# BOB vs. BAGS

## Comparison of In Situ and In Vitro Baiting Assays for *Phytophthora ramorum* Survey of Waterways in the SUSA 2011-2012

S.W. Oak[1], J. Hwang[2], and S.N. Jeffers[2]
[1] USDA Forest Service, Southern Region FHP
[2] Clemson University

# Acknowledgements


**State Cooperators- Field and Laboratory**

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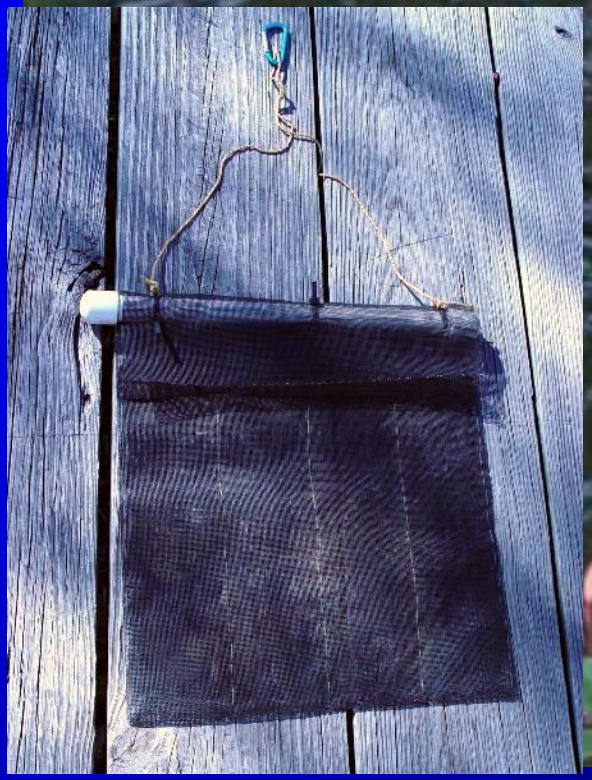
- WA: Dan Omdal, Jennifer Falacy

**Regional Diagnostics**

- East: SeongHwan Kim, PA Dept. of Agriculture
- West: Melodie Putnam, OR State University
- USFS Southern Region FHP
  - Christina Waldroup
  - Ginger Shuman

