Soybean Rust Spore Identification

An Immunofluorescence Assay

Development Team

- Ohio State University - *Assay development*
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- USDA ARS - *Antigen prep, spores*
  - Doug Luster
  - Reid Frederick
- US Navy Medical Research Center - *Antibody development*
  - Jill Czarnecki

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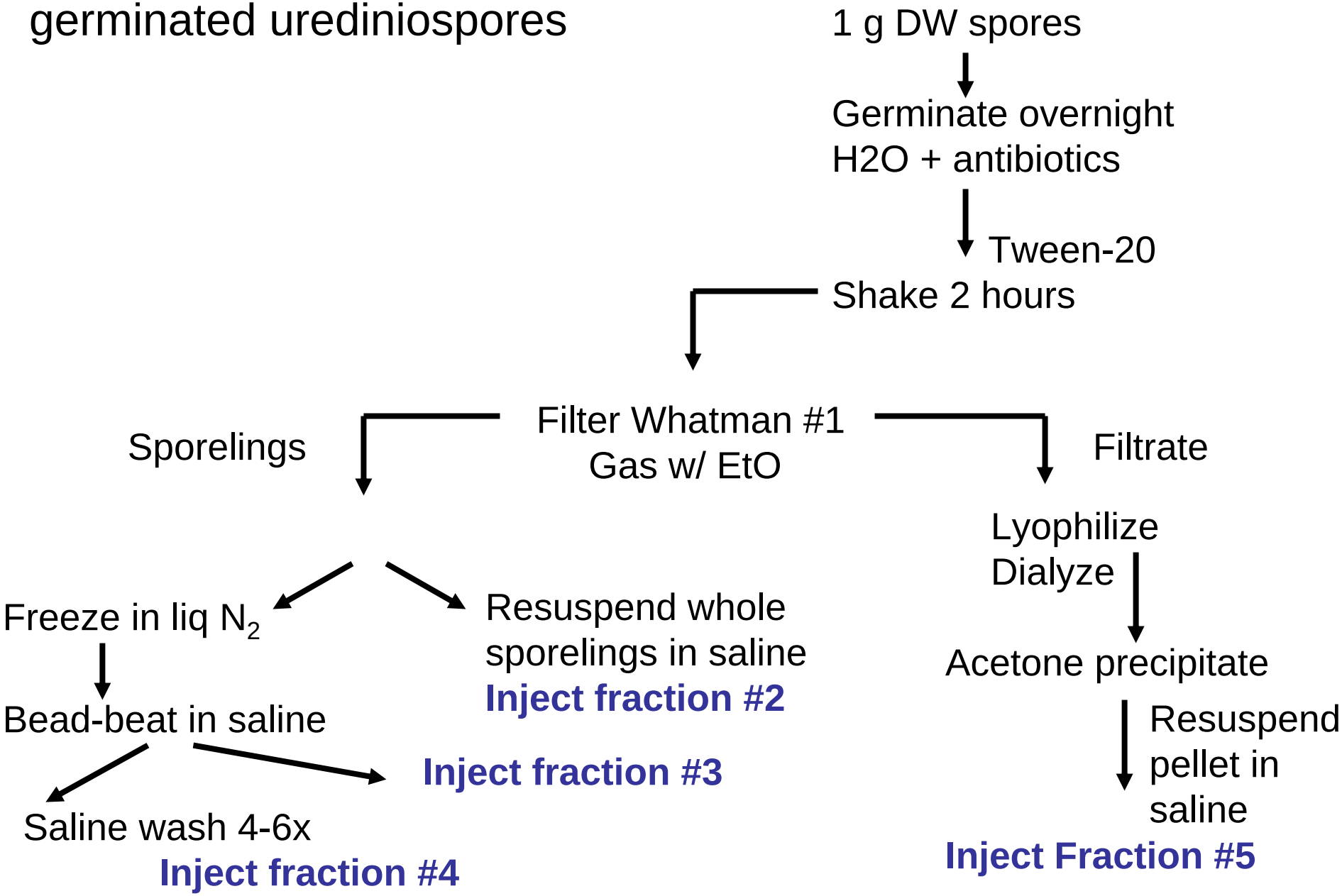
Identification of *Phakopsora* Spores

