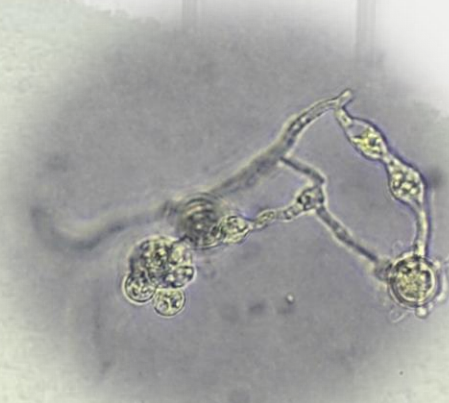
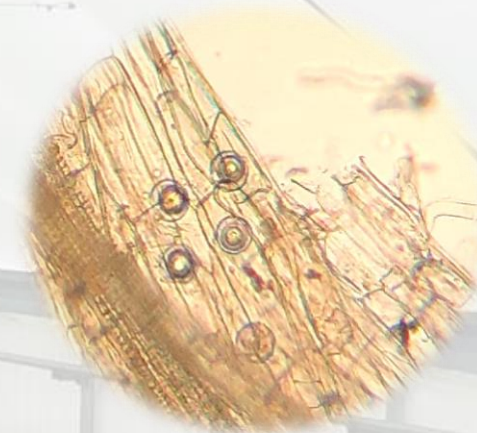
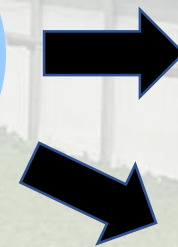
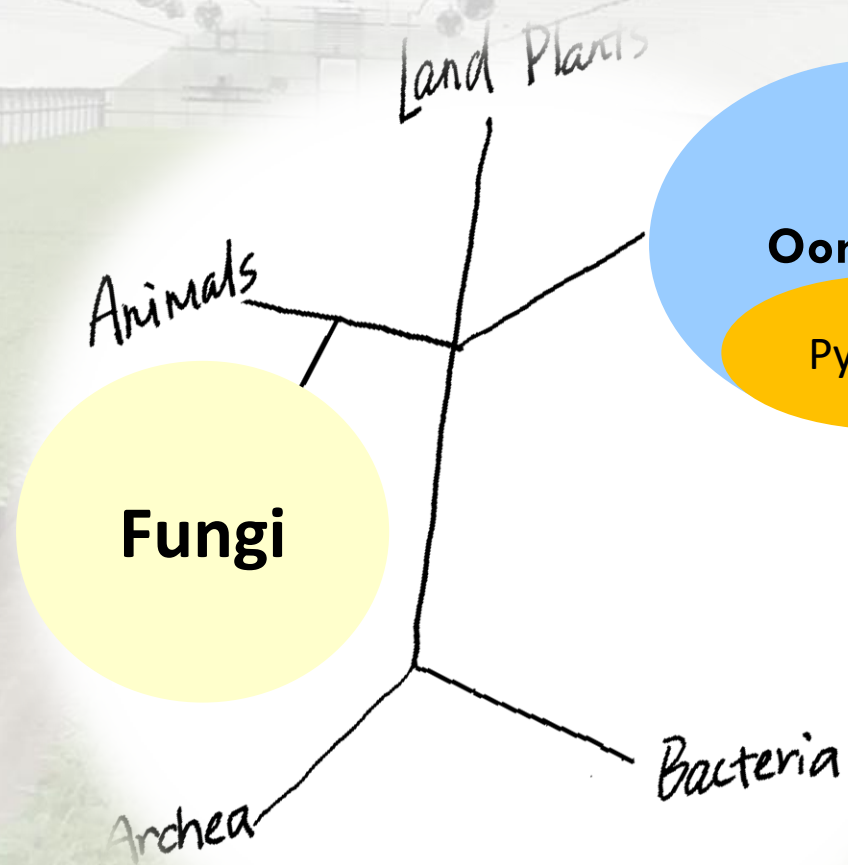




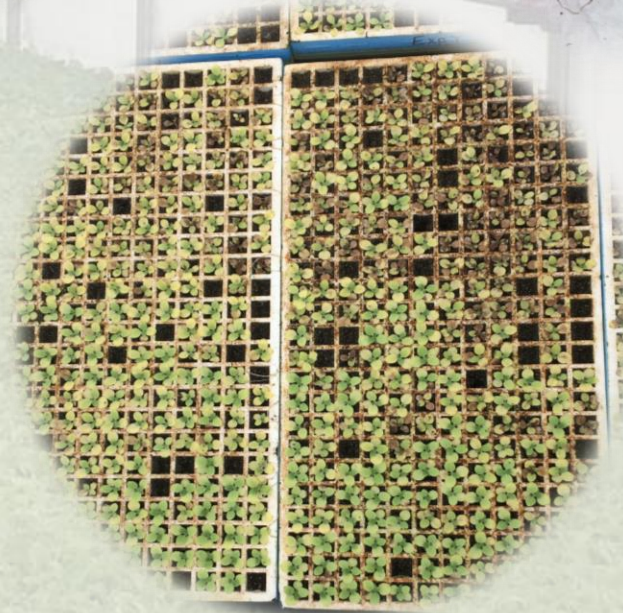
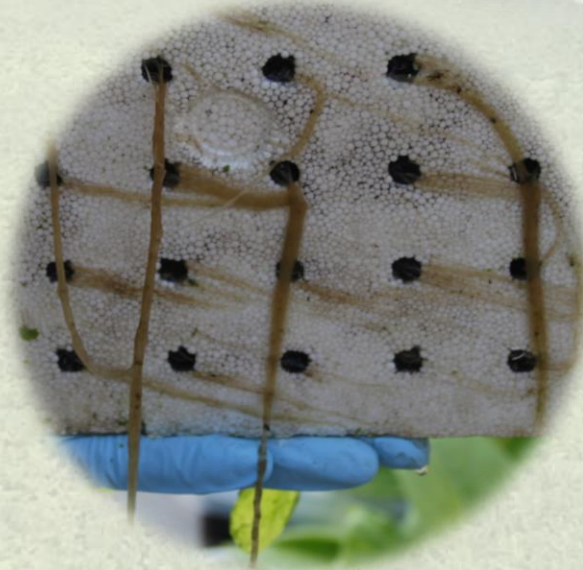
Diversity of *Pythium* species Recovered from 7 Locations Within Tobacco Greenhouses

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PhD student @ Virginia Tech
Southern Piedmont AREC
Jan 22, 2020

What is *Pythium*?



