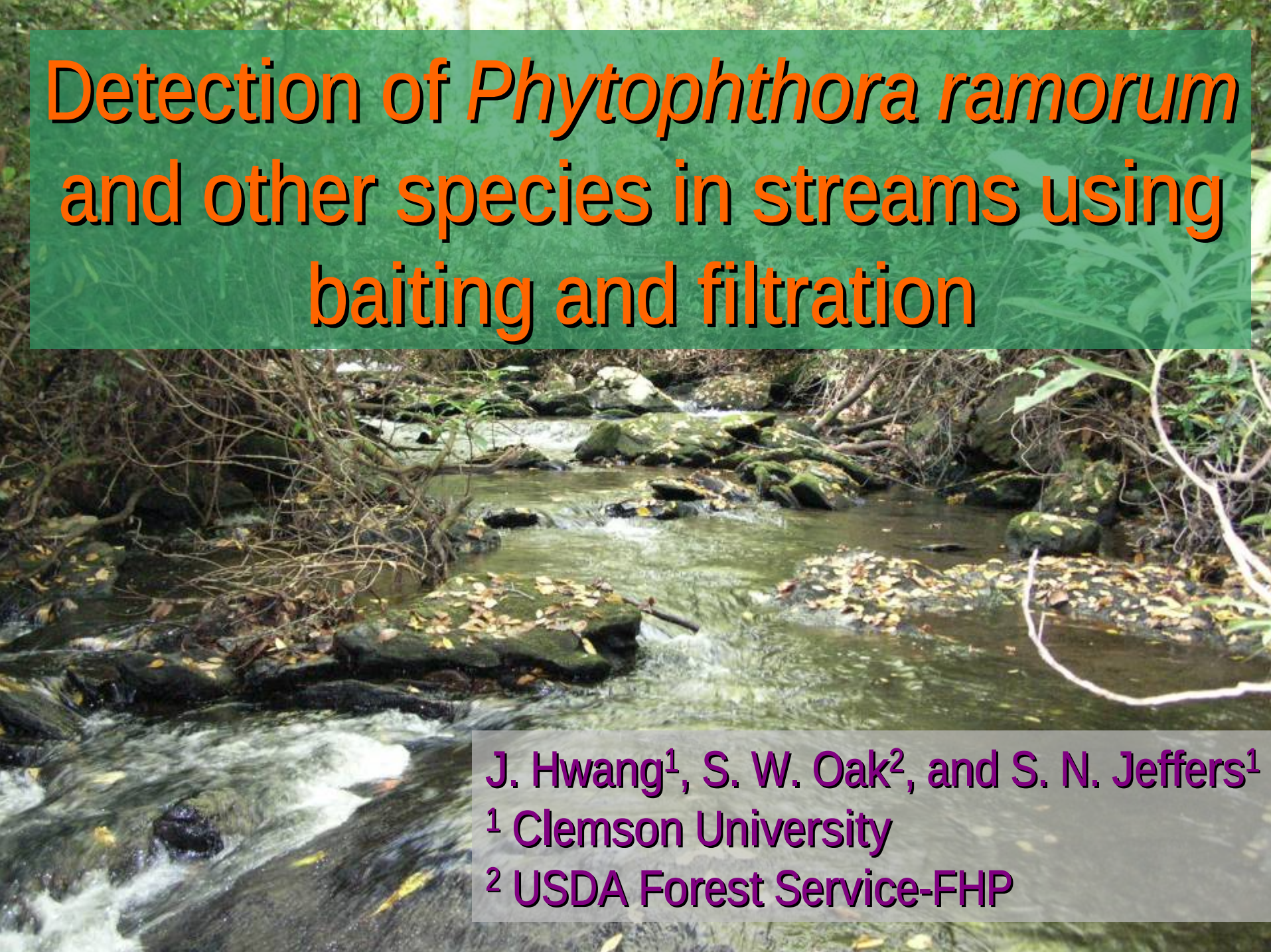
Detection of *Phytophthora ramorum* and other species in streams using baiting and filtration