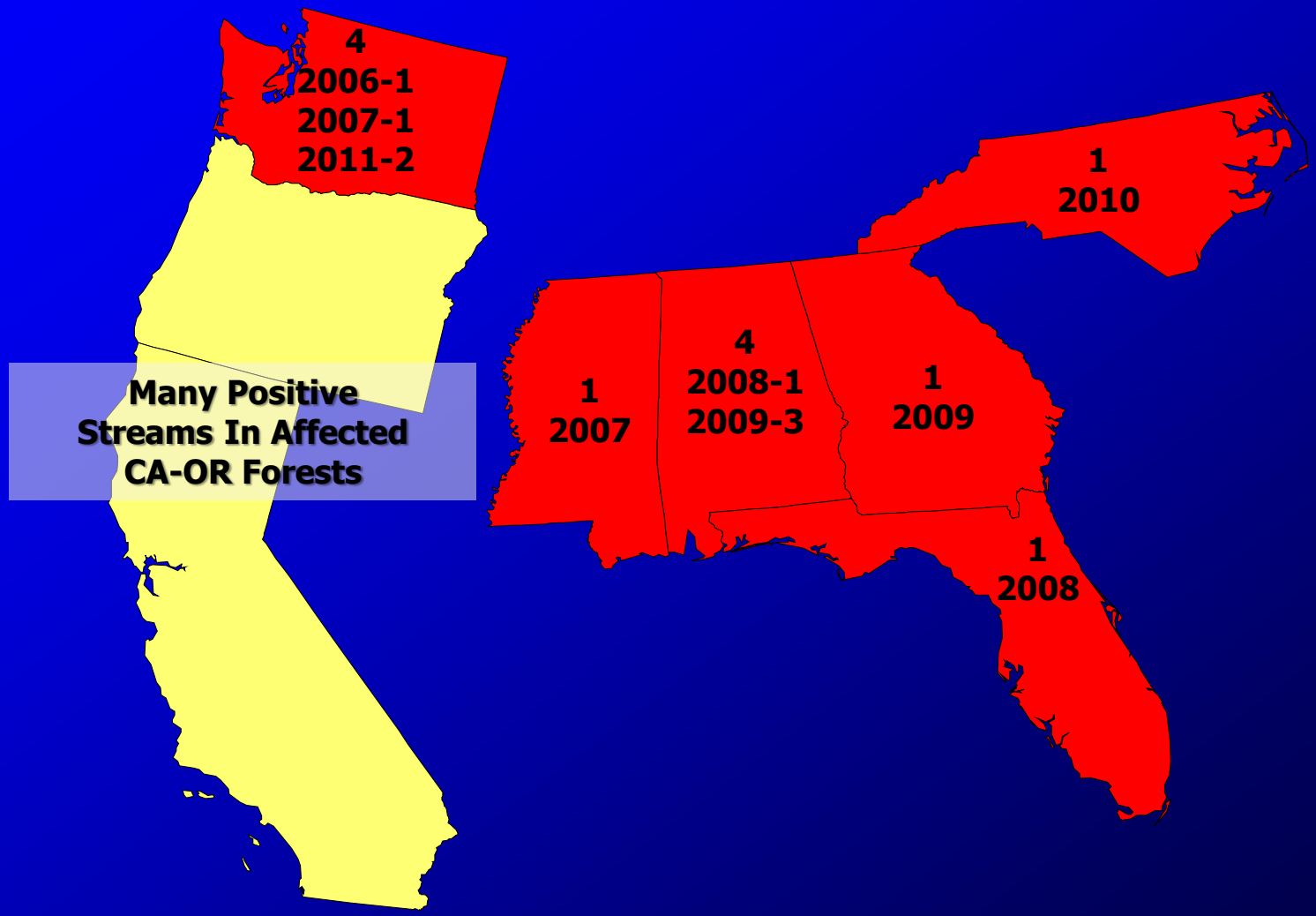
# In Situ Baiting

**National *P. ramorum* Early Detection Survey Protocol Since 2007**



# US *P. ramorum* Stream Positives

## 2006-2011 (Year of First Positive)



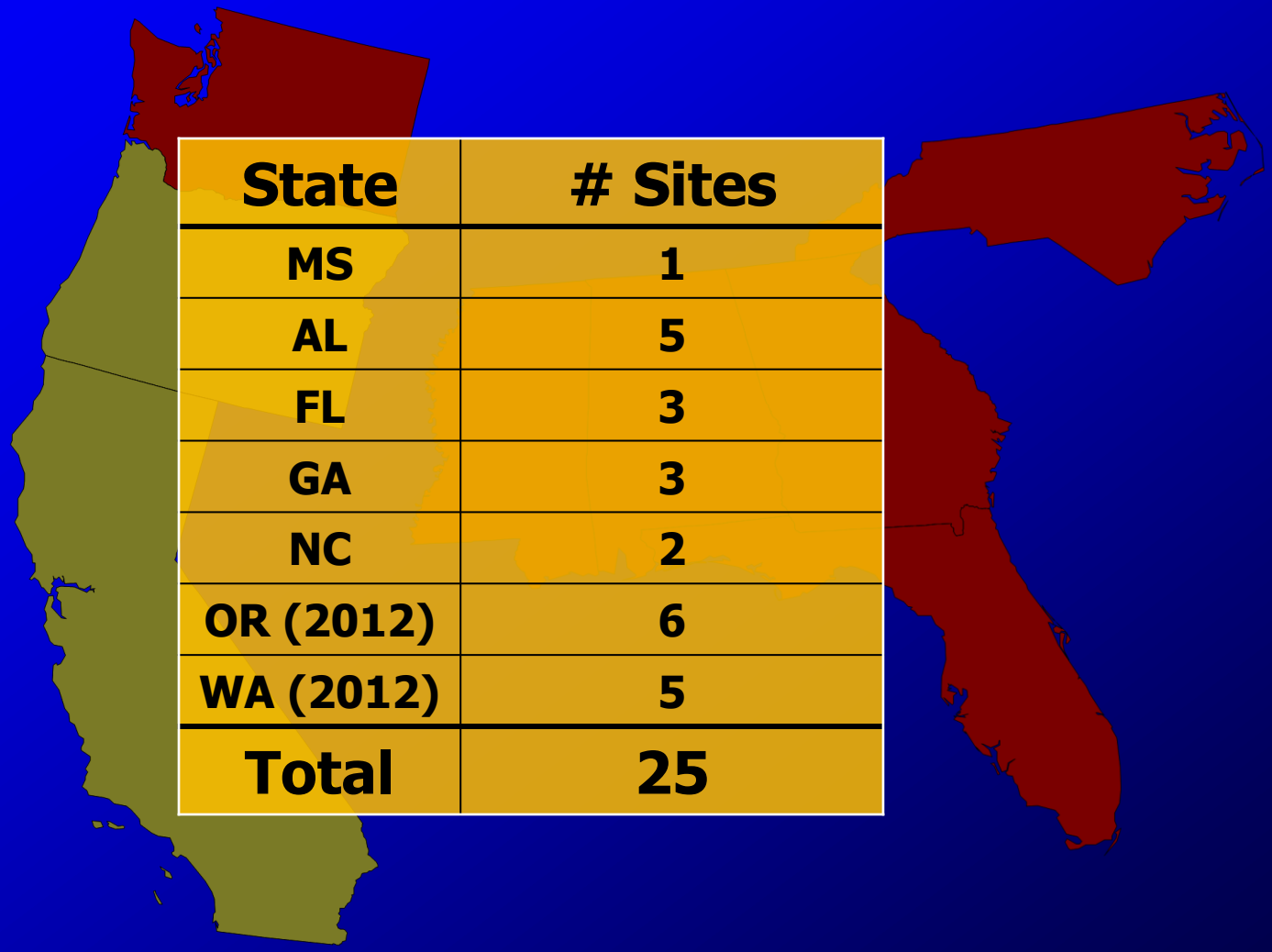
# In Situ Baiting Assay (Bags)

- Proven effective for *P. ramorum* recovery.
- Extended bait deployment allows sampling of large water volumes.
- Vulnerable to loss by vandalism & storm surges.
- Requires two visits (deploy & retrieve).
- Requires sustained water flow over the entire baiting period.

# OBJECTIVE

**Compare relative survey performance of in situ and in vitro baiting assays in *P. ramorum*-infested waterways in 7 states.**

# Sites for In Situ-In Vitro Comparison Survey, 2011-12



| State | # Sites |
|---|---|
| MS | 1 |
| AL | 5 |
| FL | 3 |
| GA | 3 |
| NC | 2 |
| OR (2012) | 6 |
| WA (2012) | 5 |
| **Total** | **25** |

# In Vitro Assay (Bottle O' Bait- BOB)

- 2-800 ml samples collected in 100 ml aliquots
- Immediately add 1 non-wounded rhododendron leaf & 20 leaf pieces
- Seal bottle, incubate in the dark on its side @ 18-22°C for 3 days
- Strain water sample to remove baits, rinse in distilled water, blot dry
- Rinse each whole leaf in running tap water followed by distilled water; incubate in a dark moist chamber @ 20°C for 7-10 days for symptom development
- Diagnose *P. ramorum* presence
  - Half of each bait type by isolation and PCR

# Incubating BOB

B
B

# In Situ vs. In Vitro *P. ramorum* Recovery

## Comparative Survey Summary Statistics

## Recovery Frequency

| | S- '11 | F- '11 | S- '12 | Overall |
|---|---|---|---|---|
| Number of Sites | 14 | 13 | 25 | 25 |
| Total Cases w/ Both Assays[1] | 36 | 33 | 89 | 158 |
| Comparable Cases[2] | 21 | 12 | 40 | 73 |
| Percent of Total | 58.3% | 36.4% | 44.9% | 46.2% |

[1]Sites * baiting periods (includes bags deployed but lost)

