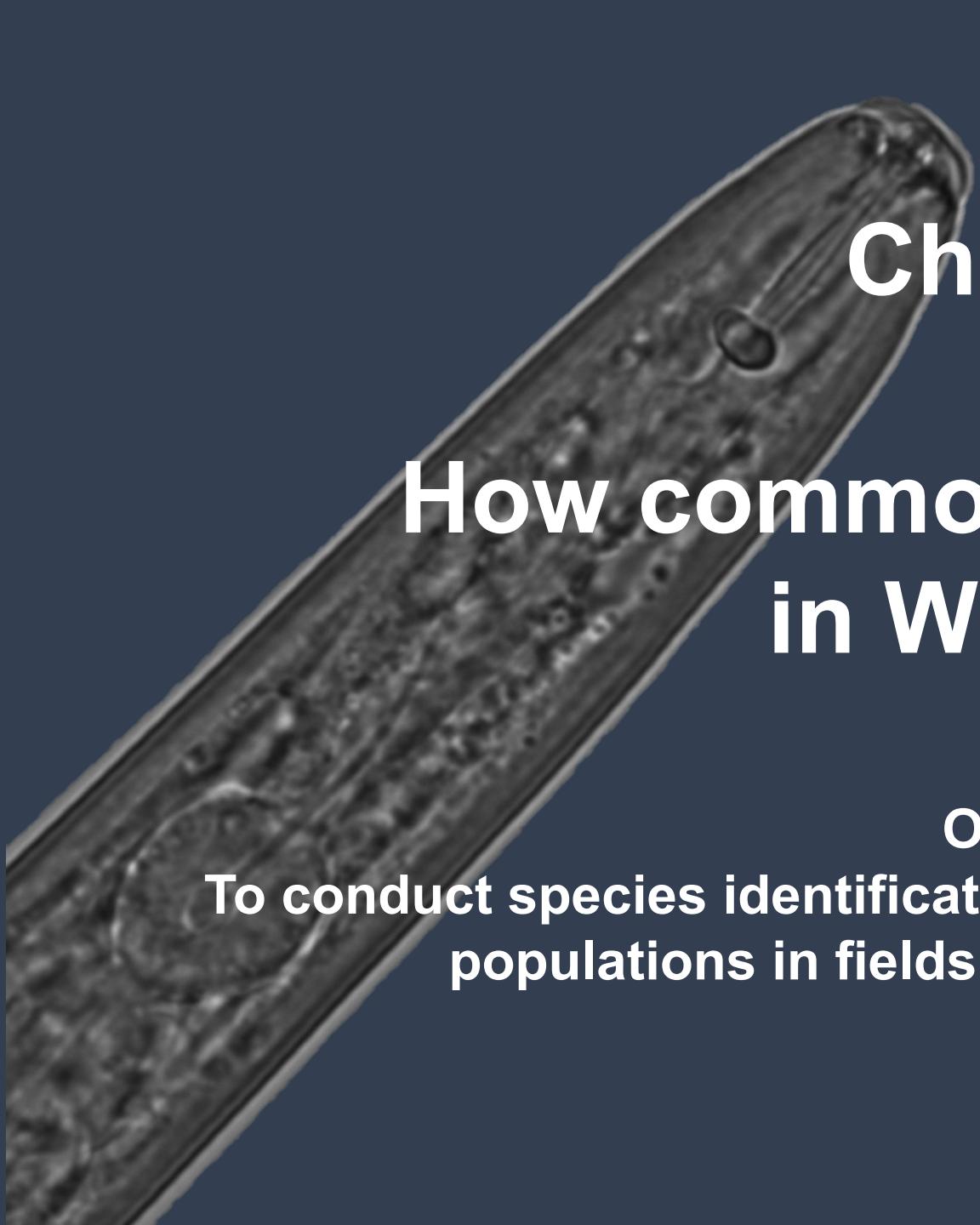


# Outline

**Chapter 1.** Soybean response to *P. penetrans* in field and greenhouse environments.

**Chapter 2.** Gonochoristic species of *Pratylenchus* in soybean cropping systems in Wisconsin.

**Chapter 3.** Gender difference of *P. penetrans* for symptom development and severity.



## Chapter 2.

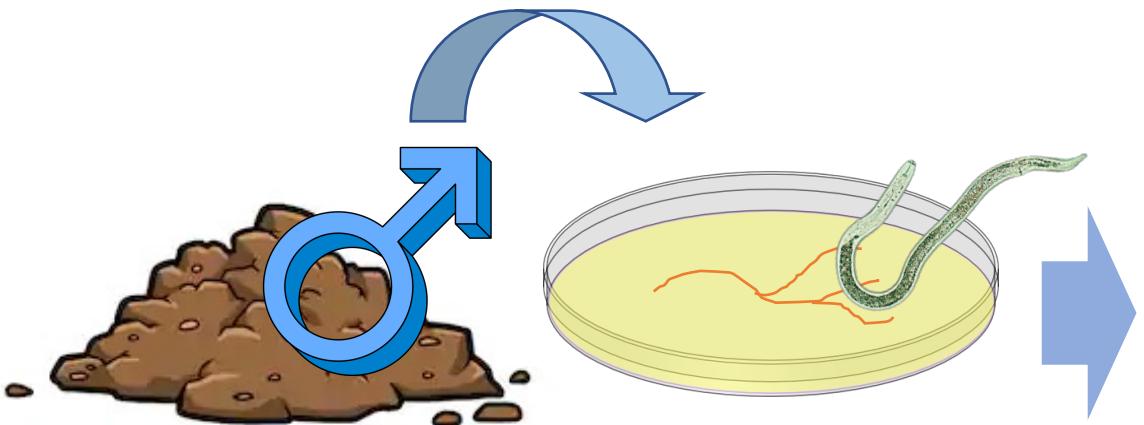
# How common is *P. penetrans* in Wisconsin.

### Objectives:

To conduct species identification of individual male nematodes from populations in fields with soybean in the rotation.