- Spore monitoring is part of an early warning system
- Spores can be trapped in various air samplers
- *Phakopsora* spores must be identified amidst other spores, etc. in traps
- Immunofluorescence is rapid, simple and definitive

Polyclonal Antibody Development

ID	*P. pachyrhizi* Antigen Preparation
1A	Ungerminated urediniospores, 20 mg DW/ml
1B	Ungerminated urediniospores, 10 mg DW/ml
2	Intact germ. urediniospores, 1.0 mg protein/ml
3	Pulverized germ. urediniospores, 1.0 mg protein/ml
4	Intact germ. spore walls, 1.25 mg protein/ml
5	Extracellular spore proteins. 0.2 mg/ml

Antigen prep from *P. pachyrhizi* germinated urediniospores



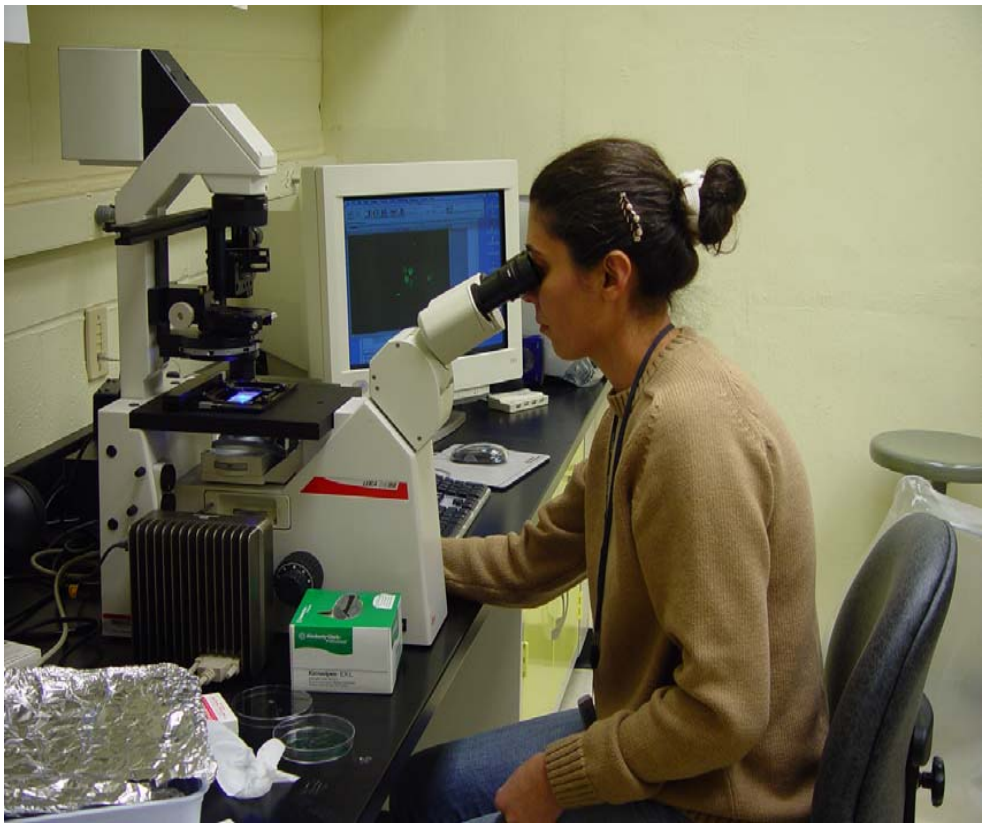
Specificity of *P. pachyrhizi* Polyclonal Abs

Extract	**Reactivity in ELISA**				
	1 A	**1B**	**2**	**3**	**4**
P. pachyrhizi spore extract	+	+	+	+	+
P. meibomiae spore extract	+	+	-	-	-
P. syringae pv. *syringae* & *phaseolicola*, *X. campestris* pv. *glycines*, *C. michiganensis* subsp. *insidiosus*, *B. cinerea*, *C. acutatum*, *C. gleosporiodes*	-	-	-	-	-
Ustilago tritici	-	-	-	-	-
Rhizoctonia solani	-	-	-	+	+
Puccinia graminis f. sp. *tritici*	-	-	-	-	+
Diseased soybean tissue: bacterial pustule, frogeye leaf spot, bacterial spot, Septoria leaf spot and powdery mildew	-	-	-	-	-
Symptomless soybean tissue: 183 cultivars	-	-	-	-	-