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Objectives

1. Monitor occurrence and distribution of *Phytophthora* spp. in natural ecosystems

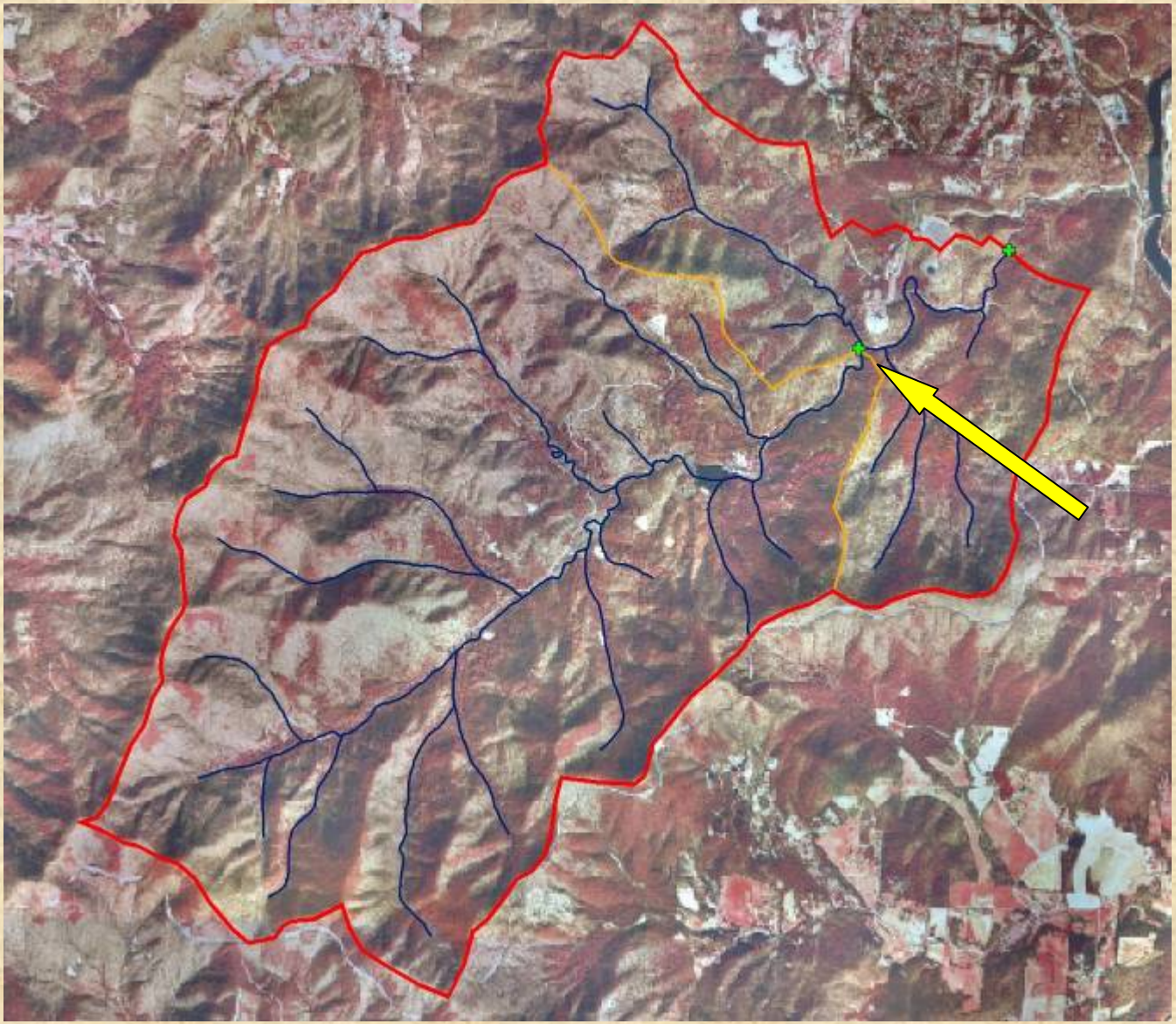
2. Evaluate detection methods (baiting and filtering) for isolating *Phytophthora* spp. in forest streams

3. Recommend a protocol for detecting *Phytophthora ramorum* in forest streams for the USDA Forest Service National Survey

Experimental Design

- Pisgah National Forest in western NC: high risk area for SOD—based on host plant species, moderate climate, nursery businesses
- 5 independent streams in 3 watersheds sampled monthly: April 2005 - March 2006
- Three different detection techniques were compared:
  - non-wounded rhododendron leaves
    - long exposure for 1-2 wk
  - wounded rhododendron leaves
    - short exposure for 72 hr
  - filtration—within 12 hr of collection