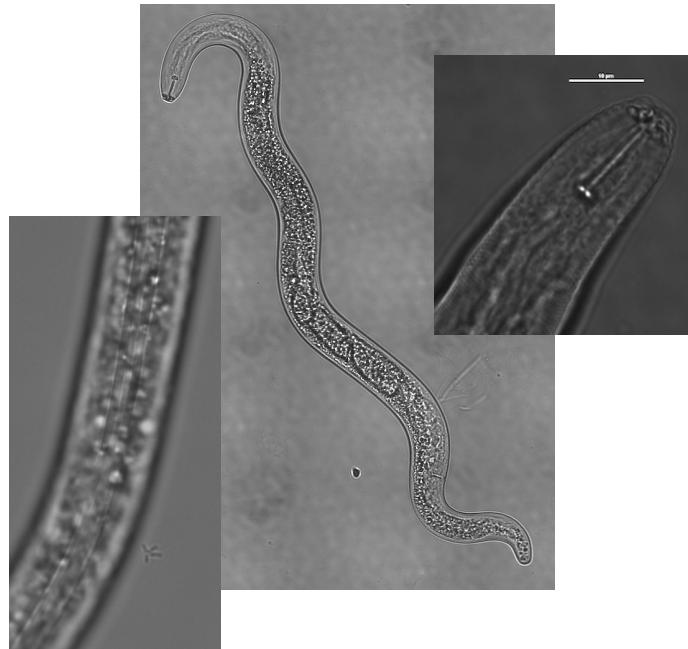
# RLN isolation and characterizations

## 1. RLN isolation



15 isolates (11 counties) +  
2 Wisconsin isolates  
of *P. penetrans*

## 2. Morphological characterization

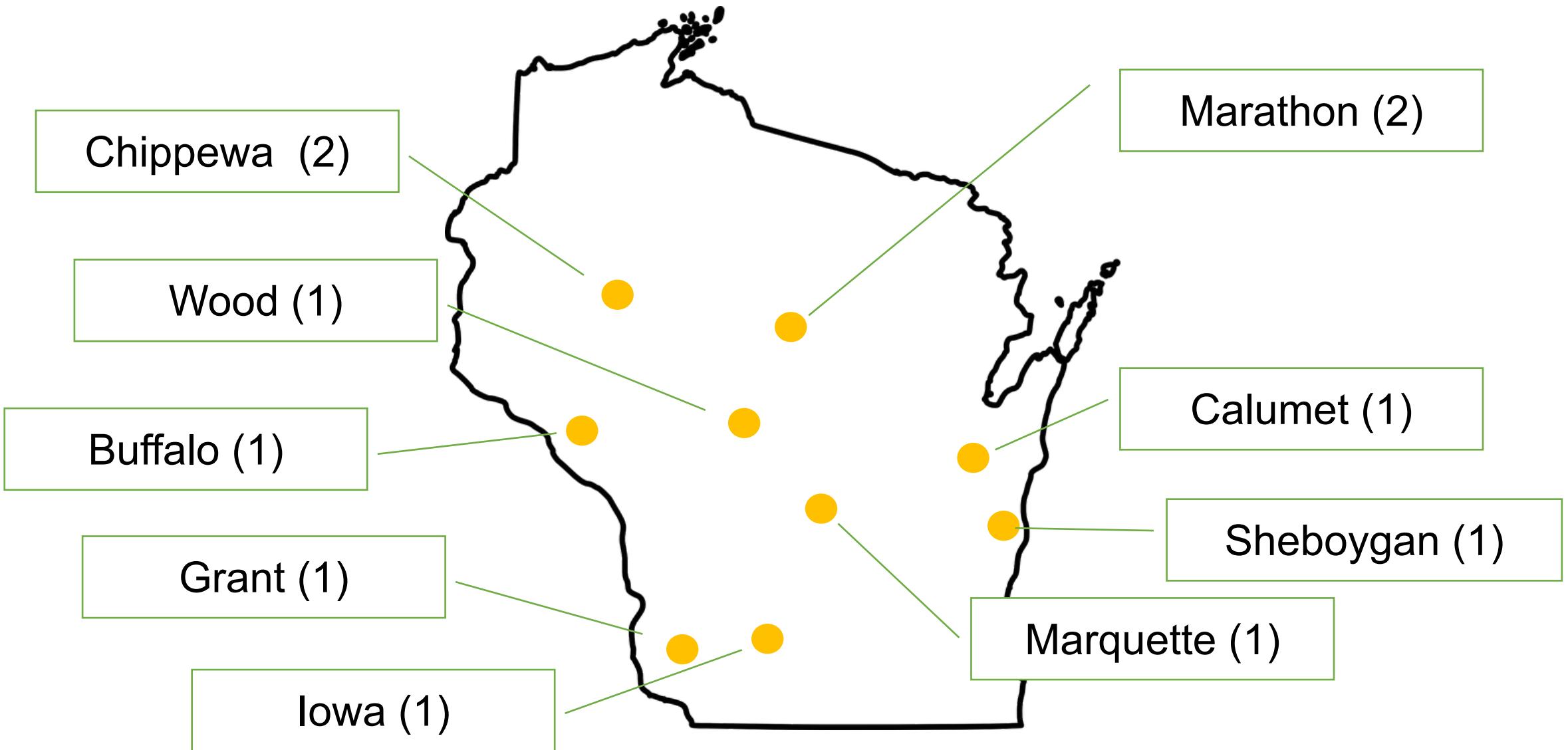


## 3. Molecular characterization

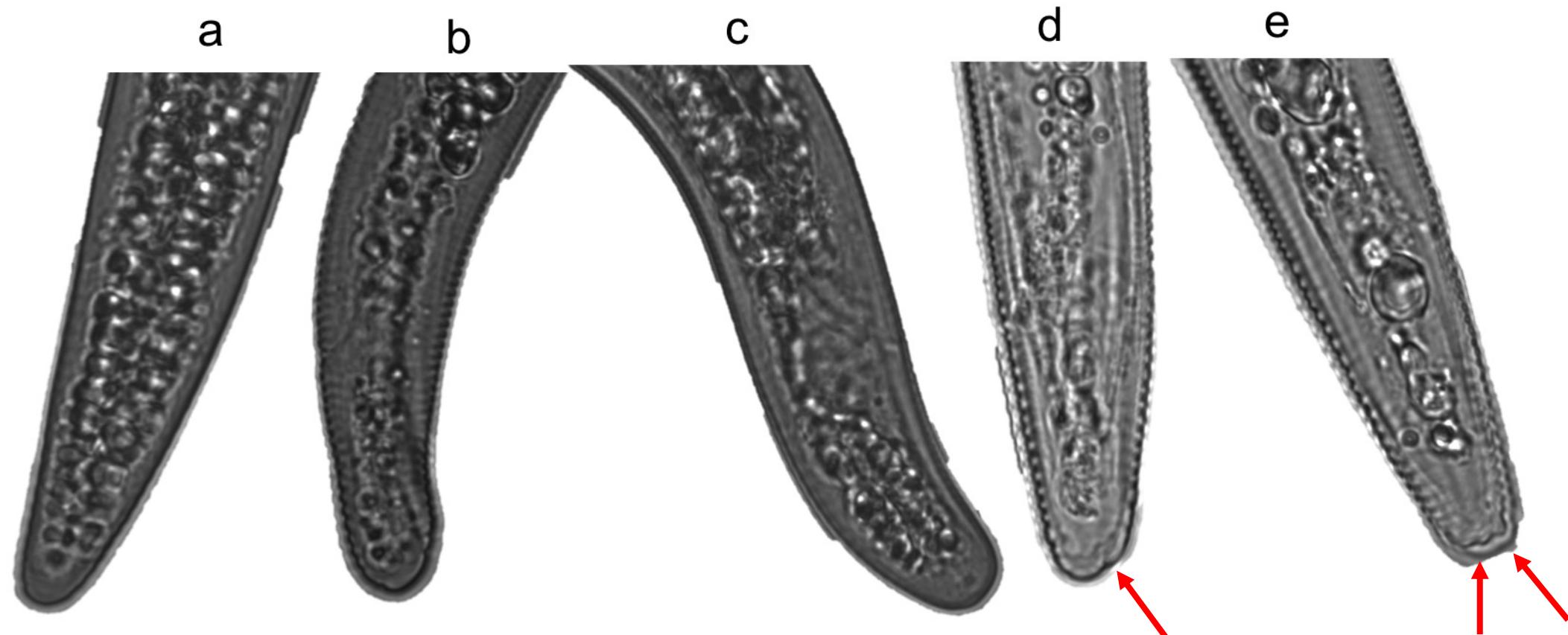


18S, 28S rDNA  
COI mtDNA

# Cultured isolates – *P. penetrans*



# Variations in tail shapes of *P. penetrans*

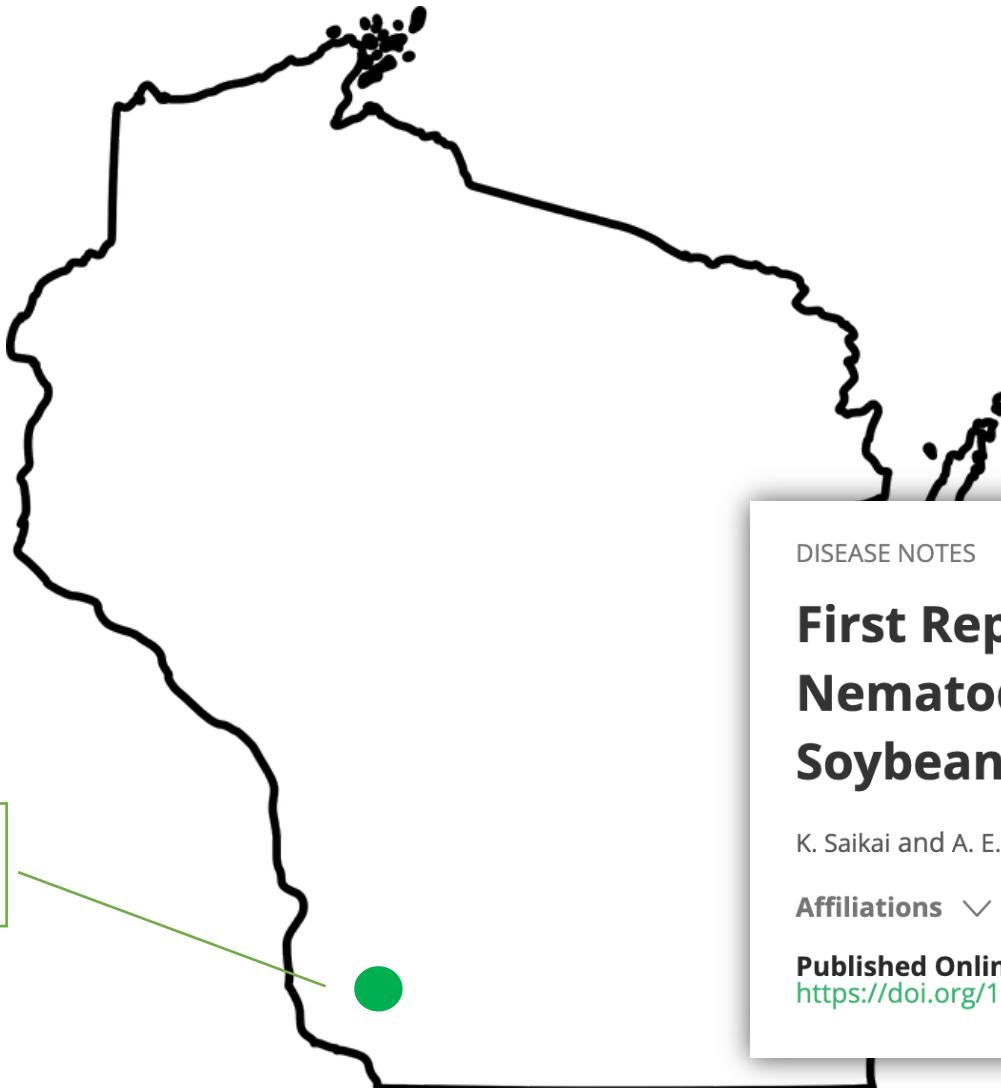


10  $\mu\text{m}$