SBR Immunofluorescence Assay

- Demonstrate specific antibody binding to spores
  - Worked with antibodies 1A and 2
- Adapt to slide capture
- Optimize indirect immunofluorescence assay
- Test using field samples

Epifluorescence Microscopy



Dr. Fulya Baysal-Tustas

- Leica DM IRB microscope
- Optronics Magnafire camera/digital imaging system
- Filter

  I3 Blue, Excitation= 450-490, Emission=515
- Exposure: 1.037 sec

Immunoassay Parameters

Parameter	Variable	Optimal result
1° Antibody	Incubation time (1, 2, 3 hr)	2 hr incubation
1° Antibody	Incubation temp: 22°C/37 ° C	22°C
Blocker	2% BSA + 5% goat serum	No blocking step
Washing step	PBS-Tween or water	PBS-Tween
2° Antibody	Incubation time (1, 2, 3 hr)	1 hr incubation
2° Antibody	Incubation temp: 22°C/37 ° C	22°C

Immunofluorescence Assay

Apply 1° Antibody 1A or 2 (1:500) in PBS-Tween

↓

Incubate 2 hr, 22C

↓

Wash 6X, PBS-Tween

↓

Apply 2° Antibody (Alexa Fluor 488, 1:500) in PBS-Tween

↓

Incubate 1 hr, 22 C in dark

↓

Wash 6X, PBS-Tween

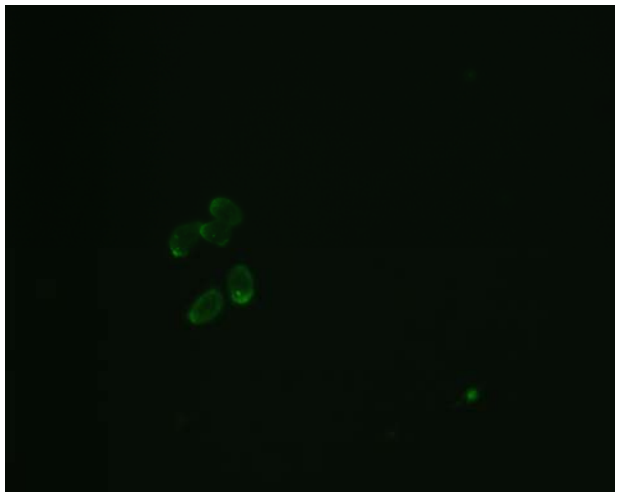
Secondary Antibody

- Alexa Fluor 488 goat anti-rabbit IgG (H+L), 2 mg/ml
  - Excited at 488 nm
  - Maximal emission at 519 nm
  - Brighter and more photostable than FITC

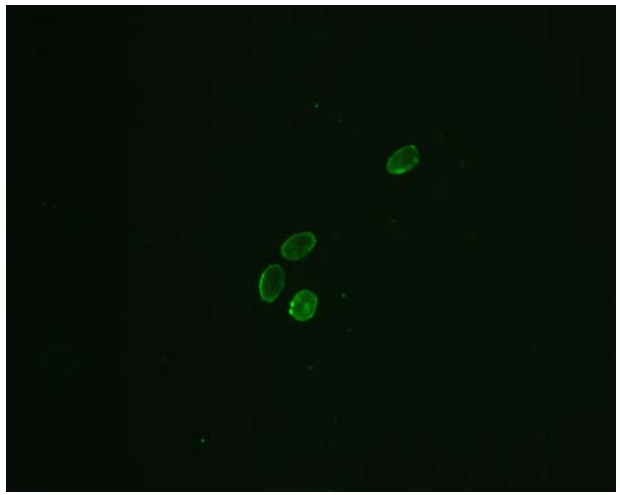
Phakopsora pachyrhizi Immunofluorescence Detection