***Pythium* Diseases on Tobacco Seedlings**



Objectives

- Identify the species of *Pythium* living in tobacco greenhouses
- Identify the sources of *Pythium* species
Where were they from? Isolation frequency?
- Identify the effect of individual species on tobacco in greenhouses
Pathogen or not? Variation of symptoms?

2017 *Pythium* Survey

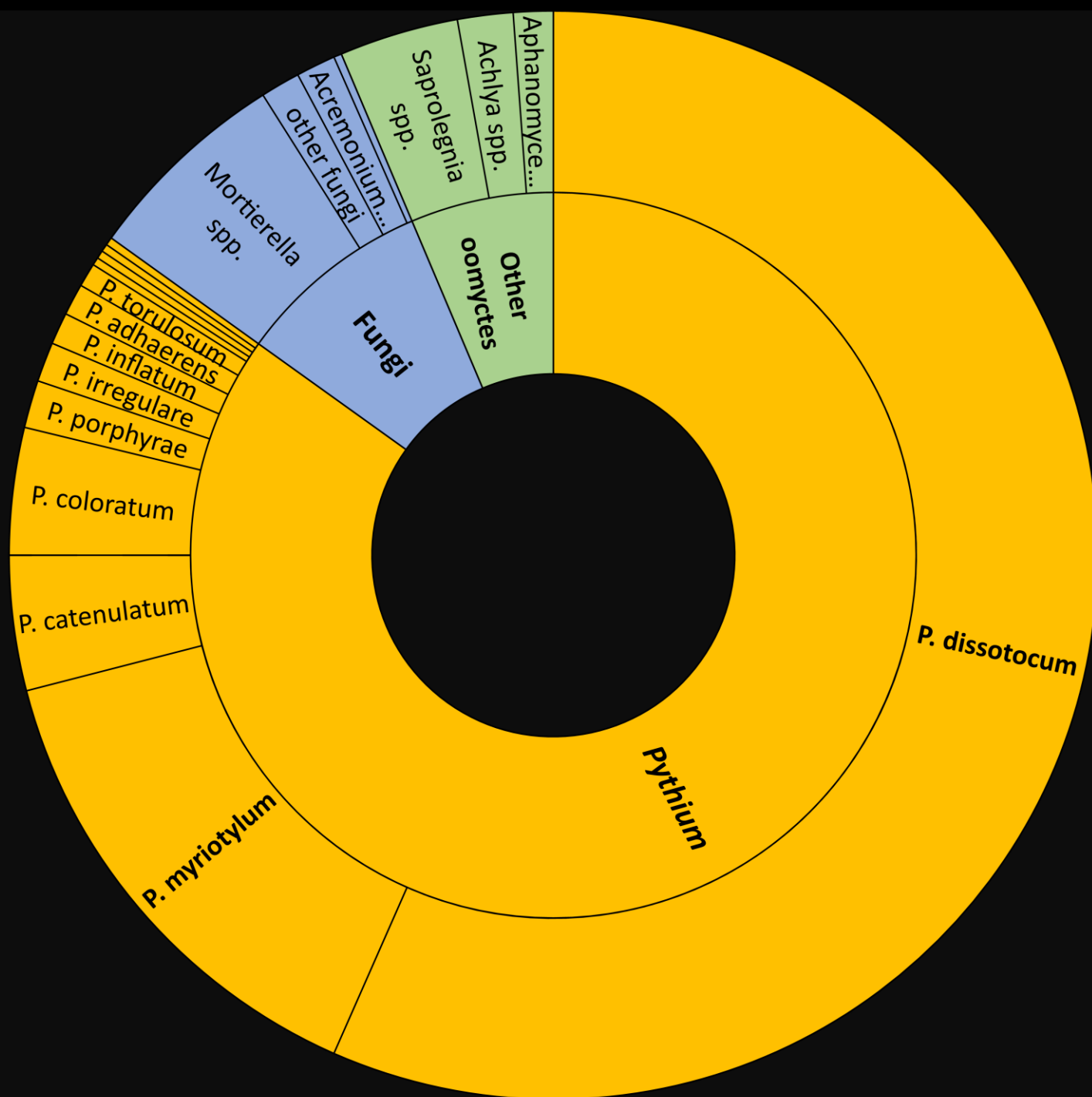
- ✓ 41 greenhouses & 4 states
(PA: 3, MD: 4, GA: 3, VA: 31)
- ✓ 7 environments within tobacco greenhouses
 - ❑ Tobacco seedlings (218 samples)
 - ❑ Bay water (145 samples)
 - ❑ Weeds (29 samples)
 - ❑ Walkway (11 samples)
 - ❑ Growth medium in tray cells (7 samples)
 - ❑ Used float-water trays (7 samples)
 - ❑ Bay surface (7 samples)