[2]*P. ramorum* positive by one or both assays

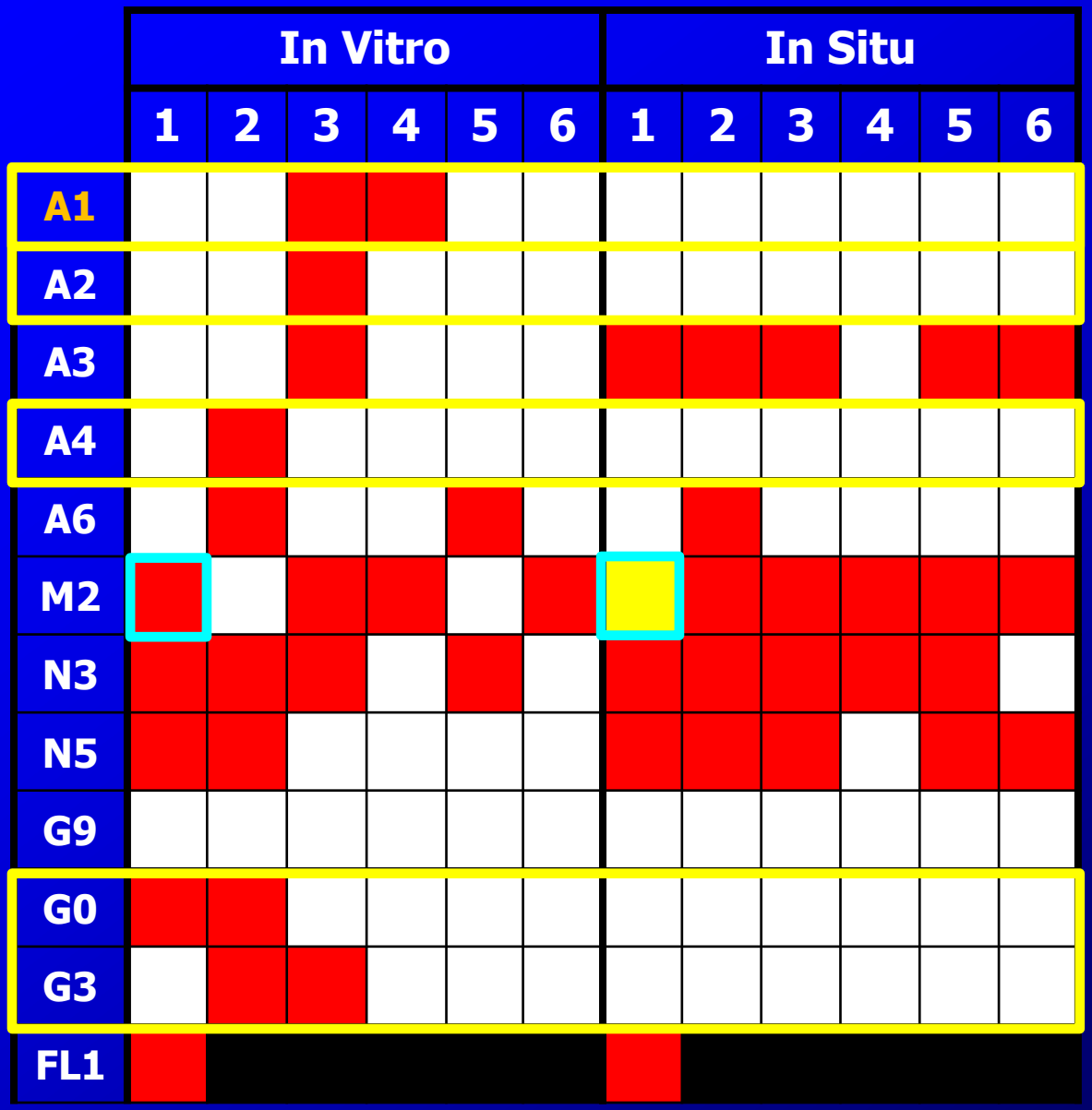
| Stream Code | Date | In Vitro Diagnosis | | | | | | | | In Situ Diagnosis | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A Bottle | | | | B Bottle | | | | | |
| | | Pieces | | Whole | | Pieces | | Whole | | | |
| | | Isolate | PCR | Isolate | PCR | Isolate | PCR | Isolate | PCR | Isolate | PCR |
| AL3-1 | 3/22 | N | N | N | N | N | N | N | Y | N | Y |
| AL3-2 | 3/29 | N | N | N | N | N | N | N | Y | N | N |
| AL3-3 | 4/5 | Y | Y | N | N | N | N | N | N | LOST | |