# Cultured isolates – *P. alleni*



Grant



## DISEASE NOTES



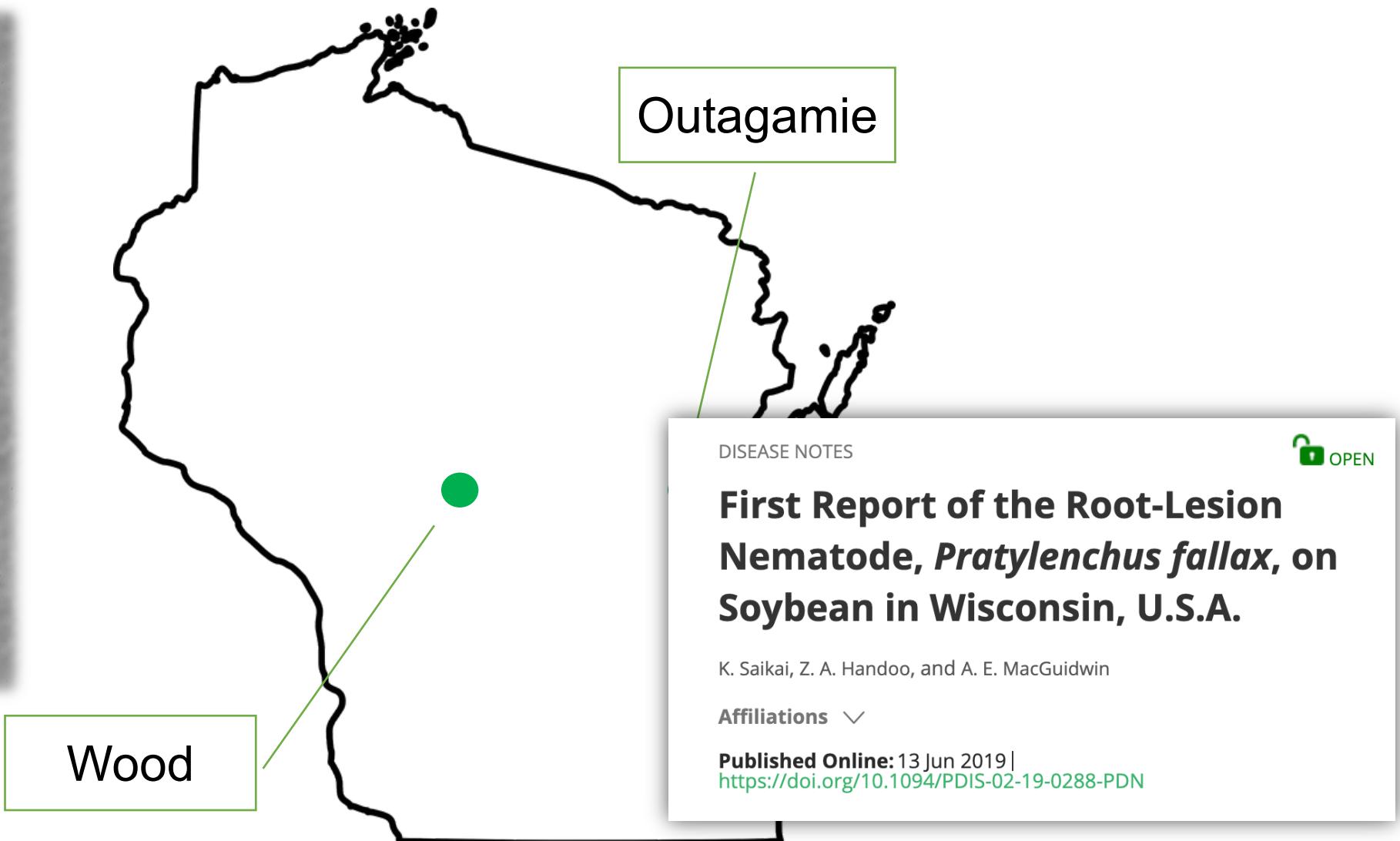
**First Report of the Root-Lesion Nematode, *Pratylenchus alleni*, on Soybean in Wisconsin, U.S.A.**

K. Saikai and A. E. MacGuidwin

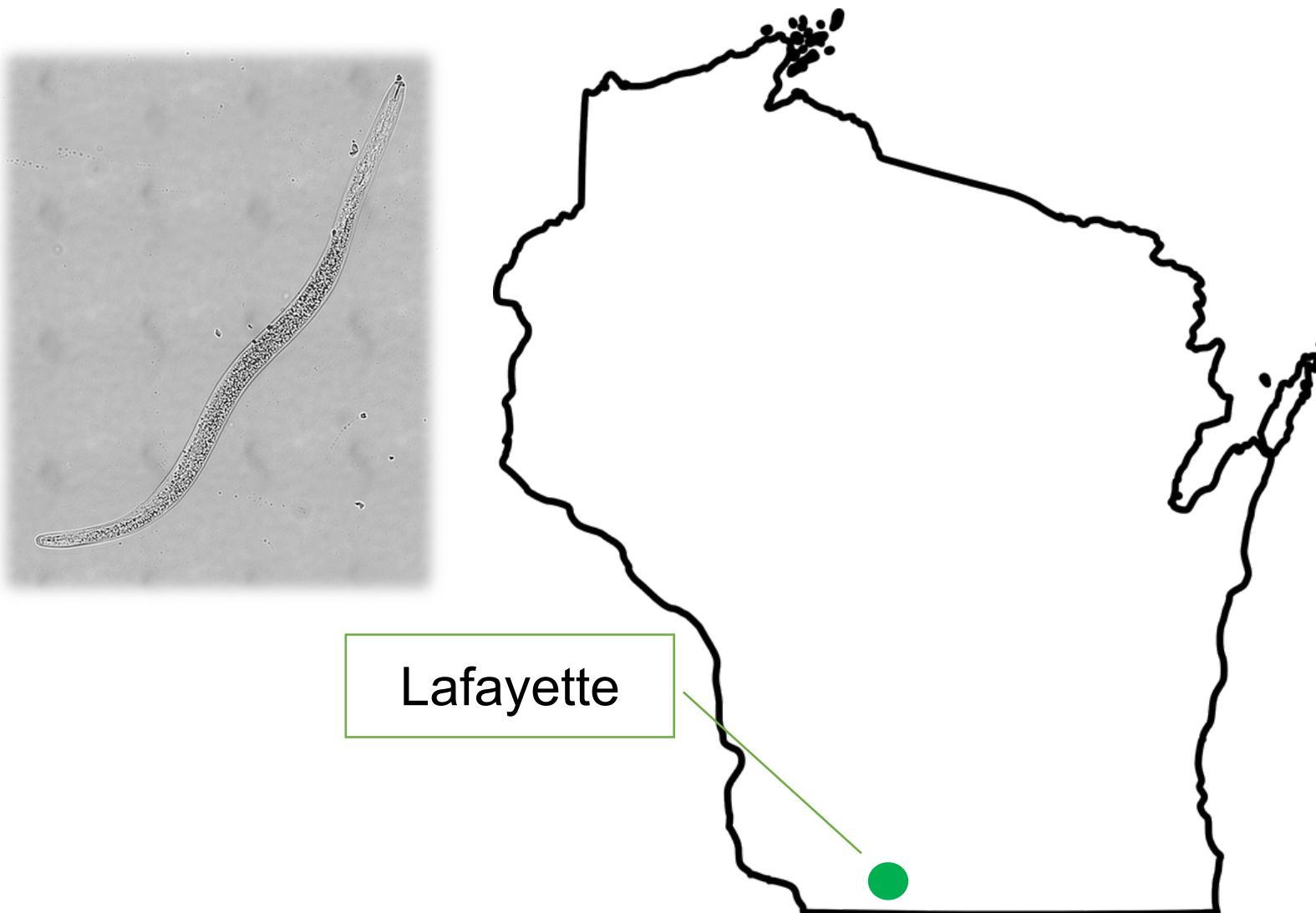
Affiliations ▾

Published Online: 13 Jun 2019 |  
<https://doi.org/10.1094/PDIS-03-19-0501-PDN>

# Cultured isolates – *P. fallax*

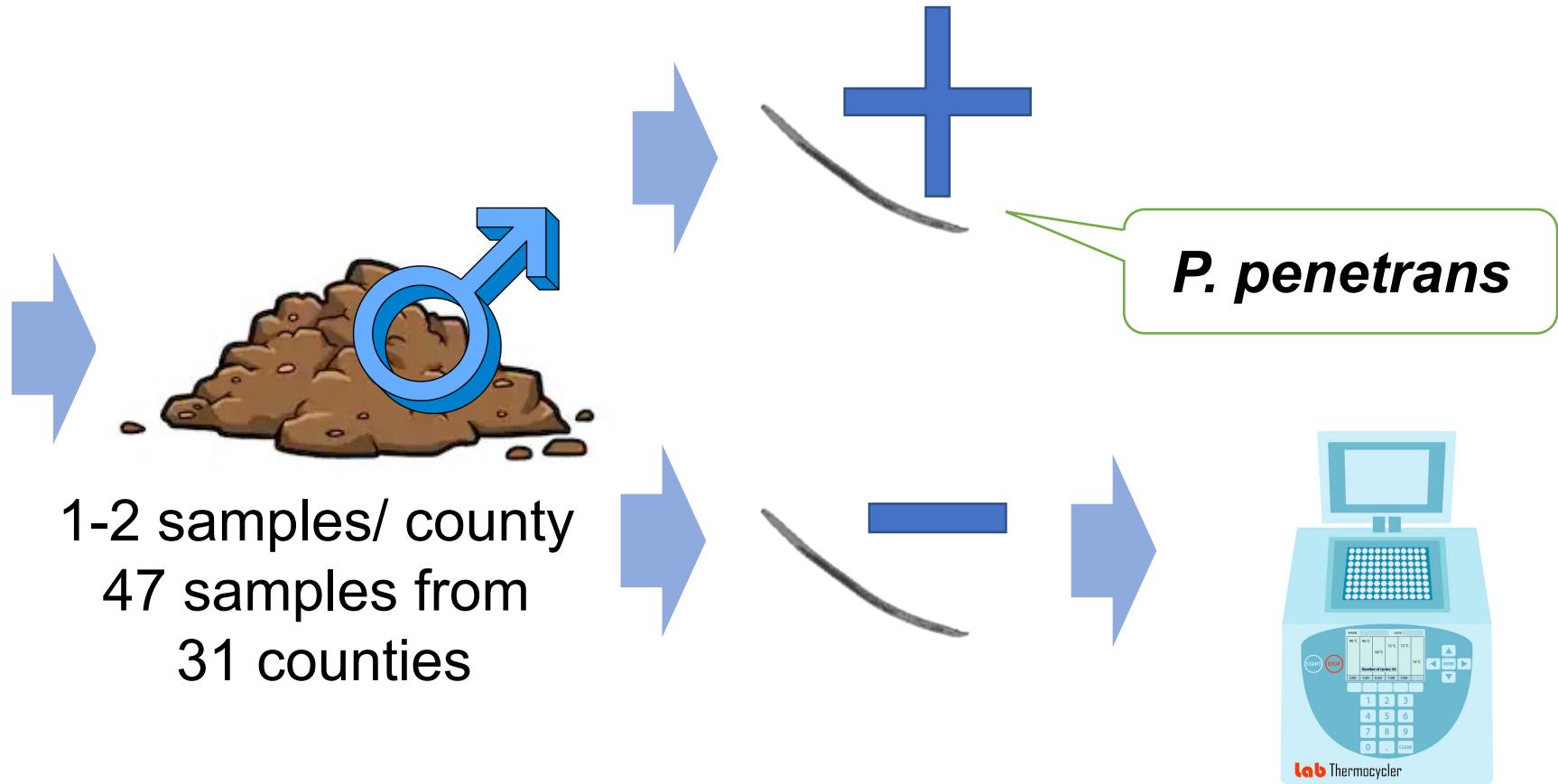
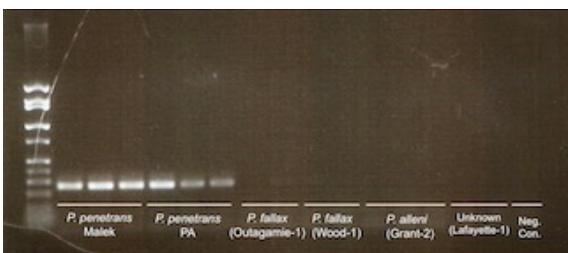