Isolate Collection

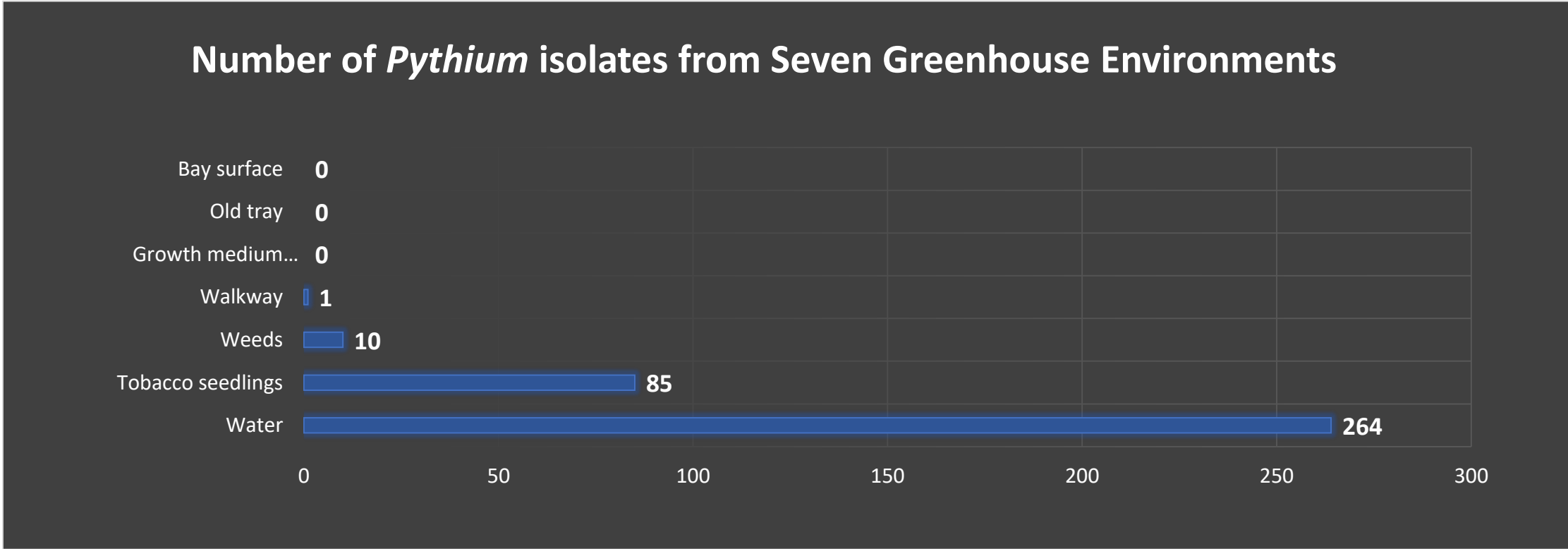
Selective medium V8-PARP:

oomycete (including *Pythium* and other genera) and a few true fungal species.



Groups	Species	Number of isolates
Pythium	<i>P. dissotocum</i>	240
Pythium	<i>P. myriotylum</i>	61
Pythium	<i>P. catenulatum</i>	17
Pythium	<i>P. coloratum</i>	16
Pythium	<i>P. porphyrae</i>	6
Pythium	<i>P. irregulare</i>	5
Pythium	<i>P. adhaerens</i>	4
Pythium	<i>P. inflatum</i>	4
Pythium	<i>P. torulosum</i>	3
Pythium	<i>P. aristosporum</i>	1
Pythium	<i>P. attrantheridium</i>	1
Pythium	<i>P. pectinolyticum</i>	1
Pythium	<i>Pythium</i> sp.	1
Other oomycetes	<i>Saprolegnia</i> spp.	15
Other oomycetes	<i>Achlya</i> spp.	7
Other oomycetes	<i>Aphanomyces</i> spp.	5
Fungi	<i>Mortierella</i> spp.	26
Fungi	<i>Acremonium</i> spp.	5
Fungi	other fungi	5
Fungi	<i>Epicoccum</i> spp.	1