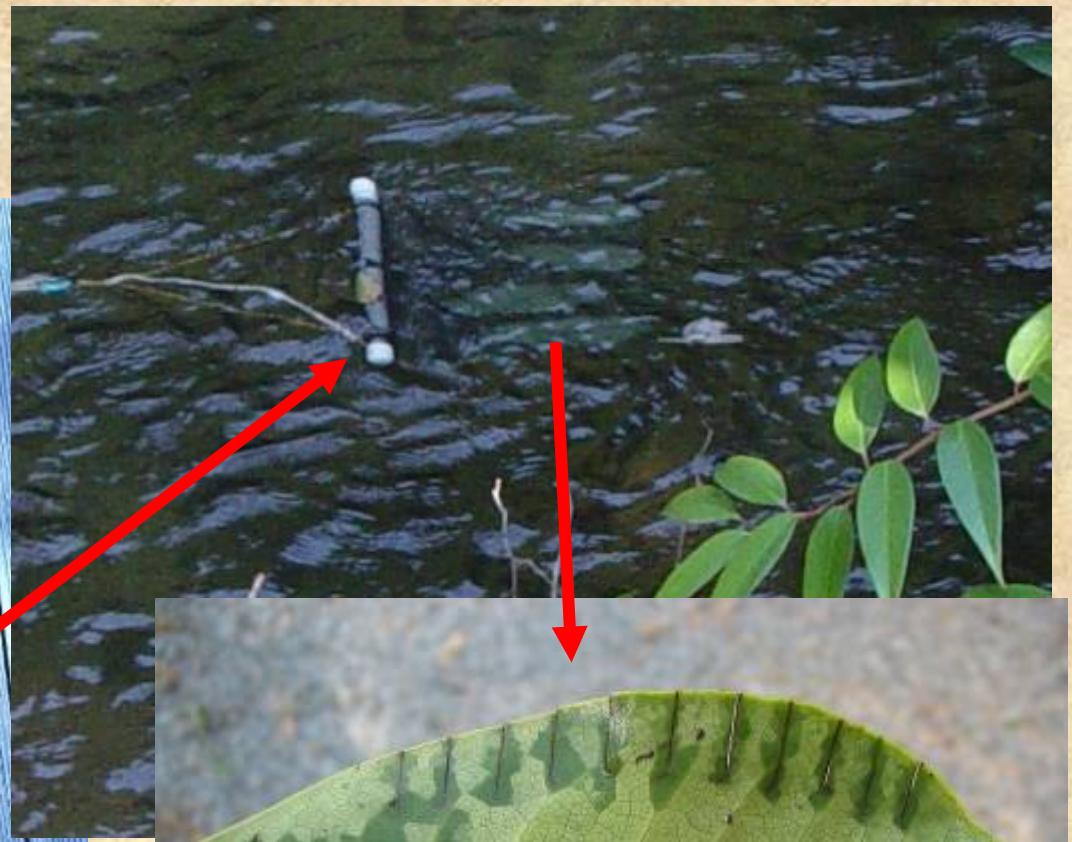
Bent Creek Watershed- 17.8 km^2

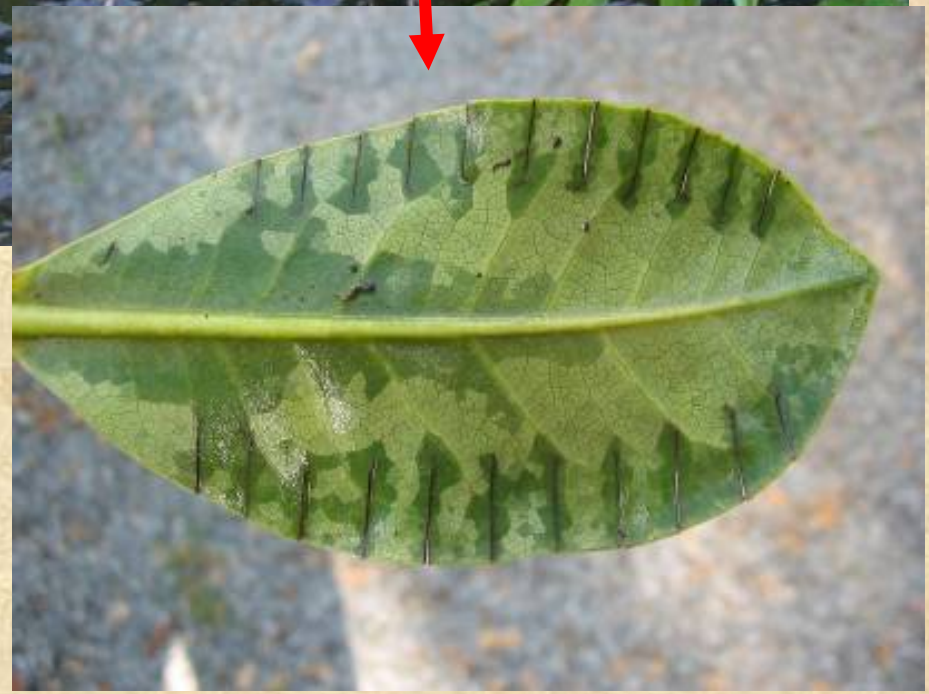


Leaf Baiting

- *Rhododendron maximum* leaves
  - largest leaves & geographic range
  - available year-round
- Bait bags: 4 leaves/bag per stream
  - easy to make and use
  - nylon screen, staples, PVC pipe, nylon rope
- Non-wounded leaves: wait for lesion development (1-2 wk)
- Wounded leaves: remove after 72 h
  - 10 tabs/side: plate one side, retain one side
- Duration: from Apr 2005 to Nov 2005