# Cultured isolates – unknown



# Survey Process

Test species-specific primer

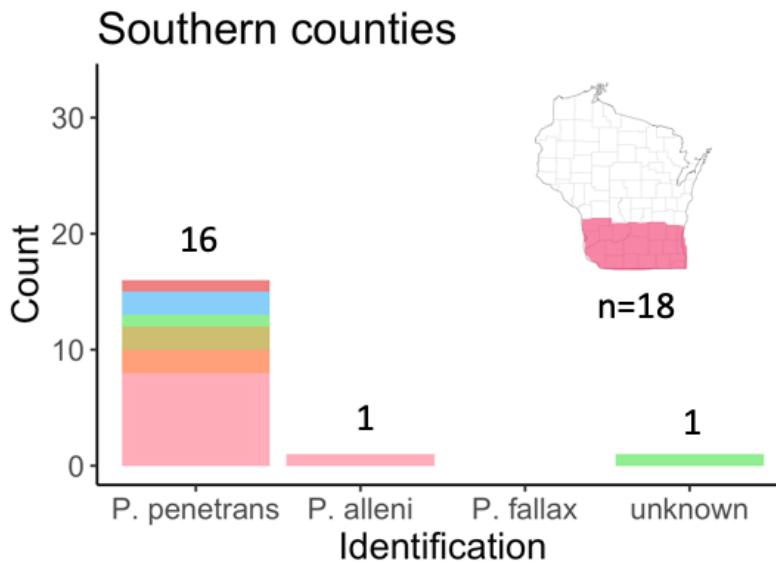
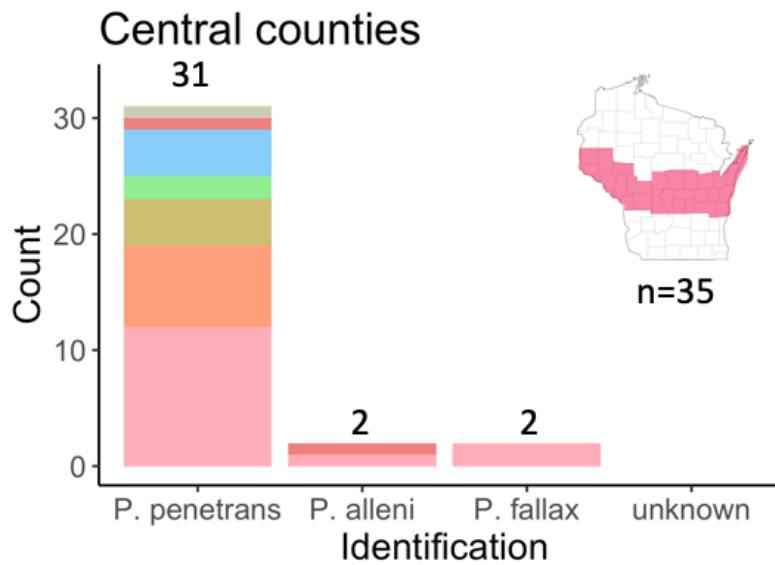
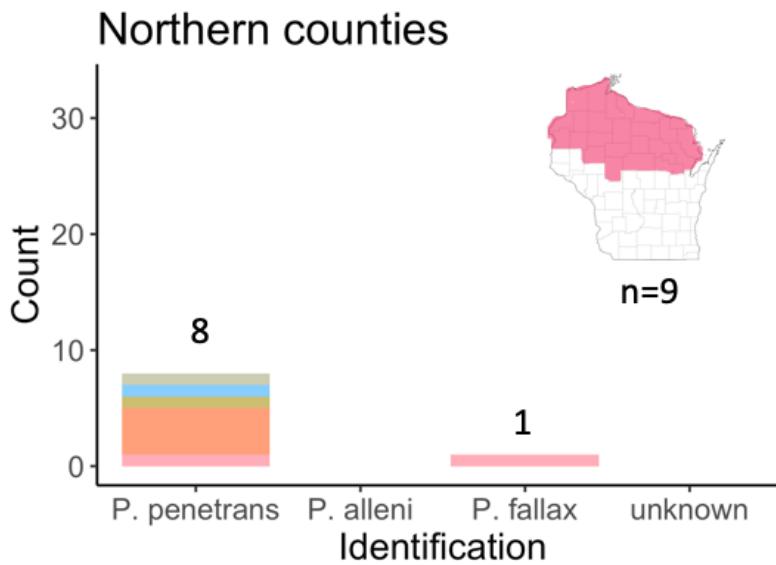


1-2 samples/ county  
47 samples from  
31 counties



28S rDNA  
COI mtDNA

# Survey Results

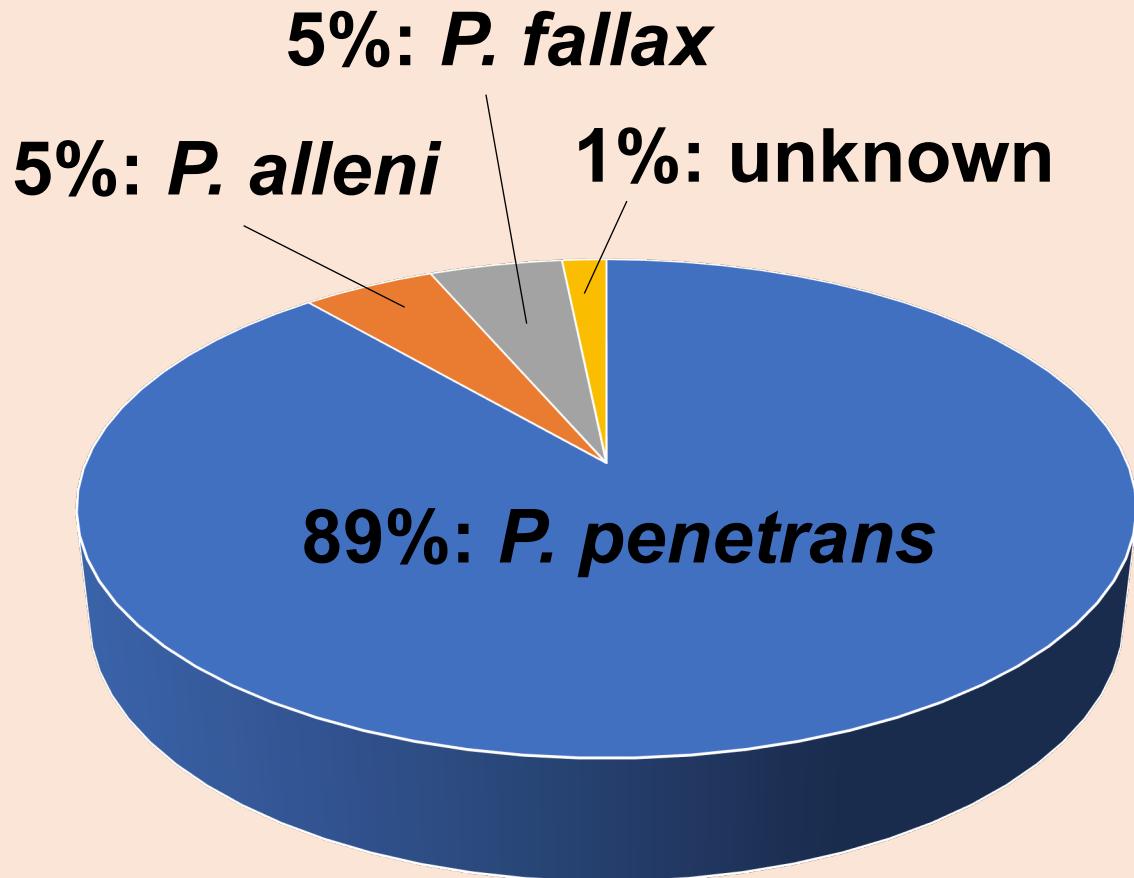


Soil type

- clay
- loam
- loamy sand
- no report
- sand
- sandy loam
- silt loam

62 samples,  
61 farms in  
41 counties in WI.

# Chapter 2 Summary



A good rule of thumb for diagnostic labs to assume that a **sample with males is likely to be *P. penetrans*.**

*P. penetrans* was found from 42 counties on all soil textures.