AB 1A

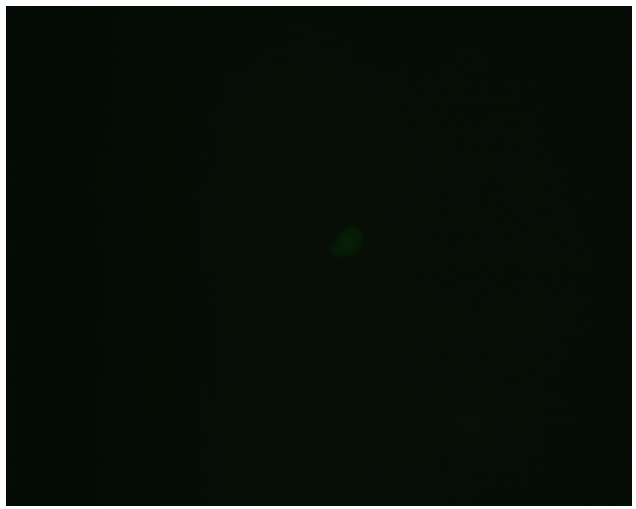
AB 2



1:500
1º AB;
1:500
2º AB



No 1º
AB;
1:500
2º AB

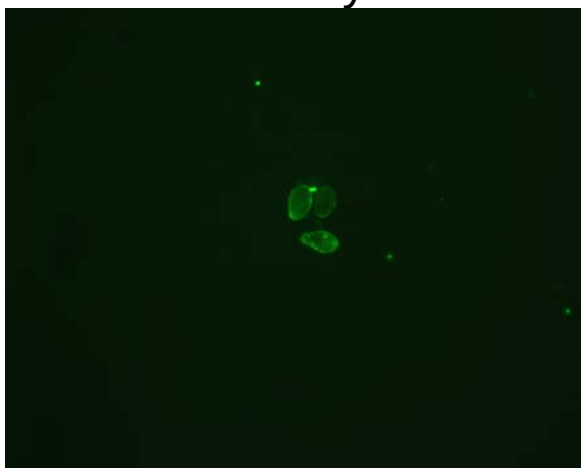


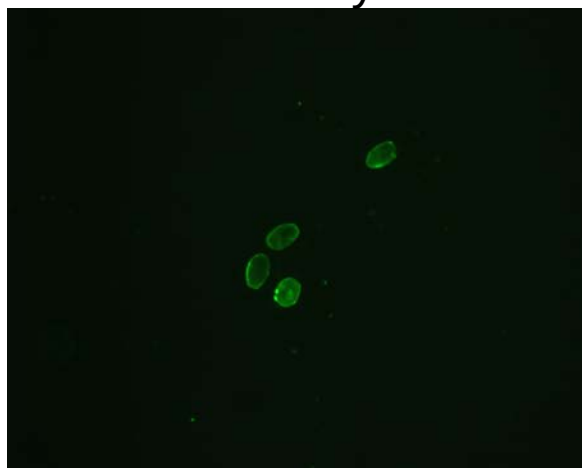
Reaction with *P. pachyrhizi* and *P. meibomiae*