Leaf Baiting…



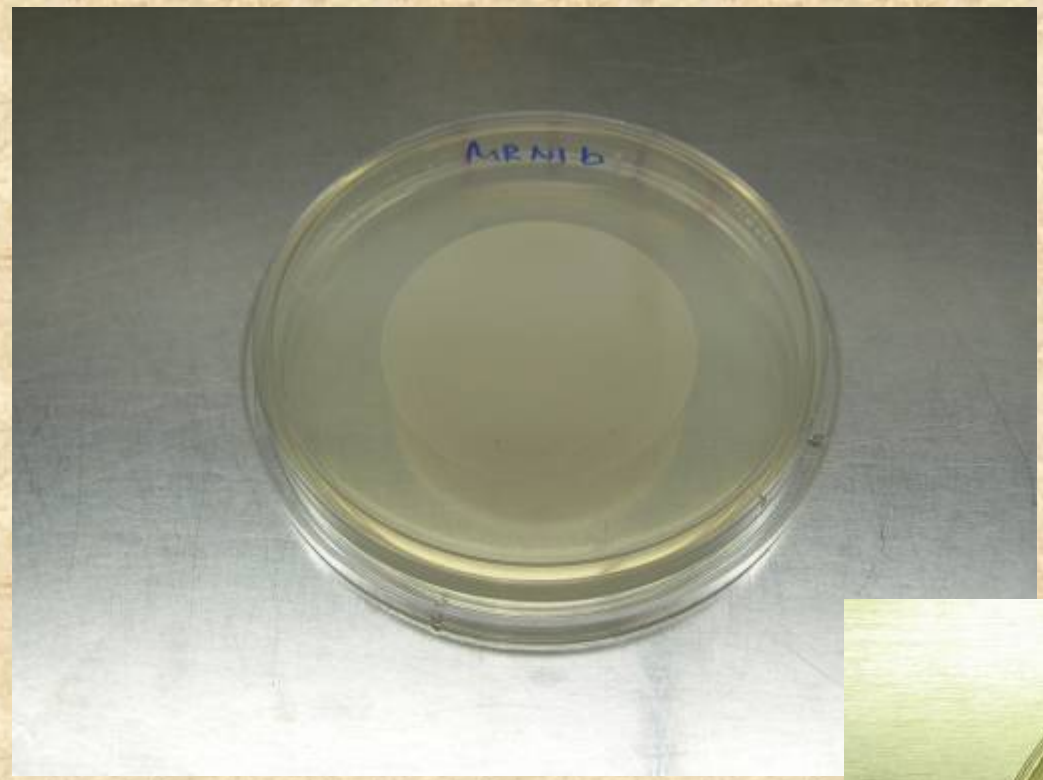


Filtering Technique

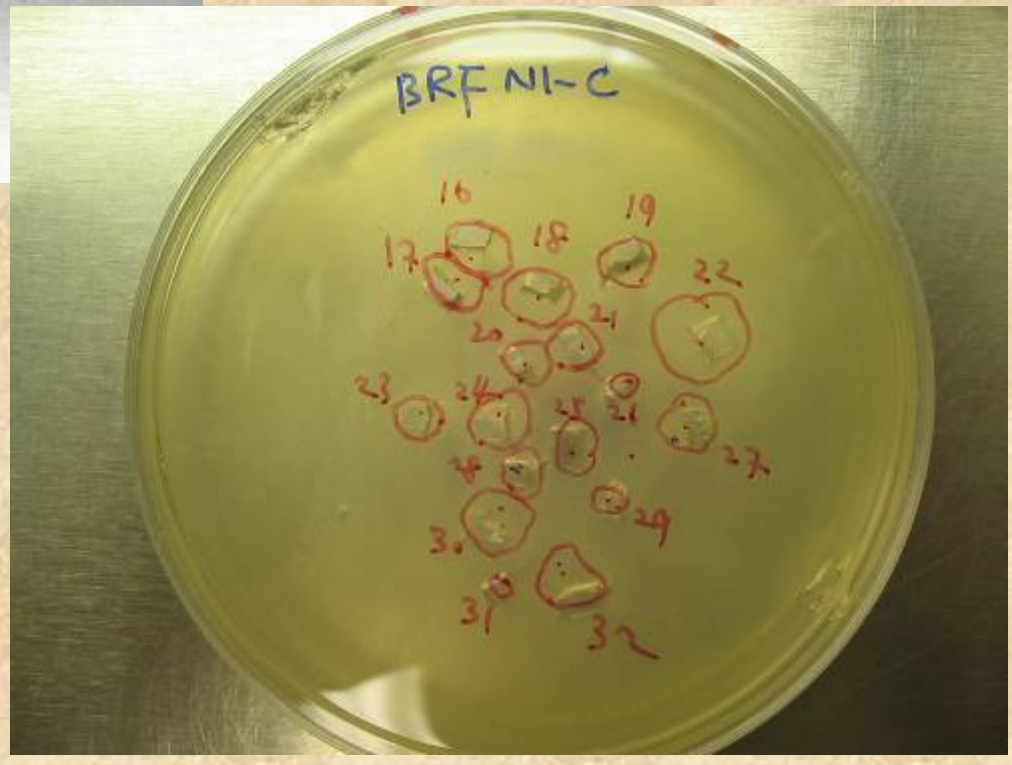
- Propagules
  - sporangia, zoospores, chlamydospores
  - hyphal fragments
  - colonized organic matter
- Three different membrane filters
  - Nuclepore 1 µm (Whatman)
  - Nuclepore 3 µm (Whatman)
  - Durapore 5 µm (Millipore)
- Use a 100-ml aliquot per filter
- Three replicates of each filter type/sample
- Total for each stream site:
  - 1000 ml water collected, 9 filters used, 900 ml processed
- Duration: from Apr 2005 to Mar 2006

Filtration Method…

Filtration Method…



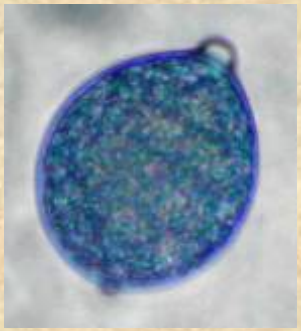
- incubate for 48-72 h at 20°C in the dark

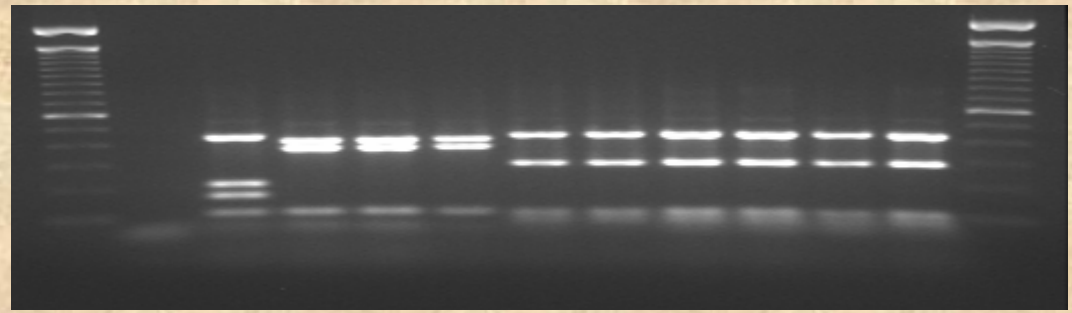


- remove filters
- rinse agar surface under running water
- count colonies
- subculture all unique colony types
- continue to incubate & look for new colonies