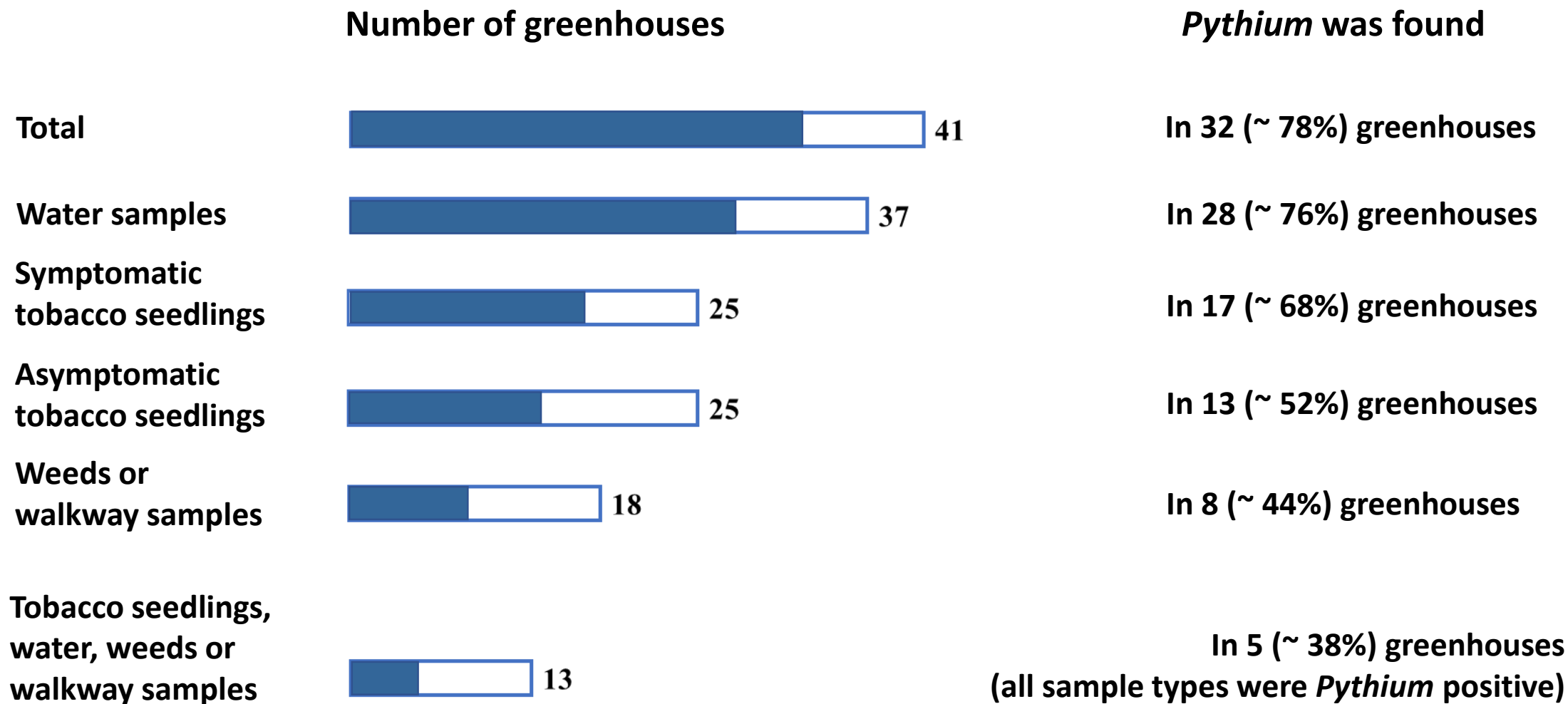
Pythium Sources in Tobacco Greenhouses



Pythium spp. were found in **walkway, weeds, tobacco seedlings** and **bay water** in this survey, which could be potential contamination sources harboring *Pythium* in tobacco greenhouses.

How Common Is *Pythium*?

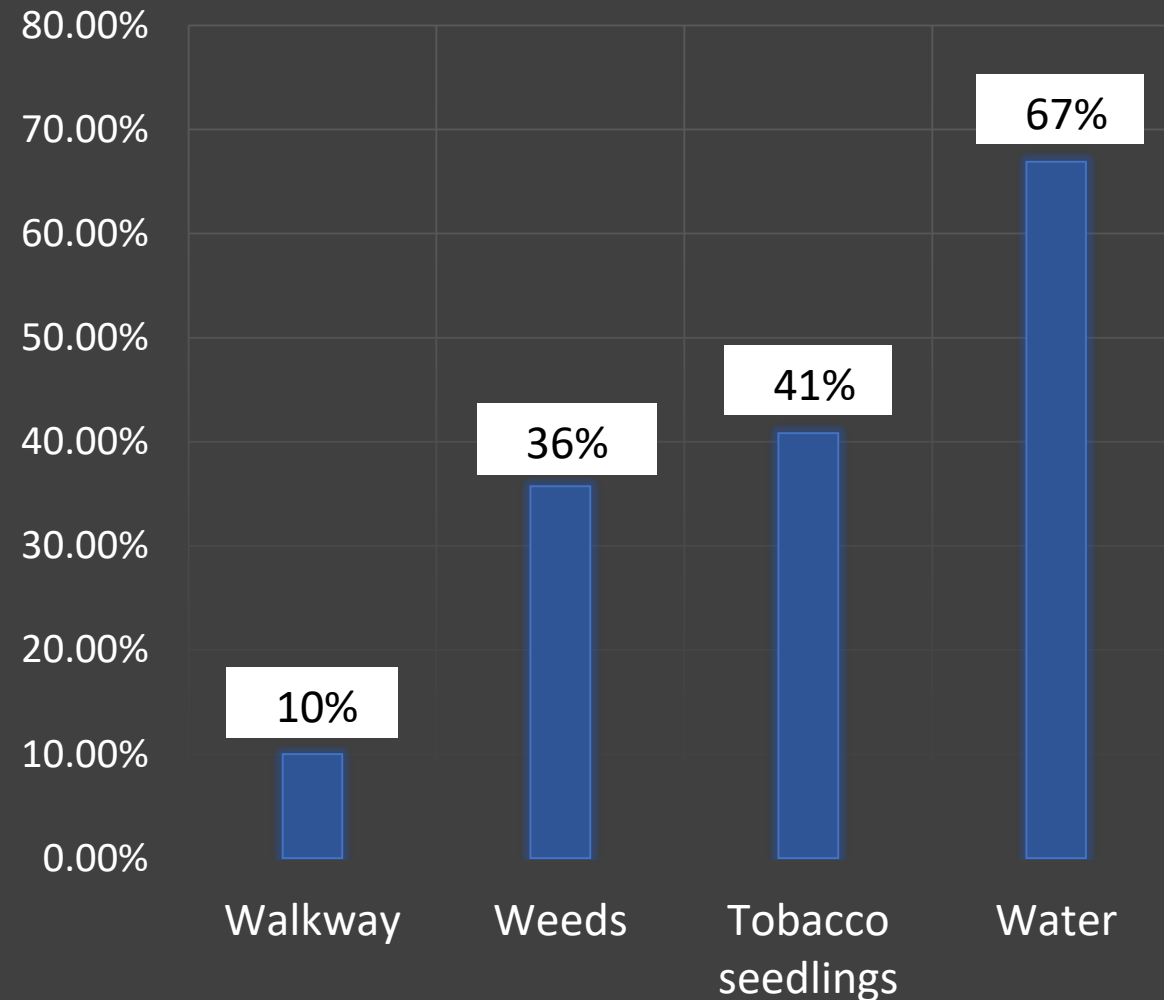
- In greenhouses
- In different environments/ sample types



* Percentage calculation was not based on the total number of surveyed greenhouses (41), but the number of greenhouses where each sample type was collected from.

Isolation Frequency of *Pythium*:

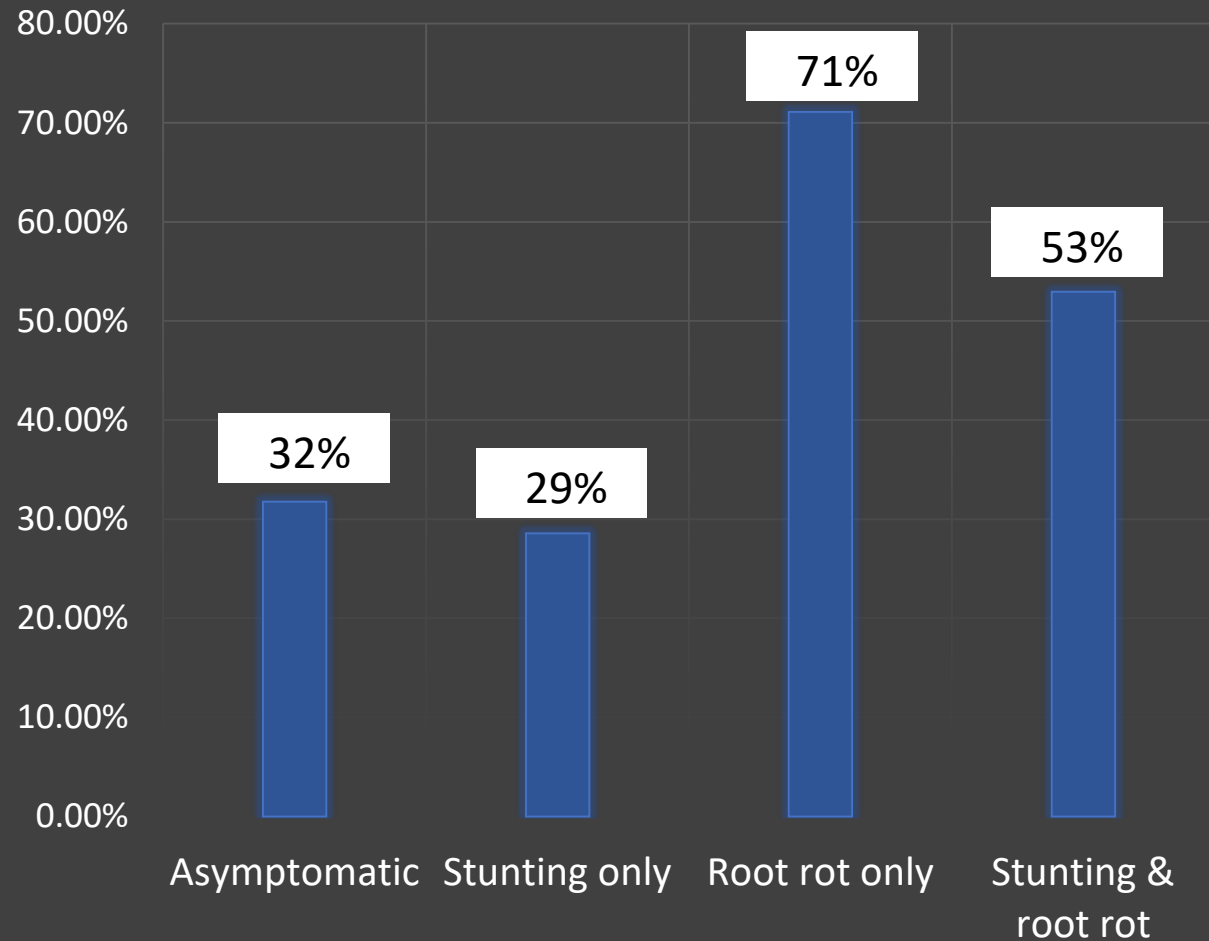
The chance of getting *Pythium* isolates from different sample types



Highest in water, followed by tobacco seedlings

Isolation Frequency of *Pythium*:

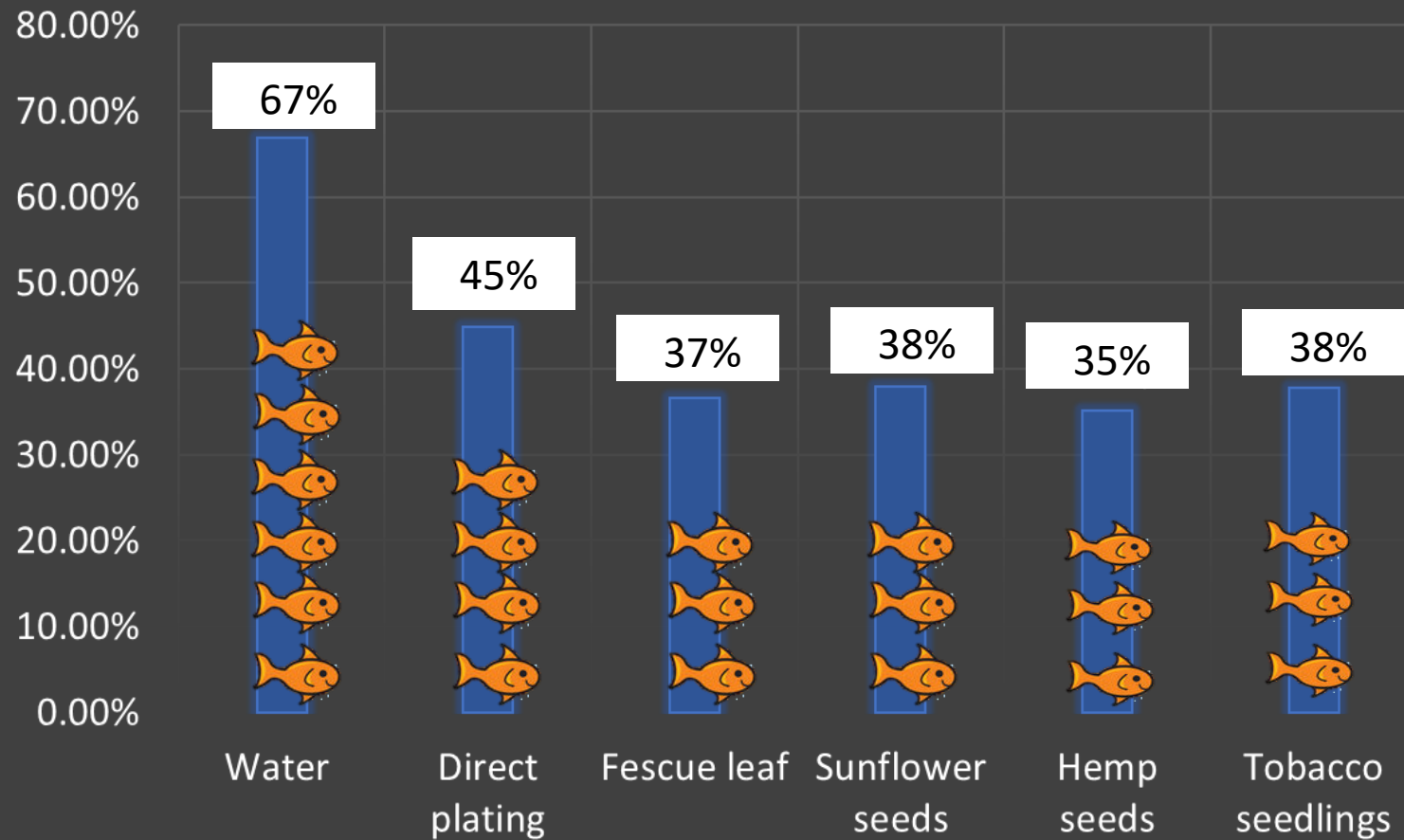
The chance of capturing *Pythium* isolates from different types of tobacco seedlings