1° Antibody 1A

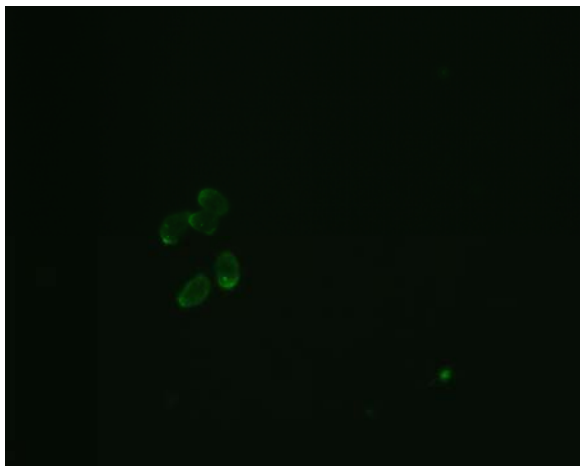
1° Antibody 2

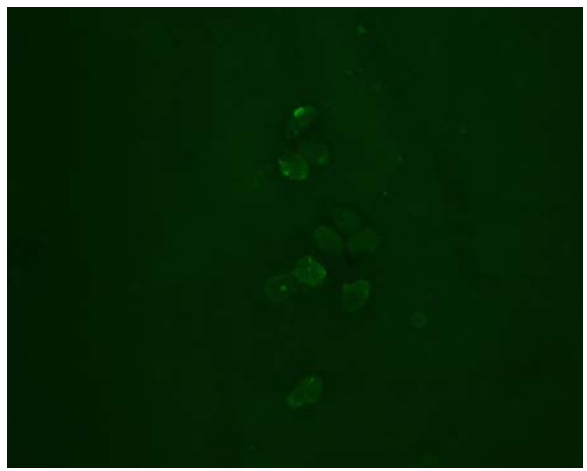
P. pacchryzi





P. meibomiae

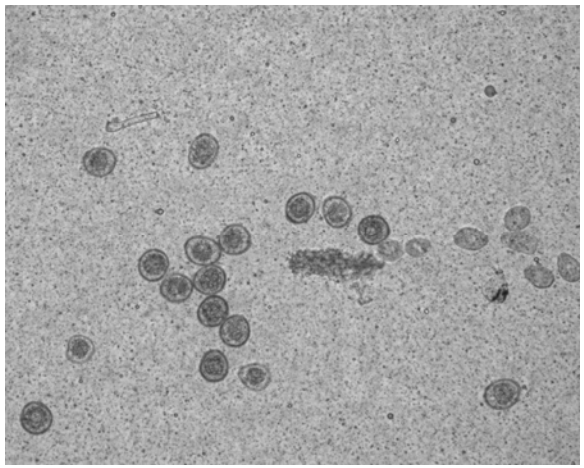


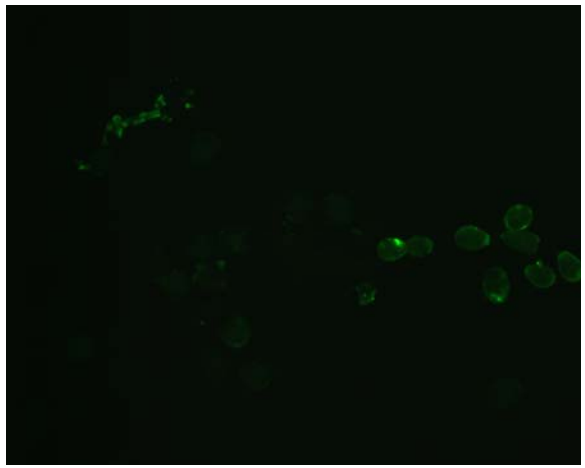


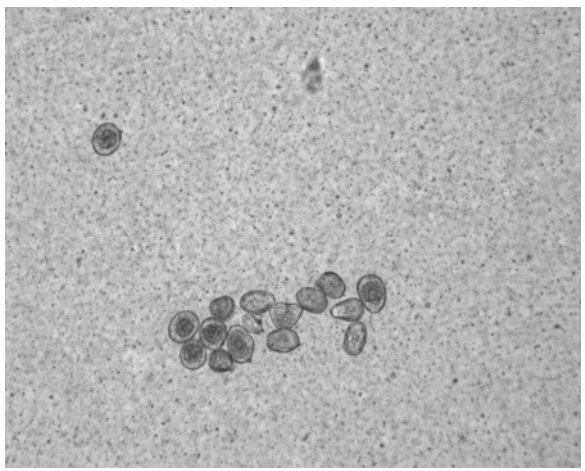
1:500 1:500

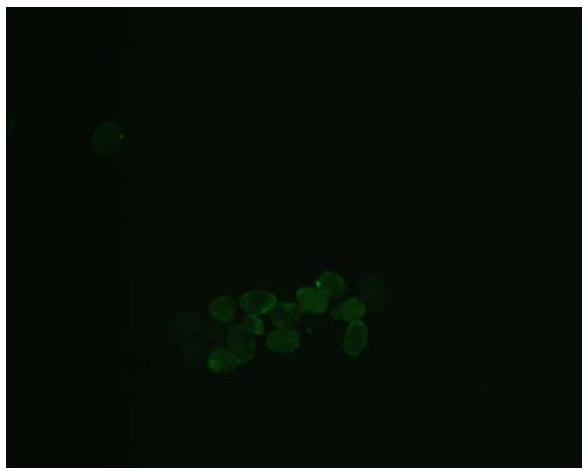
Antibody Specificity

P. pachyrhizi and P. graminis with 1° AB 1A (1:500 1:500)