Species Identification

- Morphological characteristics: physical appearance
- Genetic characteristics: RFLP analysis & sequencing
  - ITS & *cox* primers





Results

- 1181 *Phytophthora* isolates in 14 species/groups were collected from five NC streams from April to March
  - 7 known species; *P. cambivora, P. cinnamomi, P. citricola, P. citrophthora, P. gonapodyides, P. heveae,* ***P. pseudosyringae*** (first report in eastern US)
  - 7 other genetically & morphologically distinct "groups" (new species??)
- *Phytophthora* spp. found in all 5 streams in all months
- No. of species detected varied by month, site, & detection method
  - July (11) vs February (1)
  - Filtering (13) vs baiting (8)
  - South Mills River (10) vs Big Creek (4)

Isolation of *Phytophthora* from Streamside Plants & Soils

- *Rhododendron* & *Kalmia* plants with typical symptoms were observed along all five streams
  - only *P. citricola* & *P. heveae* were isolated
  - first report on these native plant species
- Soils along all five streams collected & baited
  - only *P. cinnamomi* & *P. heveae* were isolated
- Where did others come from?

Species/Month

Species	Month							
	April	May	June	July	August	September	October	November
P. cambivora				+				
P. cinnamomi				+				
P. citricola	+	+	+	+	+	+	+	
P. citrophthora			+	+	+	+	+	
P. gonapodyides	+	+	+	+	+	+	+	+
P. heveae				+	+			
P. pseudosyringae	+	+	+	+		+	+	+
Group A	+							
Group B			+				+	
Group C				+		+	+	
Group F	+			+	+		+	
Group I			+					
Group J				+				
Group L				+				

Species/Location

Species	Location				
	Davidson River	South Mills River	Big Creek	Fletcher Creek	Bent Creek
P. cambivora		+			
P. cinnamomi		+			
P. citricola	+	+	+	+	+
P. citrophthora	+	+		+	+
P. gonapodyides	+	+	+	+	+
P. heveae		+			
P. pseudosyringae	+	+		+	+
Group A			+		
Group B					+
Group C	+	+			+
Group F	+	+		+	+
Group I					+
Group J		+			
Group L			+		

Species/Method

Species	Method		
	Filter	Wounded Leaf	Unwounded Leaf
P. cambivora	+		
P. cinnamomi		+	
P. citricola	+	+	+
P. citrophthora	+	+	+
P. gonapodyides	+	+	+
P. heveae	+		+
P. seudosyringae	+	+	+
Group A	+		
Group B	+		
Group C	+	+	+
Group F	+	+	+
Group I	+		
Group J	+		
Group L	+		

Phytophthora Calendar: December to March—Filtration Only

Location	Isolation	December 05	January 06	February 06	March 06
Davidson River	No. *Phytophthora*	14	8	22	24
	No. Species	1	1	1	1
	Species ID	14*gonapodyides*	8*gonapodyides*	22*gonapodyides*	24*gonapodyides*
South Mills River	No. *Phytophthora*	39	28	24	38
	No. Species	1	2	1	2
	Species ID	39*gonapodyides*	27*gonapodyides*, 1*pseudosyringae*	24*gonapodyides*	36*gonapodyides*, 2*pseudosyringae*
Big Creek	No. *Phytophthora*	3	7	7	6
	No. Species	1	2	1	1
	Species ID	3*gonapodyides*	6*gonapodyides*, 1*pseudosyringae*	7*gonapodyides*	6*gonapodyides*
Fletcher Creek	No. *Phytophthora*	26	22	28	62
	No. Species	1	1	1	1
	Species ID	26*gonapodyides*	22*gonapodyides*	28*gonapodyides*	62*gonapodyides*
Bent Creek	No. *Phytophthora*	52	14	18	70
	No. Species	2	2	1	3
	Species ID	48*gonapodyides*, 4*pseudosyringae*	13*gonapodyides*, 1*pseudosyringae*	18*gonapodyides*	1*citricola*, 66*gonapodyides*, 3*pseudosyringae*
Total	No. *Phytophthora*	134	79	99	200
	No. Species	2	2	1	3
	Species ID	130*gonapodyides*, 4*pseudosyringae*	76*gonapodyides*, 3*pseudosyringae*	99*gonapodyides*	1*citricola*, 194*gonapodyides*, 5*pseudosyringae*