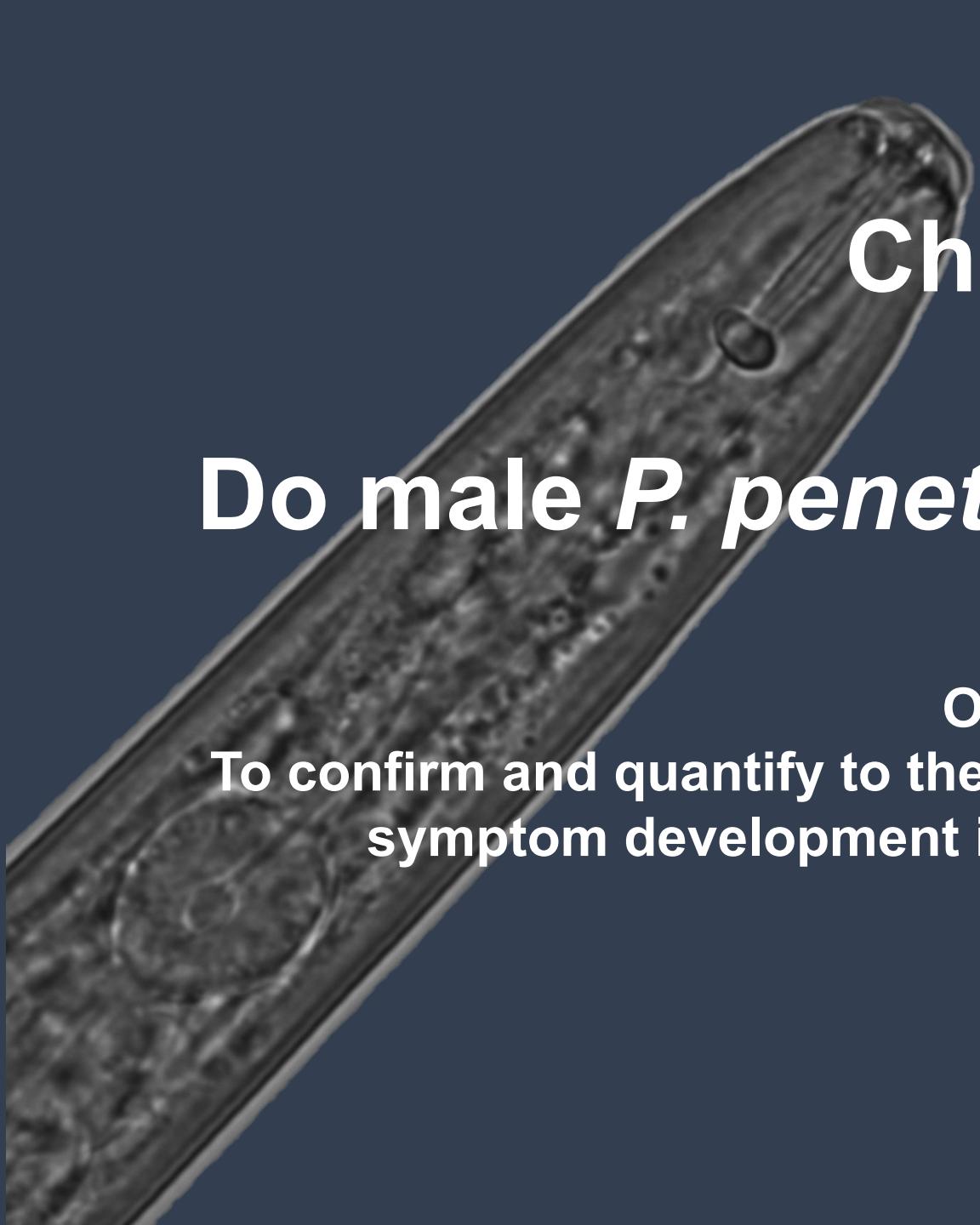
Need further studies on the damage potential of *P. alleni* and *P. fallax*.

# Outline

**Chapter 1.** Soybean response to *P. penetrans* in field and greenhouse environments.

**Chapter 2.** Gonochoristic species of *Pratylenchus* in soybean cropping systems in Wisconsin.

**Chapter 3.** Gender difference of *P. penetrans* for symptom development and severity.



## Chapter 3.

# Do male *P. penetrans* induce lesions?

**Objectives:**

**To confirm and quantify to the observation of gender differences for symptom development in roots infected by *P. penetrans***

# Background

The diagram illustrates the life cycle and infection process of *Pratylenchus penetrans* in plant roots. On the left, a detailed anatomical drawing shows the nematode's life stages: J1, J2, J3, J4, M3, and M4. A red circle highlights the male (♂) stage. A green callout box states "Male penetrates roots less". An inset image shows a trifoliate leaf of *Medicago sativa*. Below this is a text box titled "Infectivity of *Pratylenchus penetrans* on Alfalfa<sup>1</sup>" by J. L. TOWNSEND. The text discusses the infectivity of *P. penetrans* on alfalfa seedlings, noting that the root-hair zone was the preferred zone of penetration. It details the progression from a water-soaked area at the root surface to a yellow lesion and subsequent dark-brown cell infiltration. The text also mentions that females penetrate earlier and faster than males and third-stage larvae, with maximum penetration occurring between 10 and 30°C. A red arrow points from the male penetration point in the inset to the corresponding stage in the main diagram. To the right, a sequence of four cross-sections of a plant root shows the progression of infection. The first shows a normal root. Subsequent sections show a lesion developing, followed by a secondary infection where the root tissue becomes severely damaged and discolored. A green callout box states "Male exit roots more often". A red arrow points from the male exit point in the final section to the corresponding stage in the main diagram. Below this is a text box titled "Differences in Egress of Male and Female *Pratylenchus penetrans* from Pea Roots<sup>1</sup>" by D. J. WIXTED AND A. E. MACGUIDWIN<sup>2</sup>. The text discusses the differences in egress behavior between males and females, noting that males exit roots more frequently than females. A small illustration of a pea plant is shown. At the bottom right, there is additional text about inoculation conditions and keywords.

**Infectivity of *Pratylenchus penetrans* on Alfalfa<sup>1</sup>**  
J. L. TOWNSEND<sup>2</sup>

*et al.*: The infectivity of *Pratylenchus penetrans* on alfalfa seedlings cv. Du Puits was studied. The root-hair zone was the preferred zone of penetration by females, males, and third-stage larvae. A lesion initially appeared as a water-soaked area at the root surface, becoming yellow apical as the nematode entered the cortex, with dark-brown cells later appearing in the region as the nematode fed. At 20 °C, females penetrated roots earlier, faster, and in greater numbers than either males or third-stage larvae. Females penetrated roots at temperatures from 5 to 30 °C, with maximum penetration between 10 and 30 °C, while males and third-stage larvae penetrated roots only between 10 and 30 °C with maximum penetration at 20 °C. Penetration by females, males, and third-stage larvae increased after storage of 5 °C for 35 days, but decreased after storage of 140 days or more. Combinations of the three life stages in pairs neither enhanced nor inhibited penetration of roots by individual life stages; males were not attracted to females. Increasing inoculum density up to 20 nematodes/seedling did not affect penetration.

*Key Words:* root-lesion nematode, penetration, lesion, *Medicago sativa*.

**Differences in Egress of Male and Female *Pratylenchus penetrans* from Pea Roots<sup>1</sup>**  
D. J. WIXTED AND A. E. MACGUIDWIN<sup>2</sup>

*Keywords:* attraction, behavior, egress, infection, migration, *Pratylenchus penetrans*.

*Pratylenchus* spp. are migratory endoparasites of plants. Despite being obligate par-

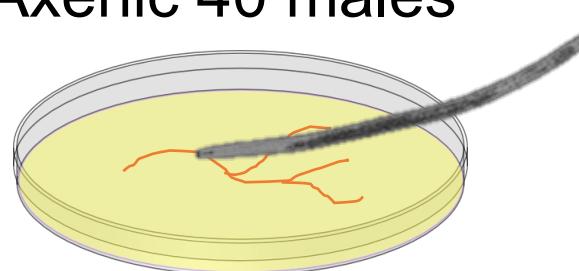
medium (2) were used. Inoculation of 40 males or 40 females was axenically transferred into a container containing 0.5 ml sterile wa

Pea seeds (*Pisum sativum* L. cv. Early Per-

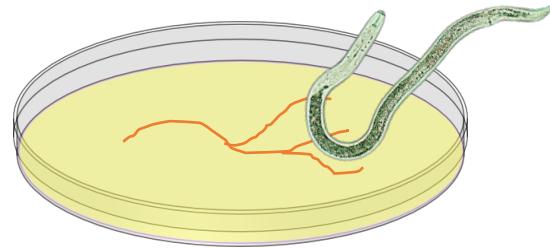
# In vitro study design

## Nematode introduction

Axenic 40 males



Axenic 40 females

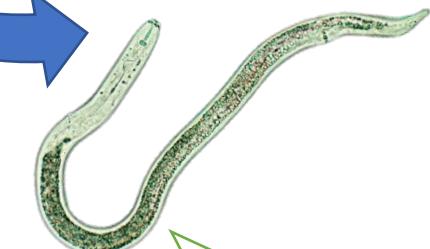
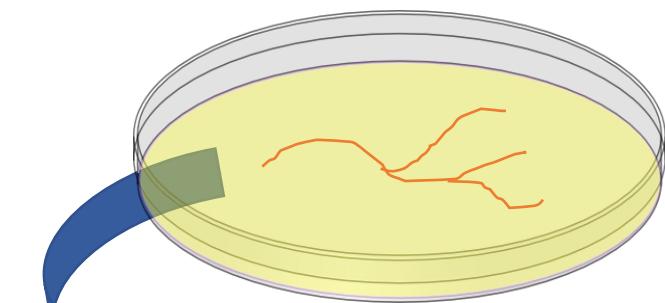