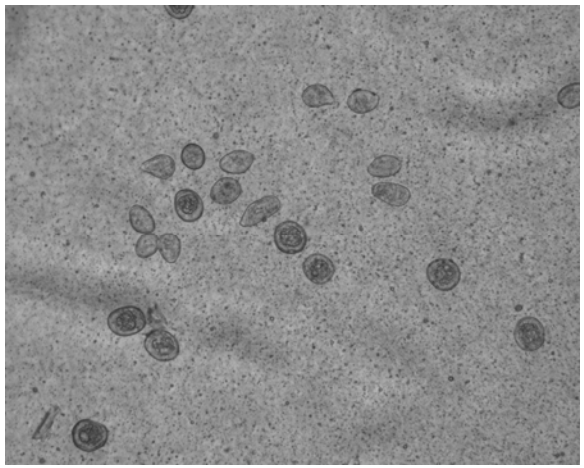


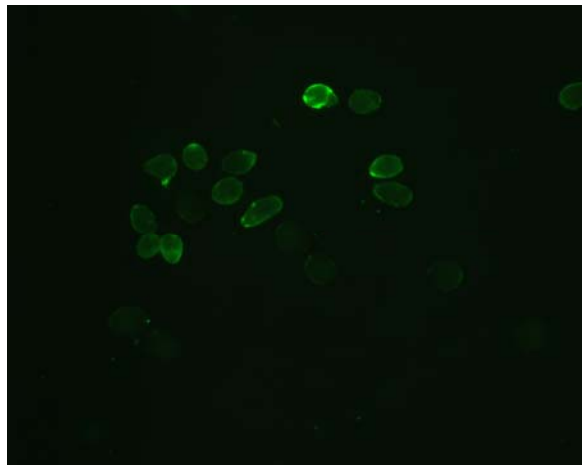


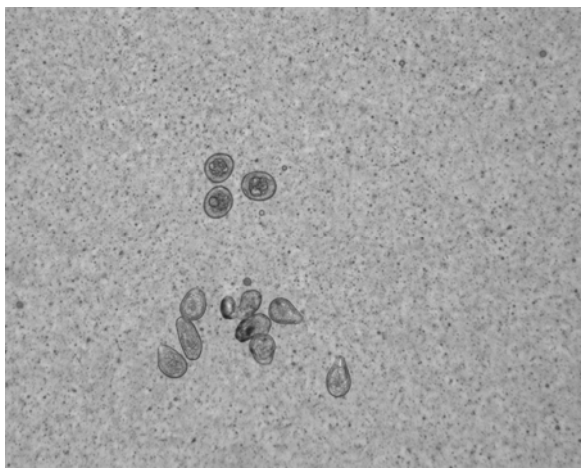
P. meibomiae and P. graminis with 1° AB 1 (1:500 1:500)

Antibody Specificity

P. pachyrhizi and P. graminis with 1° AB 2 (1:500 1:500)







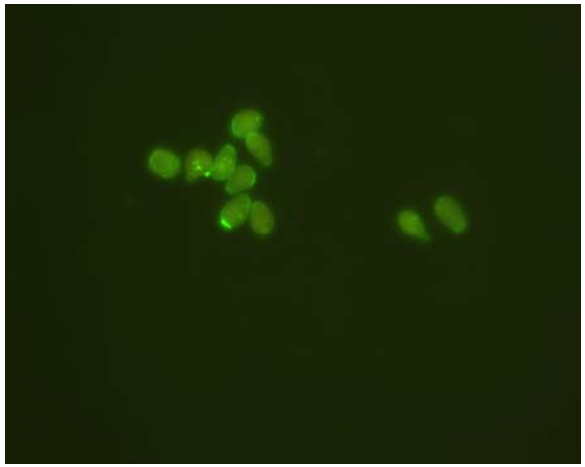
P. meibomiae and P. graminis with 1° AB 2 (1:500 1:500)