| In Vitro Diagnosis |
|---|
| Y |
| Y |
| Y |

| In Situ Diagnosis |
|---|
| Y |
| N |
| LOST |

| In Vitro Only | In Situ Only | Both |
|---|---|---|
| 2 | 0 | 1 |

# 2011 *P. ramorum* Recovery
# In Vitro vs. In Situ




| 2011 Overall | | | |
|---|---|---|---|
| | In Vitro Only | In Situ Only | Both |
| Baiting Period | 10 | 10 | 12 |
| Annual | 5 | 0 | 6 |

# 2012 *P. ramorum* Recovery In Vitro vs. In Situ

| | In Vitro | | | | In Situ | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| N3 | | | | | | | | |
| N5 | | | | | | | | |
| G9 | | | | | | | | |
| G0 | | | | | | | | |
| G3 | | | | | | | | |

| Spring 2012- NC, GA | | | |
|---|---|---|---|
| | In Vitro Only | In Situ Only | Both |
| Baiting Period | 1 | 2 | 3 |
| Seasonal | 1 | 0 | 2 |

# 2012 *P. ramorum* Recovery In Vitro vs. In Situ

| | In Vitro | | | | | | In Situ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| F99 | | | | | | | | | | | | |
| F10 | | | | | | | | | | | | |
| F1B | | | | | | | | | | | | |

| Spring 2012- FL | | | |
|---|---|---|---|
| | In Vitro Only | In Situ Only | Both |
| Baiting Period | 5 | 1 | 4 |
| Seasonal | 0 | 0 | 3 |

# 2012 *P. ramorum* Recovery In Vitro vs. In Situ

| | In Vitro | | | In Situ | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| A1 | | | | | | |
| A2 | | | | | | |
| A3 | | | | | | |
| A4 | | | | | | |
| A6 | | | | | | |
| M2 | | | | | | |

| Spring 2012- AL, MS | | | |
|---|---|---|---|
| | In Vitro Only | In Situ Only | Both |
| Baiting Period | 2 | 1 | 2 |
| Seasonal | 1 | 0 | 2 |

# 2012 *P. ramorum* Recovery In Vitro vs. In Situ

| | In Vitro | | | In Situ | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| O9 | | | | | | |
| O2 | | | | | | |
| O6 | | | | | | |
| O5 | | | | | | |
| O4 | | | | | | |
| O7 | | | | | | |
| W7 | | | | | | |
| W1 | | | | | | |
| W4 | | | | | | |
| W3 | | | | | | |
| W9 | | | | | | |

| Spring 2012- OR, WA | | | |
|---|---|---|---|
| | In Vitro Only | In Situ Only | Both |
| Baiting Period | 12 | 3 | 2 |
| Seasonal | 7 | 1 | 2 |

| | In Vitro | | | In Situ | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| O9 | | | | | | |
| O2 | | | | | | |
| O6 | | | | | | |
| O5 | | | | | | |
| O4 | | | | | | |
| O7 | | | | | | |
| W7 | | | | | | |
| W1 | | | | | | |
| W4 | | | | | | |
| W3 | | | | | | |
| W9 | | | | | | |

| | In Vitro | | | | In Situ | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| N3 | | | | | | | | |
| N5 | | | | | | | | |
| G9 | | | | | | | | |
| G0 | | | | | | | | |
| G3 | | | | | | | | |

| Spring 2012- Overall | | | |
|---|---|---|---|
| | In Vitro Only | In Situ Only | Both |
| Baiting Period | 22 | 7 | 11 |
| Seasonal | 12 | 1 | 11 |

| | In Vitro | | | In Situ | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| A1 | | | | | | |
| A2 | | | | | | |
| A3 | | | | | | |
| A4 | | | | | | |
| A6 | | | | | | |
| M2 | | | | | | |

| | In Vitro | | | | | | In Situ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| F99 | | | | | | | | | | | | |
| F10 | | | | | | | | | | | | |
| F1B | | | | | | | | | | | | |