## Lesion measurements for 14 days



Record lesion number and length

## Harvest at 14 days



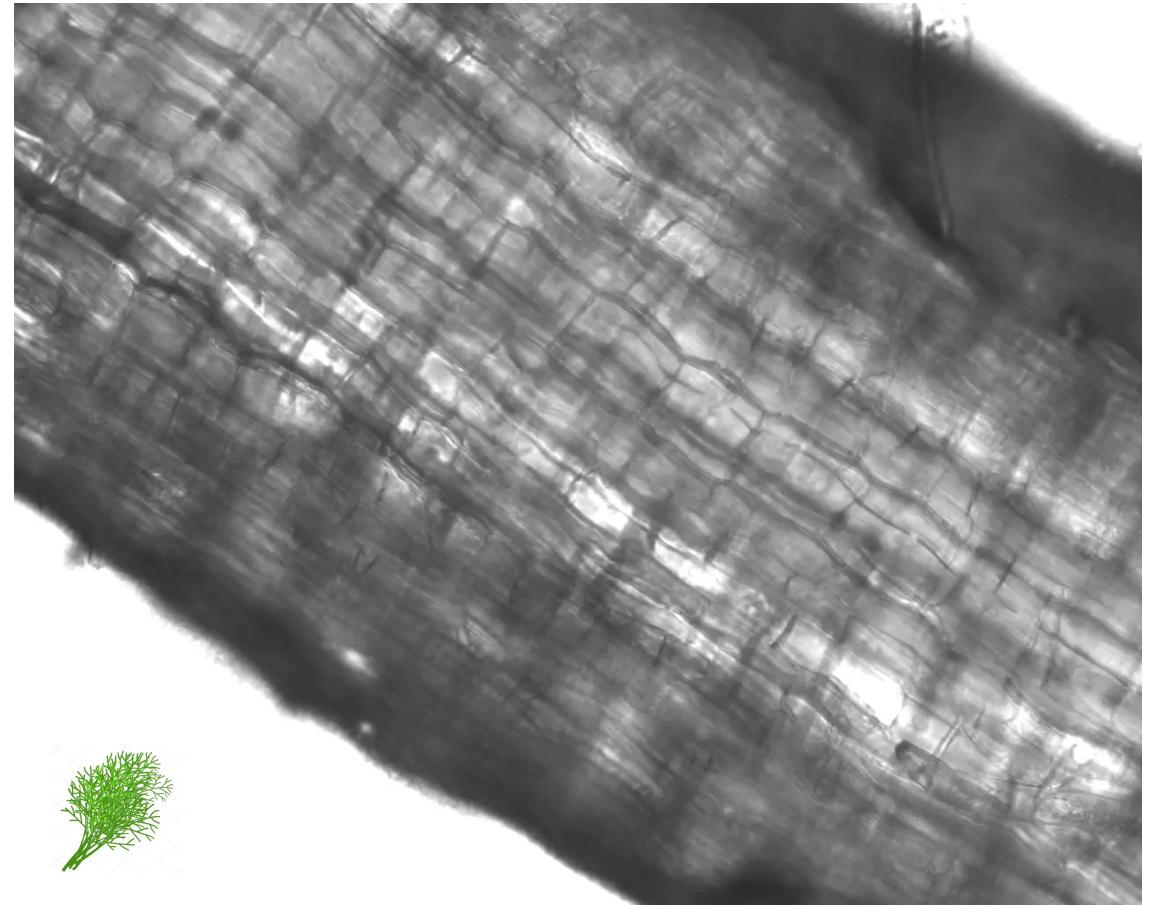
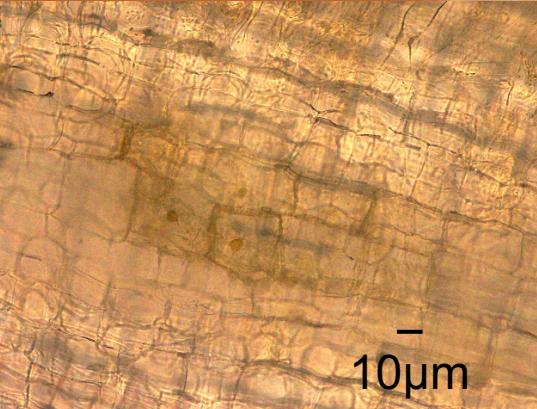
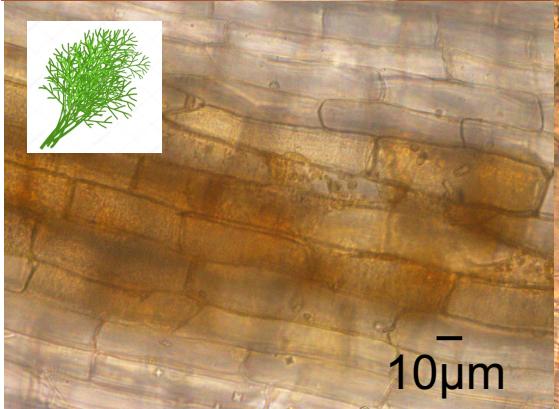
Record nematode number

# Lesions by female and male

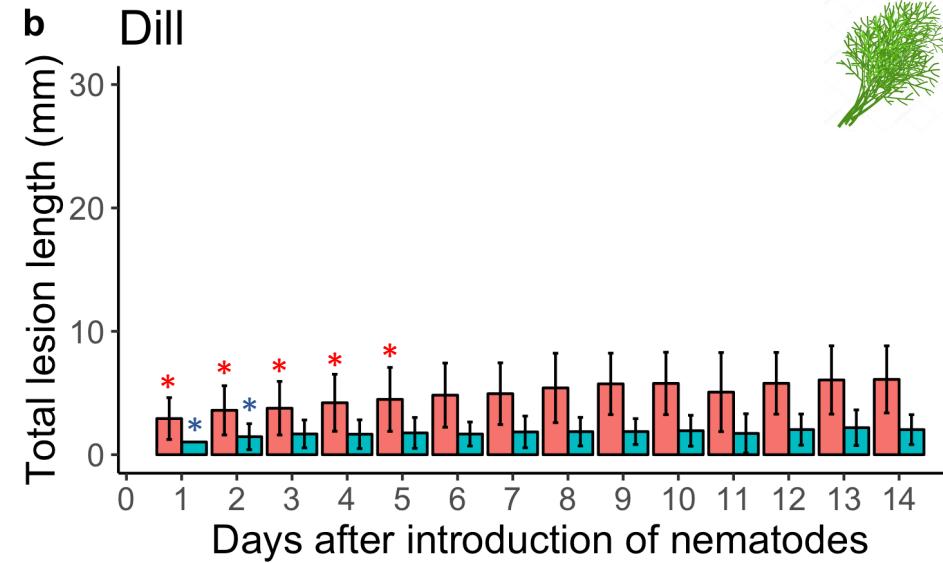
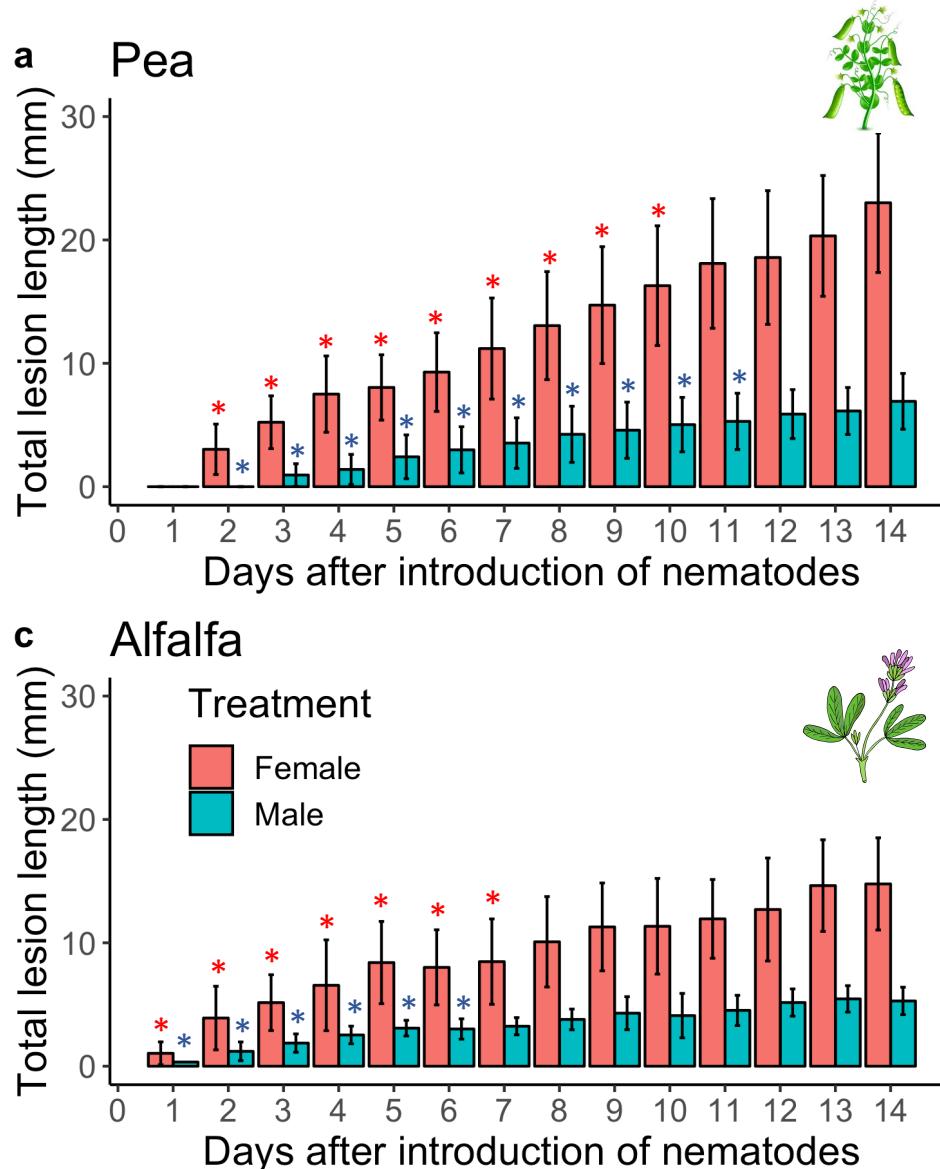
female