Assay Adaptation - Spore Traps

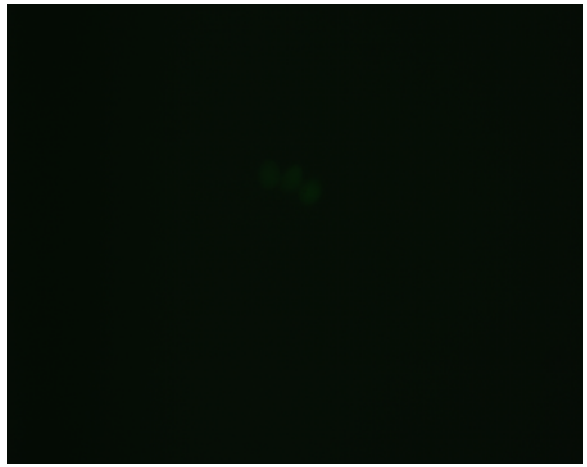
- Original assay parameters worked out using Pp spores in microfuge tubes
- Spore traps capture urediniospores on vaseline-coated slides

Double-stick tape

P. pachyrhizi with 1° AB 1A

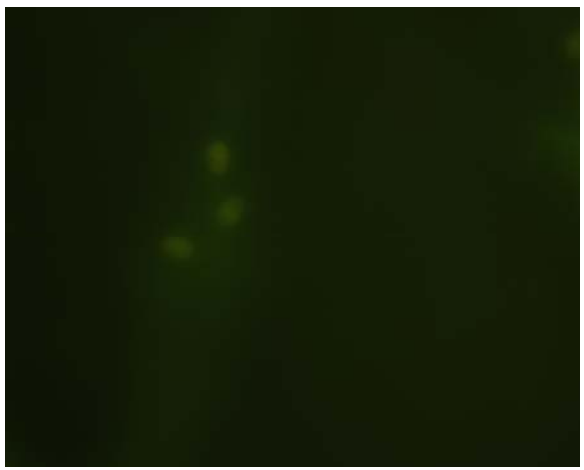


P. pachyrhizi without 1° AB



Vaseline

P. pachyrhizi with 1° AB 1A



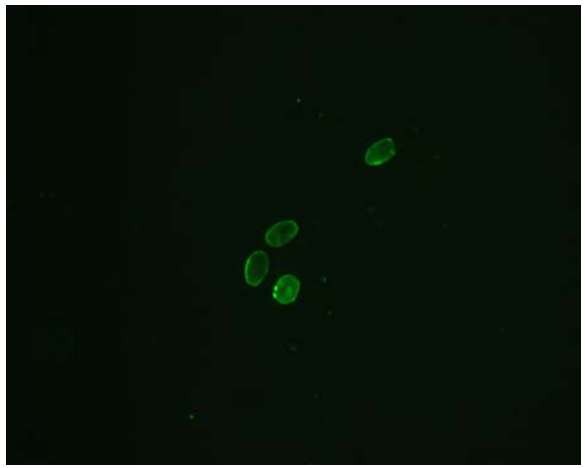
P. pachyrhizi without 1° AB

“Stickiness” of *Phakopsora* Spores to Slides

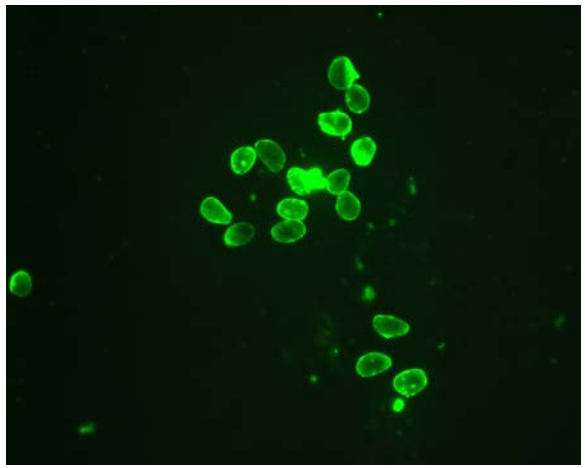
Vaseline			DoubleŠstick tape		
Slide	Initial # spores	% lost spores	Slide	Initial # spores	% lost spores
A	155	14.4	A	144	4.16
B	125	26.7	B	118	3.39
C	131	26.1	C	134	7.46
D	136	26.4	D	128	4.69
E	34	17.7	E	140	3.57
Mean		**22.2**	**Mean**		**4.7**

SBR Immunofluorescence Assay Optimization

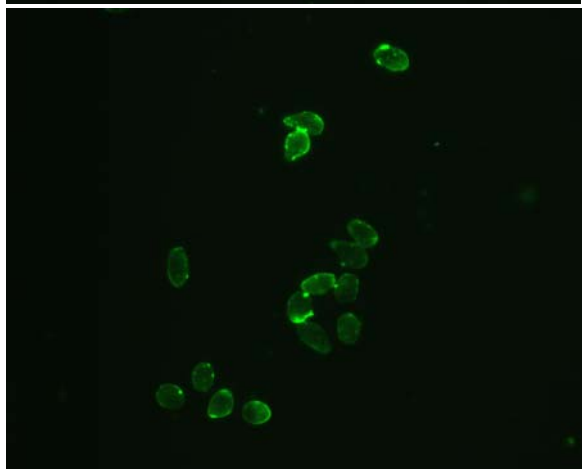
1:500 1:500



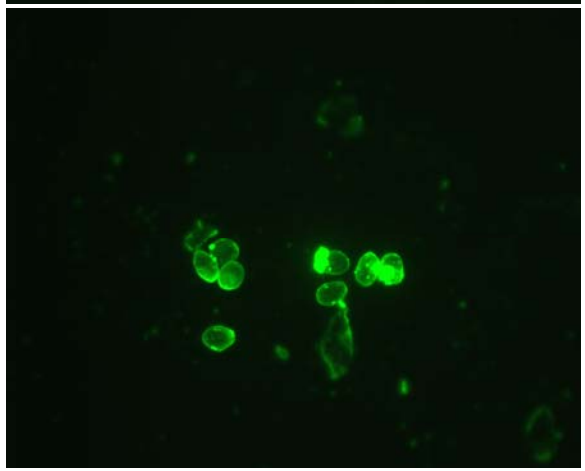
1:250 1:250



1° AB 2