Species and Filter Type

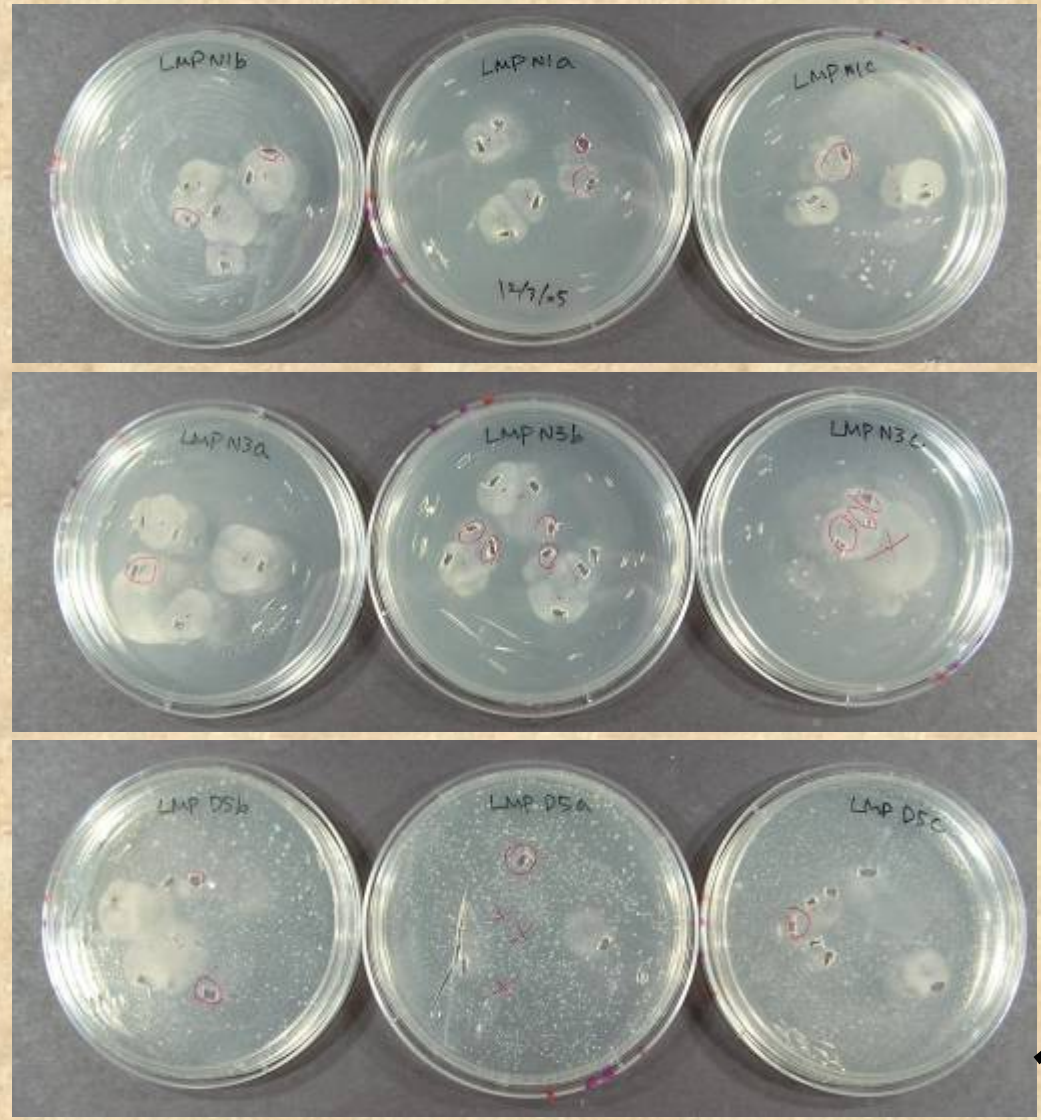
Isolation	Nuclepore 1 µm	Nuclepore 3 µm	Durapore 5 µm
No. *Phytophthora*	307	331	264
No. Species	8	9	9
Species ID	*citricola, citrophthora, gonapodyides, pseudosyringae*, A, C, F, I	*citricola, citrophthora, gonapodyides, heveae, pseudosyringae*, B, C, F, I	*cambivora, citricola, citrophthora, gonapodyides*, B, C, F, J, L