male



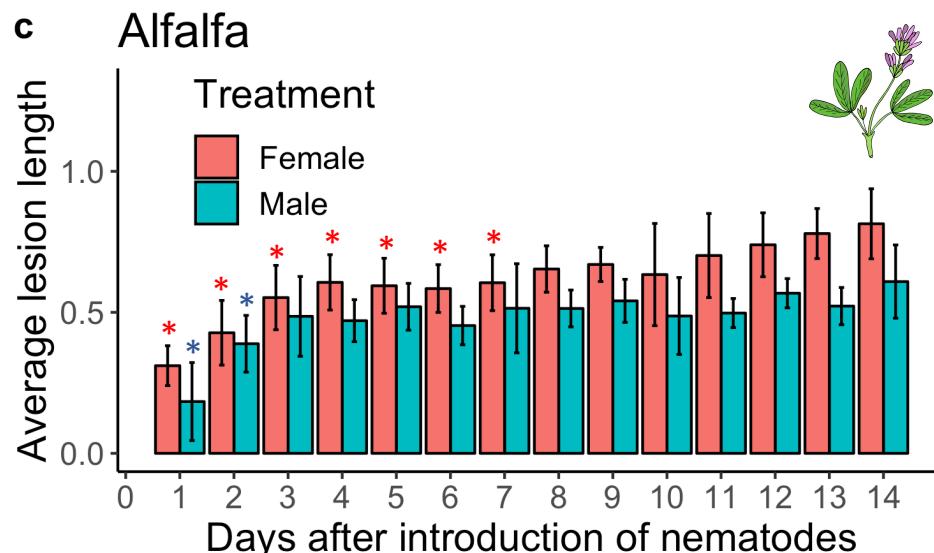
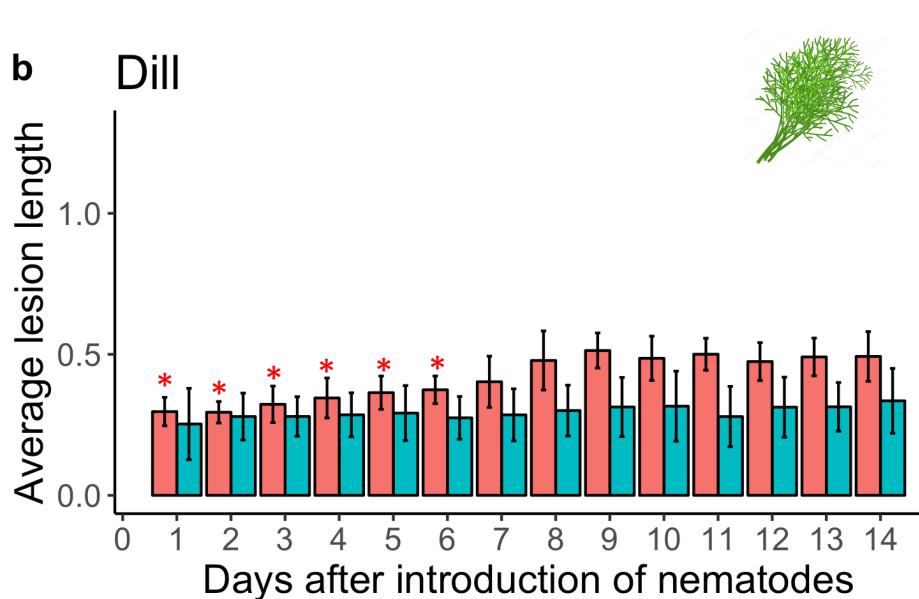
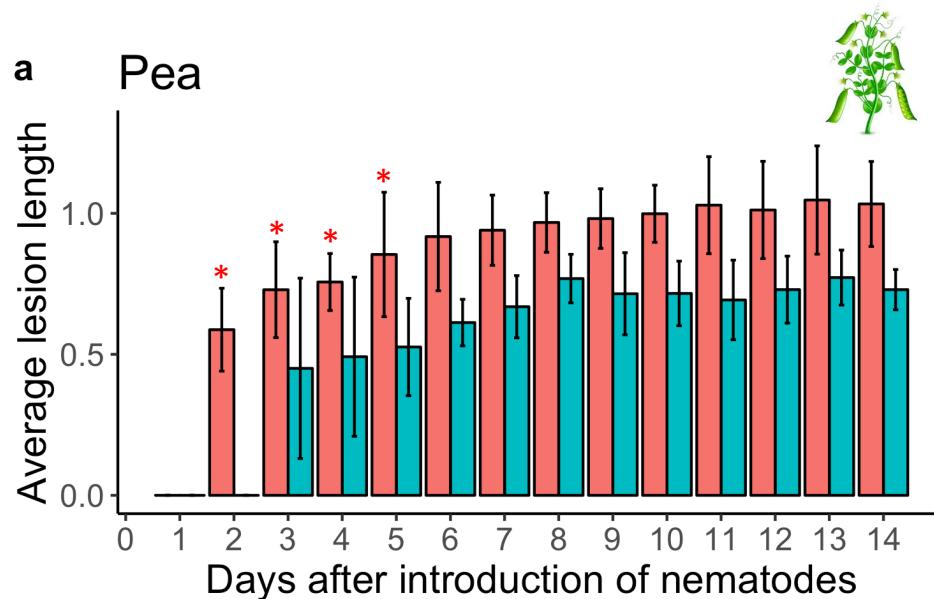
# Gender effect on total lesion length



Total lesion length was greater ( $P < 0.01$ ) for females than males at **every time point**.

\* = difference ( $P < 0.05$ ) between each time point and day 14

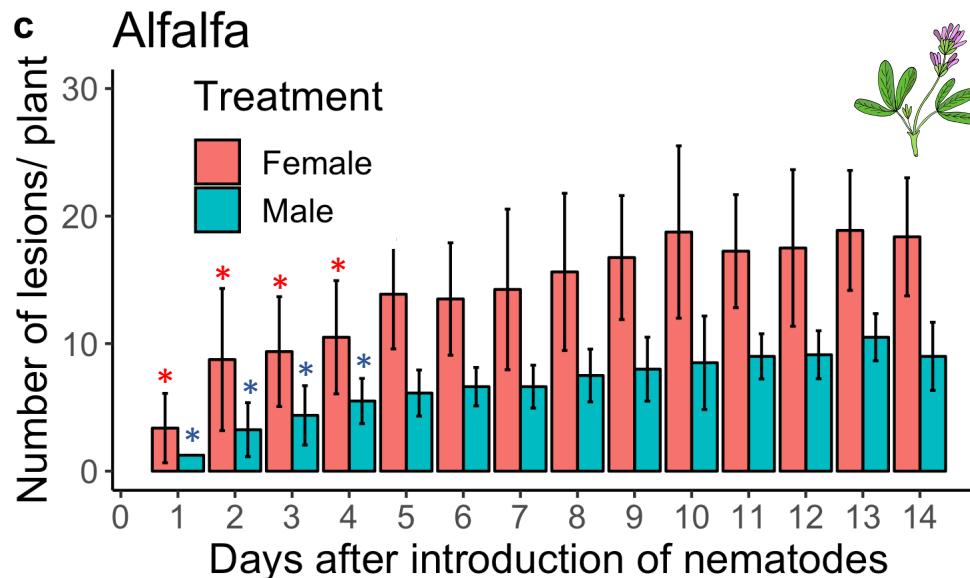
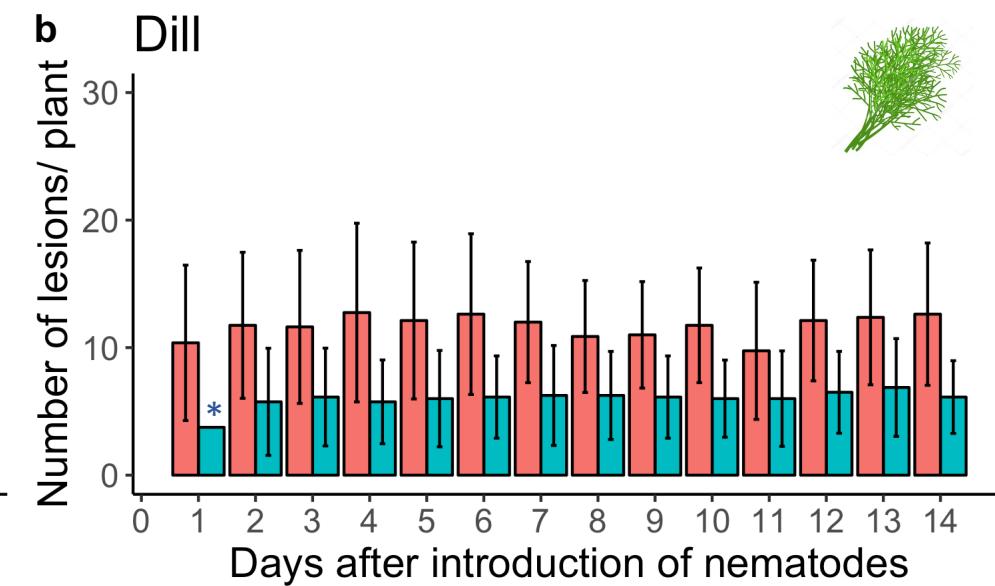
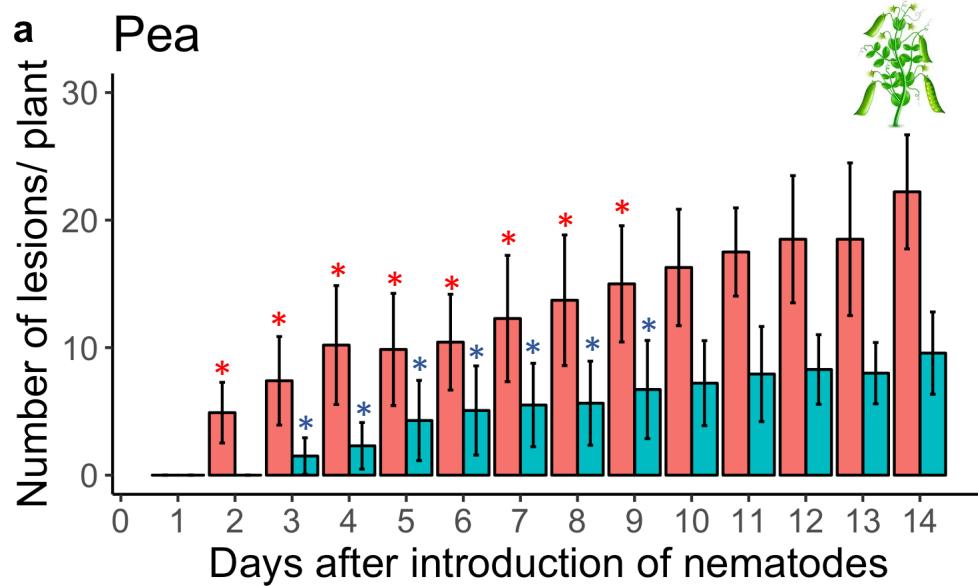
# Gender effect on average lesion length



Average lesion length was greater ( $P <0.01$ ) for female after **day 3 on pea and day 6 on dill and alfalfa**