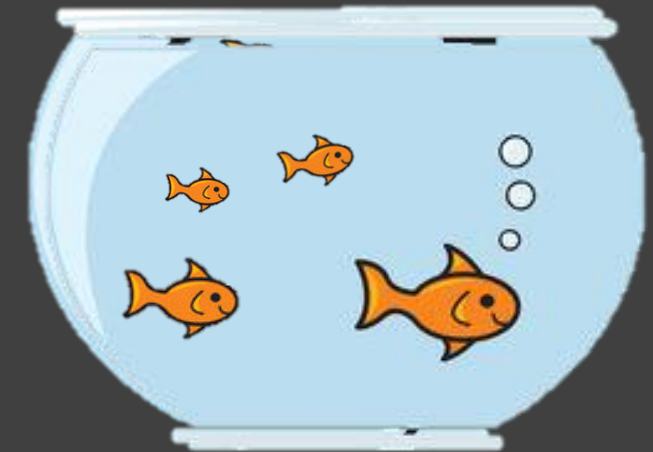
- High in root rot seedlings.
- Even healthy-looking seedlings can have *Pythium* species!

Isolation Frequency of *Pythium*:

The chance of getting *Pythium* isolates from water samples



Direct plating was more efficient, but baiting was necessary

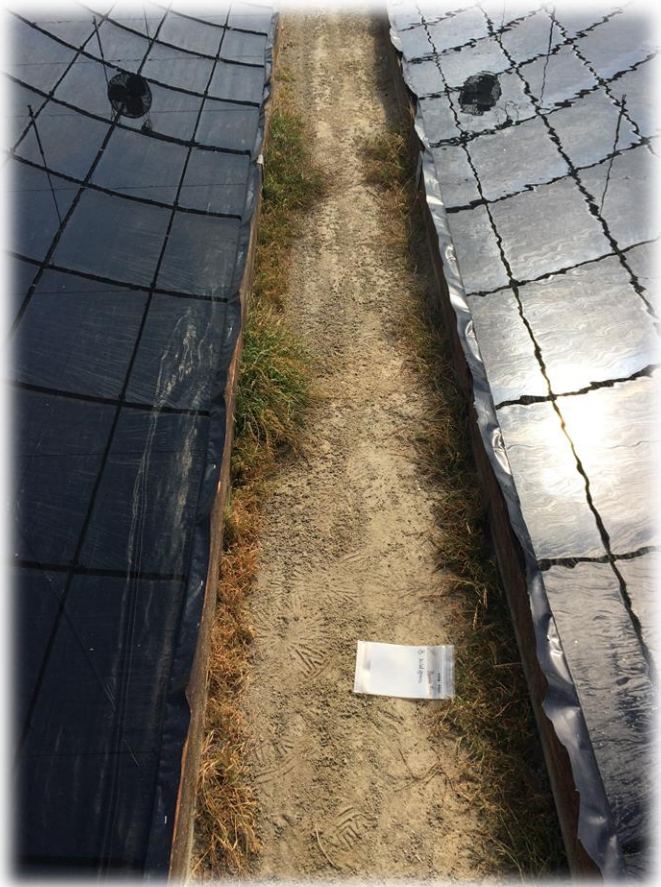


Where Did We Find Individual *Pythium* Species?

Table 1. Presence of *Pythium* spp. at different locations within tobacco greenhouses.

Species	Walkway	Weeds	Tobacco seedlings	Water
<i>P. attrantheridium</i>		X		
<i>P. aristosporum</i>		X		
<i>P. irregulare</i>	X	X		
<i>P. adhaerens</i>				X
<i>P. pectinolyticum</i>				X
<i>P. inflatum</i>				X
<i>P. torulosum</i>				X
<i>P. porphyrae</i>			X	X
<i>P. catenulatum</i>			X	X
<i>P. coloratum</i>			X	X
<i>P. dissotocum</i>			X	X
<i>P. myriotylum</i>		X	X	X

X indicates the presence of individual *Pythium* species.



Finding *Pythium* Species from Seedlings and Water

Table 2. Presence of *Pythium* spp. on different types of tobacco seedling samples and different types of baits in water samples.

Species	tobacco seedlings				water				
	asymptomatic	stunted	root rot	stunted & root rot	direct plating	fescue leaf	sunflower seed	hemp seed	tobacco seedling
<i>P. pectinolyticum</i>									X
<i>P. torulosum</i>						X	X		X
<i>P. inflatum</i>						X	X	X	X
<i>P. adhaerens</i>					X			X	
<i>P. porphyrae</i>		X			X		X		
<i>P. catenulatum</i>		X			X	X	X	X	X
<i>P. coloratum</i>	X	X			X	X	X	X	X
<i>P. dissotocum</i>	X	X	X		X	X	X	X	X
<i>P. myriotylum</i>		X	X	X	X	X	X	X	X

X indicates the presence of individual *Pythium* species.



Pythium = pathogens?



Pathogenicity test in lab:

- Inoculated seeds &
- 10-day-old seedlings

Test in greenhouse:

- Inoculated at seeding
- 10 days after seeding (when seeds germinated)
- 4 weeks after seeding (when water roots emerged)

Pythium groups

Lab & greenhouse results

⇒ *Pythium* spp. do not have the same level of virulence.

strong pathogens

always causing damages



nonpathogens

no effect on tobacco seeds or seedlings



Don't shoot!
I'm just a simple country boy!

weak pathogens

jumped in between.