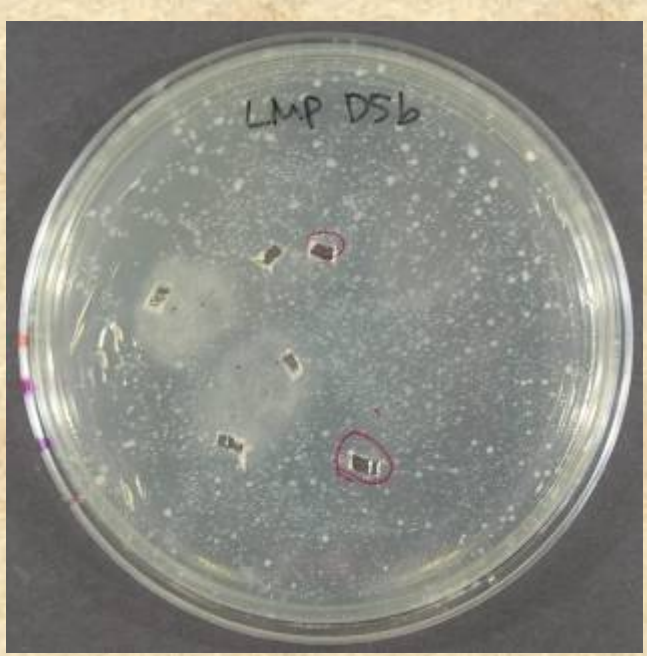
Filter Type: Bacterial contamination

N1

N3

D5





Evaluation of Detection Methods for *Phytophthora ramorum*

- Comparing filtering & baiting with wounded leaves for detection of *P. ramorum* in California streams known to be naturally infested

- 3 streams in Santa Cruz Co. sampled in May 2005:
  - Lompico Creek
  - Bean Creek
  - Branciforte Creek

- 8 streams sampled in Dec 2005 to validate filtration assay
  - Santa Cruz Co.
  - Monterey Co.
  - Marin Co.
  - Sonoma Co.

Detection of *P. ramorum* in California Streams: May 2005

Location	Filter		Wounded Leaf	
	No. *Phytophthora*	No. *P. ramorum*	No. *Phytophthora*	No. *P. ramorum*
Bean Cr	35	**10**	17	**2**
Branciforte Cr	60	**2**	7	**1**
Lompico Cr	70	**36**	8	**4**

Detection of *P. ramorum* in California Streams: Dec. 2005

Location	N1		N3		D5		Total	
	#Phy	#ram	#Phy	#ram	#Phy	#ram	#Phy	#ram
Bean Cr	16	0	25	0	38	1	79	1
Lompico Cr	15	0	20	2	17	0	52	2
Willow Cr	2	0	6	0	4	0	12	0
Pfeiffer-Redwood Cr	1	0	1	0	0	0	2	0
China Camp State Park	5	4	1	0	1	1	7	5
Lagunitas Cr	25	1	18	0	25	1	68	2
Novato Cr	13	0	13	0	18	0	44	0
Copeland Cr	0	0	0	0	0	0	0	0

4 of 8 streams were positive for *P. ramorum*

***Phytophthora* population level was low in some creeks**

Density of *P. ramorum* was lower in Dec. compared to May

Location	May 2005		December 2005	
	No. *Phytophthora*	No. *ramorum*	No. *Phytophthora*	No. *ramorum*
Bean Cr	35	10	79	1
Lompico Cr	70	36	52	2

Summary

- Both filtering & leaf baits successfully detected multiple species of *Phytophthora* in forest streams
- Isolation results varied by detection method, location, and month
  - selection of survey time and location
- Overall, filtering was more effective than leaf baits for detecting diverse groups of *Phytophthora* spp.

Summary

- Filtering detected *Phytophthora ramorum* in naturally infested streams in California
  - density of propagules in streams in December was significantly lower than in May
- More studies on the occurrence & distribution of *Phytophthora* species in streams, soil, and plants in natural ecosystems are needed…

Special thanks to

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  Jill Baker, NC Arboretum, Pisgah Ranger District, Pisgah National Forest
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