\* = difference ( $P <0.05$ ) between each time point and day 14

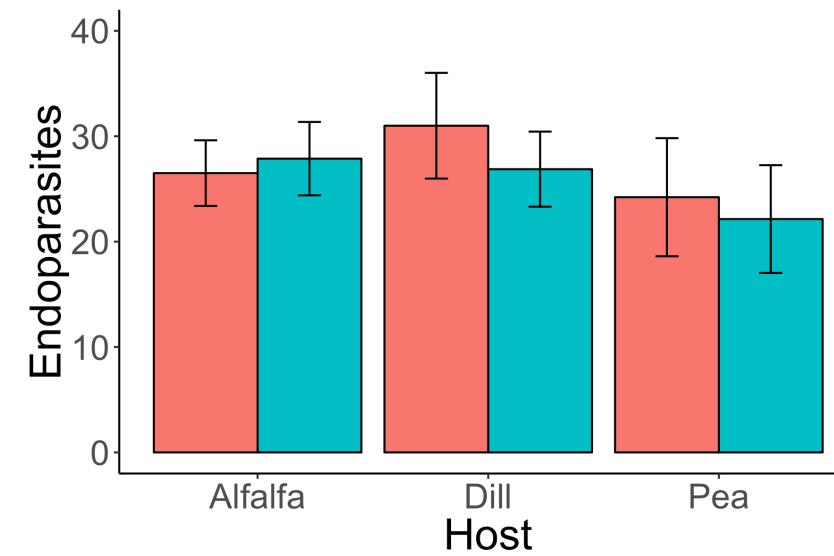
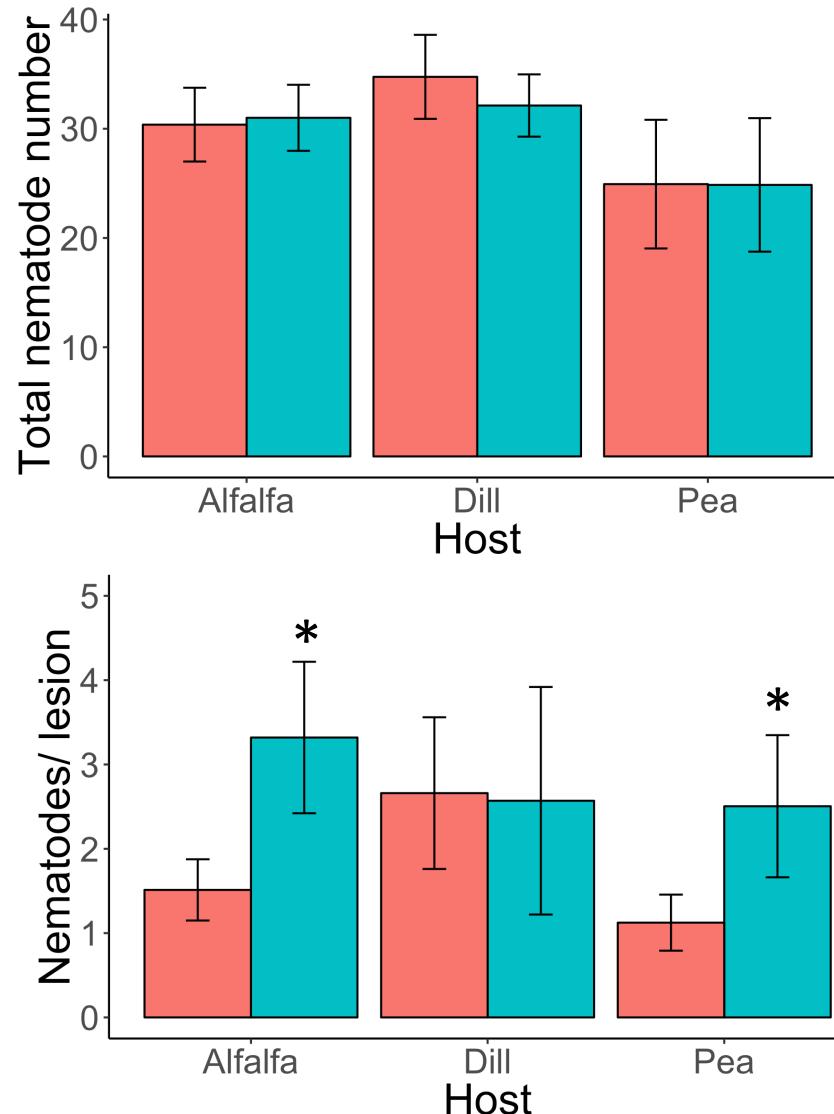
# Gender effect on lesion number



Lesion number was greater ( $P < 0.01$ ) for females than males at **every time point**.

\* = difference ( $P < 0.05$ ) between each time point and day 14

# Number of nematodes at day 14



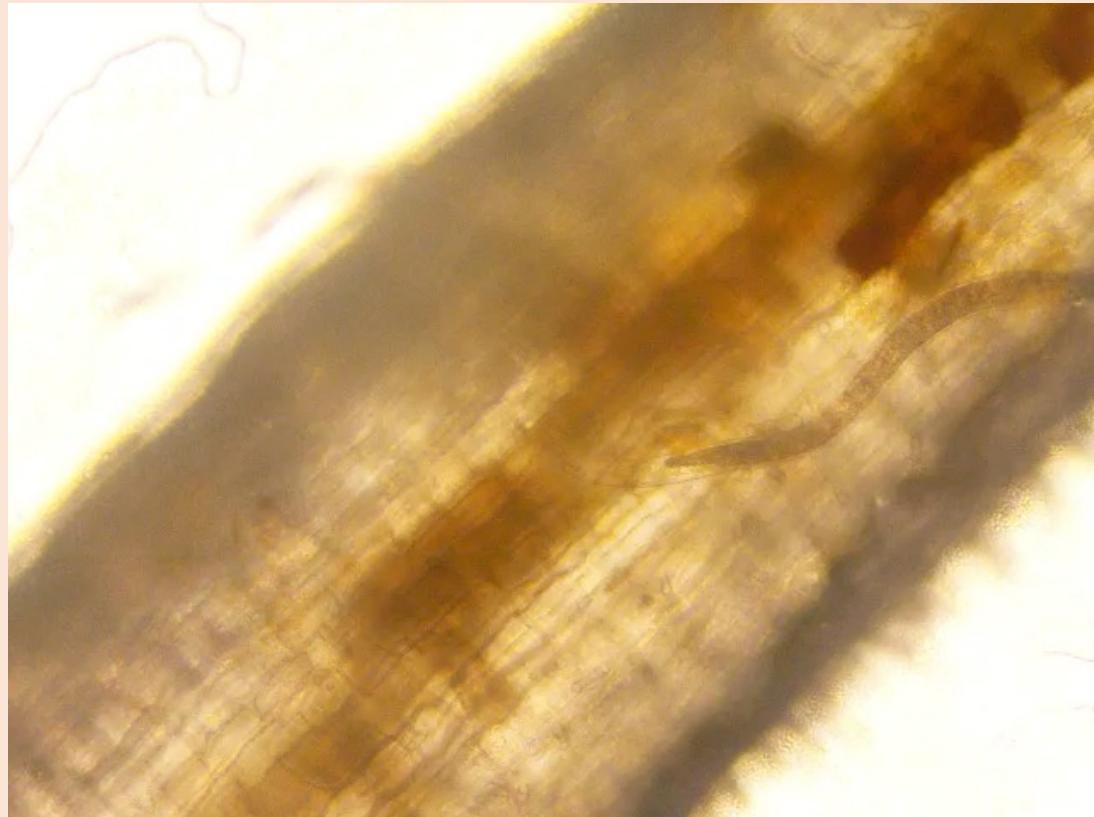
Treatment

Female  
Male

Number of total RLN and endoparasites did not differ by gender.

\* = gender difference ( $P < 0.01$ )

# Chapter 3 - Summary



**Male *P. penetrans* induced fewer lesions on the three hosts.**

Root infection and occupancy was the same for both genders, diminishing the possibility that our findings were biased by gender difference in infection competency.

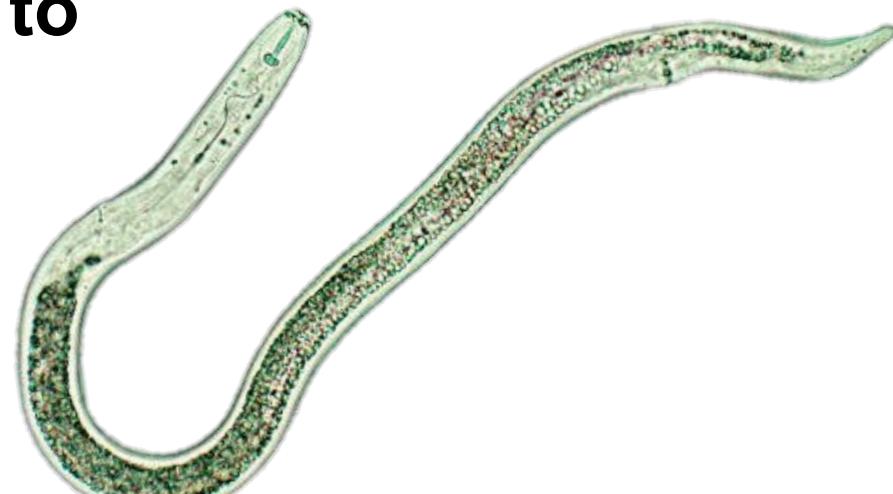
Greater lesion number and size were constantly associated with female at all time points.

# Synthesis

*Pratylenchus penetrans* is an important pathogen on soybean.

The nematode induces yield loss by reducing pod number and seed mass.

The high incidence of *P. penetrans* demonstrated in chapter 2 in combination with its damage potential based on chapter 1 suggest ***P. penetrans* is a major constraint to the soybean industry.**



# Acknowledgement

## MacGuidwin lab member:

Gary Pack, PhD  
Ibrahim Saeed, PhD  
Lauren Raasch  
(David Sundquist)  
(Bre Bender)  
(Toby Lunt)  
(Tomas R)  
All the

## Horticultural Station:



MacGuidwin (Chair)  
Conley  
Lard Lankau  
Rug Rouse  
Damon Smith  
(Dr. Murray Clayton)

## Special thanks:

Maria Kamenetsky (Statistic Consulting)  
Dr. Zafar Handoo (USDA-ARS)