# National Survey Protocol Implications

- **In Vitro = In Situ?**
  - **Yes, in absolute terms;**
  - **No, when stream x season interactions are considered.**
  - **Season pattern suggests both should be used to maximize detection.**
- **Why seasonal differences?**
  - **Long deployment is apparently advantageous under some environmental circumstances.**
- **Anecdotes**
  - **Spring flood at M2 made bag retrieval impossible, while BOB collection was possible (AND POSITIVE!).**
  - ***P. ramorum* would have been undetected in 5 infested streams without BOB.**
  - **First ever detection at A1 by in vitro.**

# In Situ vs. In Vitro *P. ramorum* Recovery

## Comparative Survey Summary Statistics

## Seasonal Recovery by Stream and Assay

| | Spring | |
|---|---|---|
| Stream Code | In Vitro | In Situ |
| A1 | Y | N |
| A2 | Y | N |
| A3 | Y | Y |
| A4 | Y | N |
| A6 | Y | Y |
| M2 | Y | Y |
| N3 | Y | Y |
| N5 | Y | Y |
| G9 | N | N |
| G10 | Y | N |
| G13 | Y | N |
| F10 | Y | Y |

***Each cell** <u>**USUALLY**</u> **represents 3 collections/baiting periods***

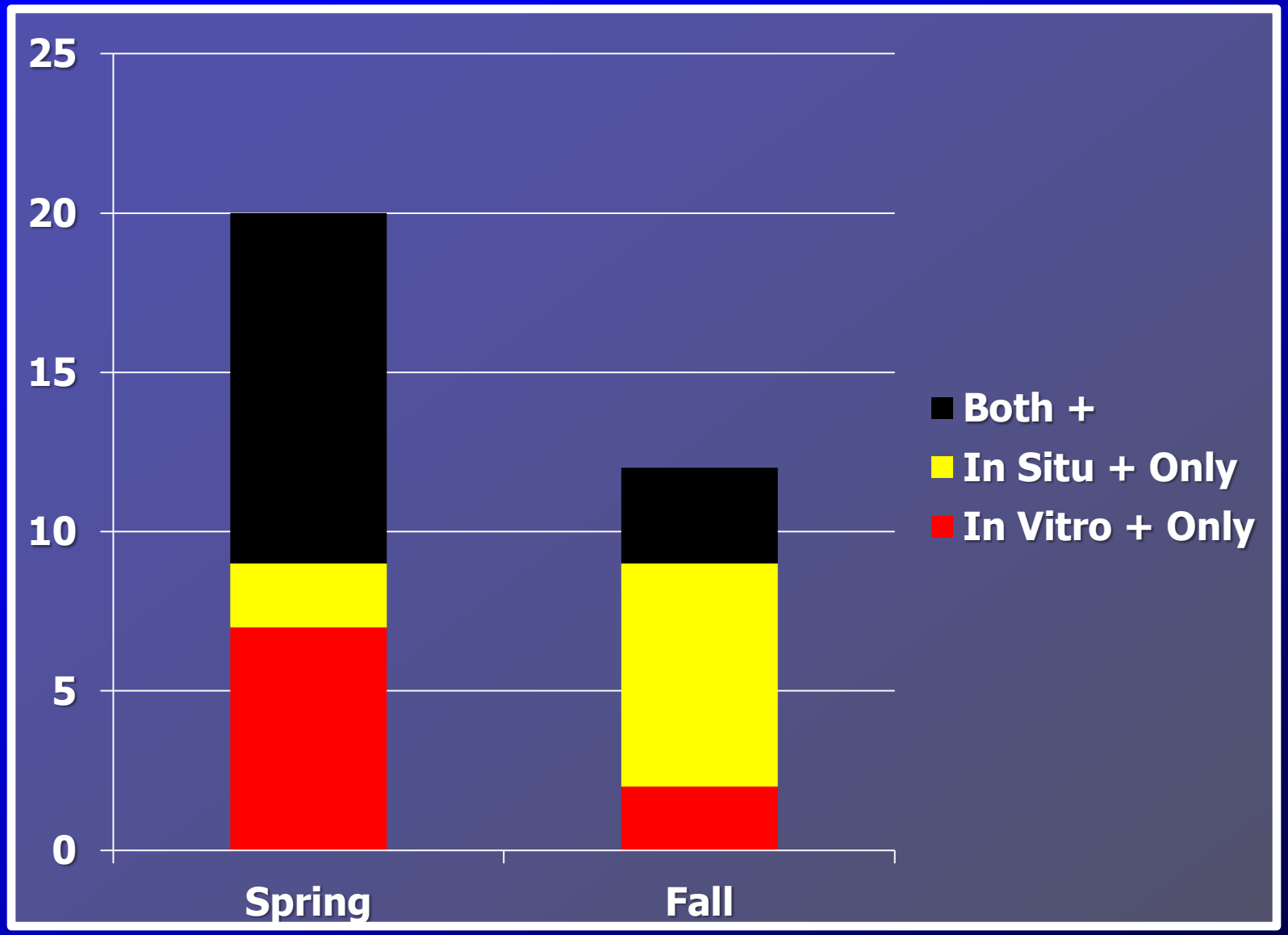
# In Situ vs. In Vitro *P. ramorum* Recovery