Lab tests: Disease incidence on 10-day-old seedlings

Table 2. Pathogenicity of representative *Pythium* isolates as indicated by disease incidence after inoculation of newly germinated seedlings of burley tobacco cultivar TN 90 in petri dish trials.

<i>Pythium</i> species	% Disease Incidence			
	Day 7		Day 10	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
<i>P. myriotylum</i>	100 a	90.6 a	100 a	100 a
<i>P. dissotocum</i> -1	100 a	25.0 de	92.5 a	64.1 c
<i>P. coloratum</i>	63.4 b	89.1 a	100 a	98.4 a
<i>P. torulosum</i>	3.1 d	81.3 a	47.5 c	93.8 ab
<i>P. irregulare</i>	29.7 c	46.9 b	10.0 d	93.8 ab
<i>P. catenulatum</i>	15.0 cd	42.2 bc	67.5 b	90.6 ab
<i>P. dissotocum</i>	0 d	7.8 f	75.0 b	9.4 de
<i>P. porphyrae</i>	0 d	28.1 cd	0 d	91.3 b
<i>P. aristosporum</i>	4.7 d	9.4 f	4.7 d	17.2 d
<i>P. attrantheridium</i>	0 d	4.7 f	0 d	4.7 de
<i>P. inflatum</i>	0 d	0 f	0 d	0 e
<i>P. adhaerens</i>	0 d	0 f	0 d	0 e
<i>P. pectinolyticum</i>	0 d	0 f	0 d	0 e
Non-infected control	0 d	0 f	0 d	0 e

Strong Pathogen

Nonpathogen

Lab tests: Disease severity on 10-day-old seedlings

Table 3. Pathogenicity of representative *Pythium* isolates as indicated by disease severity after inoculation of newly germinated seed of burley tobacco cultivar TN 90 in petri dish trials.

Pythium species	% Disease Severity			
	Day 7		Day 10	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
<i>P. myriotylum</i>	67.5 a	65.3 a	93.8 a	90 a
<i>P. coloratum</i>	21.3 b	55.0 ab	58.8 b	81.6 ab
<i>P. dissotocum</i> -1	65.0 a	11.6 cd	48.8 bc	47.8 d
<i>P. irregulare</i>	17.5 b	16.6 c	13.8 d	73.8 bc
<i>P. torulosum</i>	2.5 c	43.8 b	26.3 d	52.5 d
<i>P. catenulatum</i>	12.5 bc	19.1 c	58.8 b	63.8 c
<i>P. porphyrae</i>	0 c	9.4 cde	0 e	48.8 d
<i>P. dissotocum</i>	0 c	2.5 de	45.0 c	4.4 e
<i>P. aristosporum</i>	2.5 c	2.5 de	0 e	4.4 e
<i>P. attrantheridium</i>	0 c	0.9 de	0 e	0.9 e
<i>P. inflatum</i>	0 c	0 e	0 e	0 e
<i>P. adhaerens</i>	0 c	0 e	0 e	0 e
<i>P. pectinolyticum</i>	0 c	0 e	0 e	0 e
Non-infected control	0 c	0 e	0 e	0 e

Strong Pathogen
Nonpathogen

Lab tests: Seed Germination

Table 1. Pathogenicity of representative *Pythium* isolates as indicated by seed germination after inoculation of burley tobacco cultivar TN 90 in petri dish trials.

Pythium species	% Seed Germination			
	Day 7		Day 10	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
<i>P. dissotocum</i> -1	17.2 h	3.1 e	0 e	0 d
<i>P. coloratum</i>	28.1 gh	1.6 e	21.9 d	0 d
<i>P. irregulare</i>	40.6 g	14.1 d	3.1 e	15.6 c
<i>P. myriotylum</i>	67.2 de	95.3 ab	21.9 d	87.5 b
<i>P. aristosporum</i>	42.2 g	95.3 ab	53.1 c	93.8 ab
<i>P. torulosum</i>	46.9 fg	93.8 abc	79.7 b	90.6 ab
<i>P. inflatum</i>	64.1 ef	93.8 abc	85.9 ab	95.3 ab
<i>P. porphyrae</i>	73.4 cde	85.9 c	92.2 ab	90.6 ab
<i>P. adhaerens</i>	75.0 b-e	93.8 abc	90.6 ab	98.4 a
<i>P. dissotocum</i>	84.4 a-d	90.6 bc	92.2 ab	96.9 a
<i>P. attrantheridium</i>	93.8 ab	93.8 abc	92.2 ab	98.4 a
<i>P. pectinolyticum</i>	95.3 a	96.9 ab	95.3 a	96.9 a
<i>P. catenulatum</i>	96.9 a	93.8 abc	100 a	92.2 ab
Non-infected control	87.5 abc	100 a	95.3 ab	98.4 a

Strong Pathogen
Nonpathogen

Table 3. The effects of float water inoculation with *Pythium* species at seeding on TN LC 90 tobacco seed germination and seedling health.

Treatment	Root vigor (%)	Root rot incidence (%)	Root rot severity (%)	Spore count (per 2mm root tissue)	Root weight (g)	Root length (cm)	Seedling vigor (%)	Germination percentage (%)	Mortality percentage (%)
Uninoculated Control	99.37 a	0 a	0 a	0 a	19.45 a	20.43 a	97.50 a	95.54 ab	0 a
<i>Pythium inflatum</i>	91.41 ab	0 a	0 a	0 a	17.90 ab	19.26 a	91.63 ab	96.26 ab	0 a
<i>Pythium porphyrae</i>	88.05 abcd	0 a	0 a	0 ab	17.63 ab	18.53 a	83.81 ab	98.99 a	0 a
<i>Pythium aristosporum</i>	89.03 abc	14.64 ab	5.00 ab	1 abc	19.40 a	21.23 a	94.34 ab	94.23 abc	0 a
<i>Pythium dissotocum</i>	70.93 bcd	50.00 abc	30.00 ab	7 cd	12.65 bc	17.08 a	89.45 ab	81.95 bcd	0.01 a
<i>Pythium irregulare</i>	57.14 cde	65.81 bc	31.00 ab	14 d	12.23 bc	20.04 a	64.41 ab	98.99 a	6.62 a
<i>Pythium torulosum</i>	55.62 cde	50.00 abc	20.00 ab	3 abcd	11.43 c	19.48 a	92.70 ab	94.23 abc	0.25 a
<i>Pythium coloratum</i>	29.41 e	93.30 c	38.00 b	69 ef	11.05 c	17.86 a	58.49 ab	73.72 cde	11.10 ab
<i>Pythium dissotocum-1</i>	53.82 de	85.36 bc	35.00 b	20 def	10.28 c	19.62 a	40.01 b	68.15 de	0.89 a
<i>Pythium myriotylum</i>	0.14 f	100.00 c	100.00 c	173 f	1.23 d	7.88 b	0.14 c	53.49 e	44.30 b