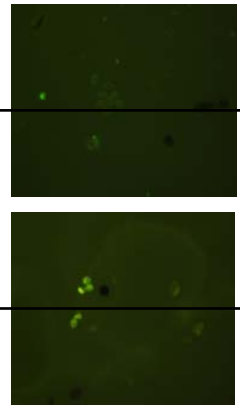


1:500 1:100



1:100 1:100

Field Test Results

Slides name	Date	Slide type	1° Antibody	2° Antibody	SBR- like spores (fluorescent)	
NW (003)	No date	Double sided tape	1A	Alexa Fluor 488	+	
NW (004)	09.05.2006-09.11.2006	Double sided tape	1A	Alexa Fluor 488	+	
NW (6)	09.05.2006	Double sided tape	1A	Alexa Fluor 488	_	
NW 1 (Wood County)	8.21.2006-8.23.2006	Double sided tape	1A	Alexa Fluor 488	_	

Next steps

- Antisera will be available to interested parties
- Complete monoclonal antisera
  - Recombinant secreted proteins (ARS)
  - Polyclonal for the secreted proteins is quite active
- Develop ELISA formats – other formats
- Develop and assess field sampling protocols

Acknowledgments

- This project was funded by soybean check-off dollars from the Iowa Soybean Promotion Board and United Soybean Board

- Antiserum and protocol can be obtained by contacting Anne Dorrance:

dorrance.1@osu.edu