## Comparative Survey Summary Statistics

## Recovery by Assay and Season

| *P. ramorum* Diagnosis Summary | | | |
|---|---|---|---|
| | Spring | Fall | Year |
| BOB + Only | 7 | 2 | 9 |
| Bags + Only | 2 | 7 | 9 |
| Both + | 11 | 3 | 14 |

# In Situ vs. In Vitro *P. ramorum* Recovery
## Comparative Survey Summary Statistics



# In Situ vs. In Vitro *P. ramorum* Recovery

## Comparative Survey Summary Statistics- 2012

## Seasonal Recovery by Stream and Assay

| | Spring | |
|---|---|---|
| Stream Code | In Vitro | In Situ |
| A1 | Y | N |
| A2 | Y | N |
| A3 | Y | Y |
| A4 | Y | N |
| A6 | Y | Y |
| M2 | Y | Y |
| N3 | Y | Y |
| N5 | Y | Y |
| G9 | N | N |
| G10 | Y | N |
| G13 | Y | N |
| F10 | Y | Y |

***Each cell USUALLY represents 3 collections/baiting periods***

# In Situ vs. In Vitro *P. ramorum* Recovery

## Comparative Survey Summary Statistics

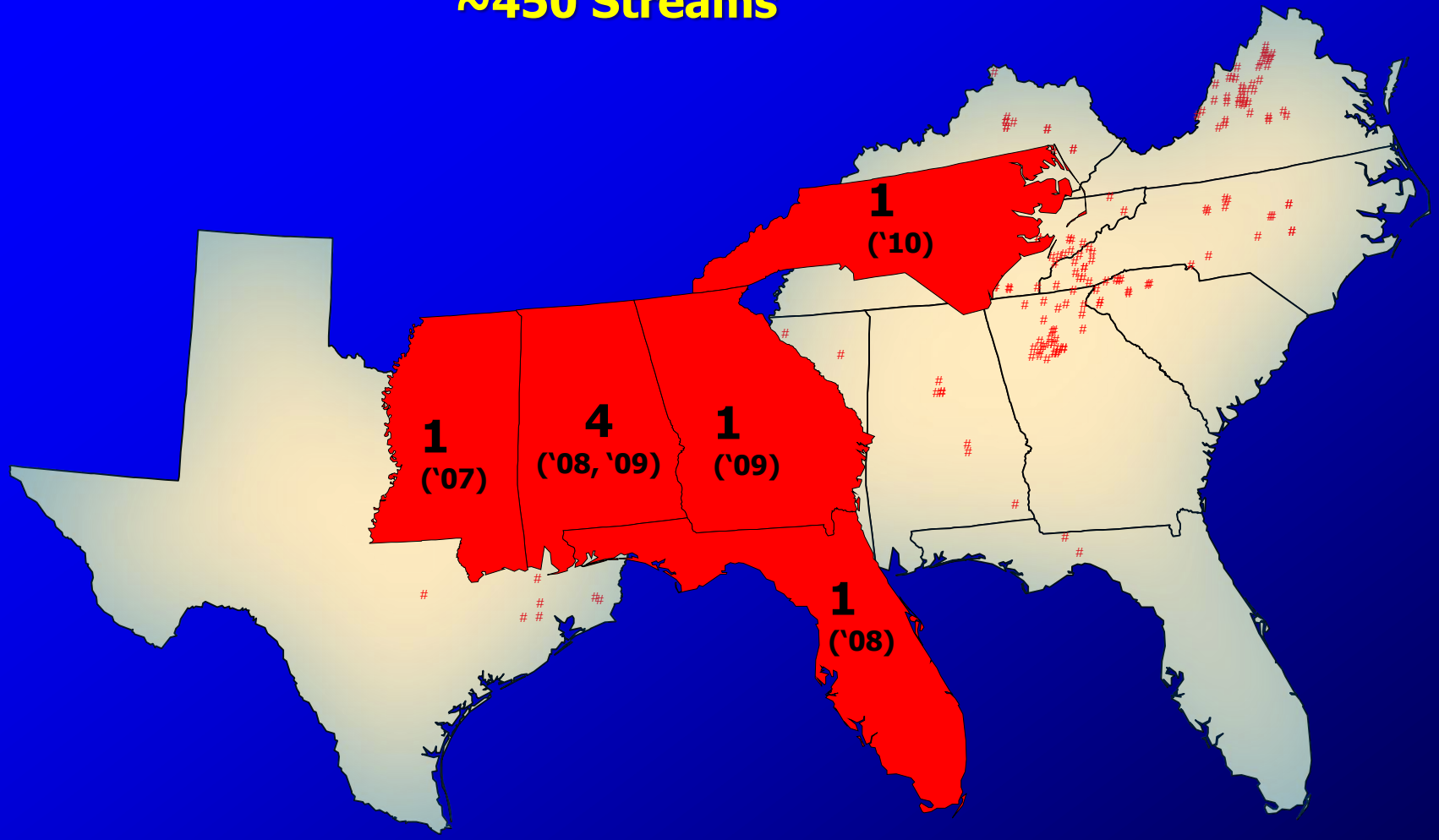
## Spring 2012 Recovery by Stream and Assay

| EAST | | |
|---|---|---|
| Stream Code | In Vitro | In Situ |
| A1 | | |
| A2 | | |
| A3 | | |
| A4 | | |
| A6 | | |
| M2 | | |
| N3 | | |
| N5 | | |
| G9 | | |
| G10 | | |
| G13 | | |
| FL10 | | |

| WEST | | |
|---|---|---|
| Stream Code | In Vitro | In Situ |
| OR9 | | |
| OR12 | | |
| OR61 | | |
| OR65 | | |
| OR74 | | |
| OR75 | | |
| WA7 | | |
| WA21 | | |
| WA24 | | |
| WA31 | | |
| WA39 | | |

***Each cell USUALLY represents 3 collections/baiting periods***

# Southern Region Stream Baiting Sites 2006-2011
## *P. ramorum* Stream Positives 2007-2011 (Year of First Positive)
## ~450 Streams



# In Situ vs. In Vitro *P. ramorum* Recovery

## Comparative Survey Summary Statistics

## Spring 2012 Recovery by Stream and Assay

| | Spring | | Fall | |
|---|---|---|---|---|
| Stream Code | In Vitro | In Situ | In Vitro | In Situ |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
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| | | | | |
| | | | | |
| | | | | |
| | | N | Y | N |
| | | N | N | N |
| | | Y | N | Y |

*represents 3 collections/baiting periods*