Treatment means followed by the same letter(s) not significantly different. Percentage data Arcsine transformed before ANOVA with Fisher's LSD or Wilcoxon ($\alpha = 0.05$). Root measurements included the entire root of individual seedlings. Root rot incidence is the percentage of tobacco seedlings with symptomatic water roots among all seedlings in a tray. Seedling vigor reflects the size and greenness of the entire seedling. Root rot severity is the percentage of water roots affected by root rot on 5 individual seedlings in a tray. Mortality rate shows the percentage of dead seedlings among germinated plants in a tray.

Table 4. The effects of float water inoculation with *Pythium* species at 10 days after seeding on TN LC 90 tobacco seedling health.

Treatment	Root vigor (%)	Root rot incidence (%)	Root rot severity (%)	Spore count (per 2mm root tissue)	Root weight (g)	Root length (cm)	Seedling vigor (%)	Mortality percentage (%)
Uninoculated control	98.75 a	0 a	0 a	0 a	22.43 ab	23.81 a	100.00 a	0 a
<i>Pythium porphyrae</i>	97.50 a	0 a	0 a	0 a	28.10 a	26.06 ab	93.75 ab	0.01 ab
<i>Pythium aristosporum</i>	96.25 a	50.00 ab	23.00 ab	0 a	23.88 ab	24.00 ab	100.00 a	0 a
<i>Pythium catenulatum</i>	82.50 a	25.00 ab	25.00 ab	12 b	23.13 ab	24.50 ab	100.00 a	0 a
<i>Pythium irregulare</i>	76.25 ab	50.00 ab	44.00 ab	32 bc	14.93 bc	23.94 a	93.75 ab	0.01 ab
<i>Pythium torulosum</i>	66.25 abc	50.00 ab	40.00 ab	32 bc	17.10 bc	24.56 ab	97.50 ab	0 a
<i>Pythium dissotocum-1</i>	46.25 bc	95.00 c	57.00 bc	12 b	11.53 cd	20.38 b	63.75 ab	2.93 bc
<i>Pythium coloratum</i>	38.75 cd	75.00 bc	65.00 bc	20 b	10.98 cd	22.44 ab	60.00 bc	7.74 cd
<i>Pythium myriotylum</i>	6.25 d	100.00 c	100.00 c	84 c	1.75 d	13.97 c	25.00 c	40.70 d

Treatment means followed by the same letter(s) were not significantly different. Percentage data were Arcsine transformed before analysis. Data analyzed using ANOVA with Fisher's LSD or Wilcoxon ($\alpha = 0.05$). All root measurements were based on the entire root ball of individual seedlings. Root rot incidence reflects the percentage of tobacco seedlings with symptomatic water roots among all the seedlings in a tray. Root rot severity shows the percentage of water roots affected by root rot on 5 individual seedlings in a tray. Mortality rate is the percentage of dead seedlings among germinated plants in a tray.

Greenhouse

Nonpathogen
Strong Pathogen

Where do *Pythium* pathogens stay?

Table 1. Presence of *Pythium* spp. at different locations within tobacco greenhouses.

Species	Walkway	Weeds	Tobacco seedlings	Water
<i>P. attrantheridium</i>		X		
<i>P. aristosporum</i>		X		
<i>P. irregulare</i>	X	X		
<i>P. adhaerens</i>				X
<i>P. pectinolyticum</i>				X
<i>P. inflatum</i>				X
<i>P. torulosum</i>				X
<i>P. porphyrae</i>			X	X
<i>P. catenulatum</i>			X	X
<i>P. coloratum</i>			X	X
<i>P. dissotocum</i> *			X	X
<i>P. myriotylum</i>		X	X	X

X indicates the presence of individual *Pythium* species.

Nonpathogen

Strong Pathogen

* Subgroup:

Virulence variation within
P. dissotocum

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TWC2020(49) - Document not peer-reviewed

Summaries

Pathogenicity results

- Strong pathogens: *P. myriotylum*, *P. dissotocum* & *P. coloratum*

Most aggressive, also in NC & Zimbabwe studies
Bean, Corn, Lettuce & Grass. Roots, seed, soil & water

- Weak Pathogens: *P. aristosporum*, *P. irregulare*, *P. torulosum*, *P. porphyrae* & *P. catenulatum*

No above-tray symptoms Diverse hosts. Soil, roots, plant debris & water

- Nonpathogens: *P. attrantheridium*, *P. adhaerens*, *P. inflatum* & *P. pectinolyticum*

Diverse hosts, mostly grass.
Soil & roots



Acknowledgement

- **Altria, JTI, PMI & Virginia Tobacco Board**
- **Spencer, Noah, Laura, Molly, Tyler, Reed Lab, and other folks @ SPAREC**
- **Elizabeth Bush, Mary Ann Hansen, Baudoin Lab and other folks on campus**

Questions

- What is *Pythium*?
- **2017 *Pythium* survey**
 - Sampling plan
 - Isolate collection
 - What were found?
 - Where were they?
 - How common are they?
 - Source of individual species
- Concerns
- **Are they all pathogens?**
 - Do they cause the same symptoms?
 - Groups and subgroups
- Where do pathogenic species stay?
- Summaries