Rapid Detection of Oak Wilt

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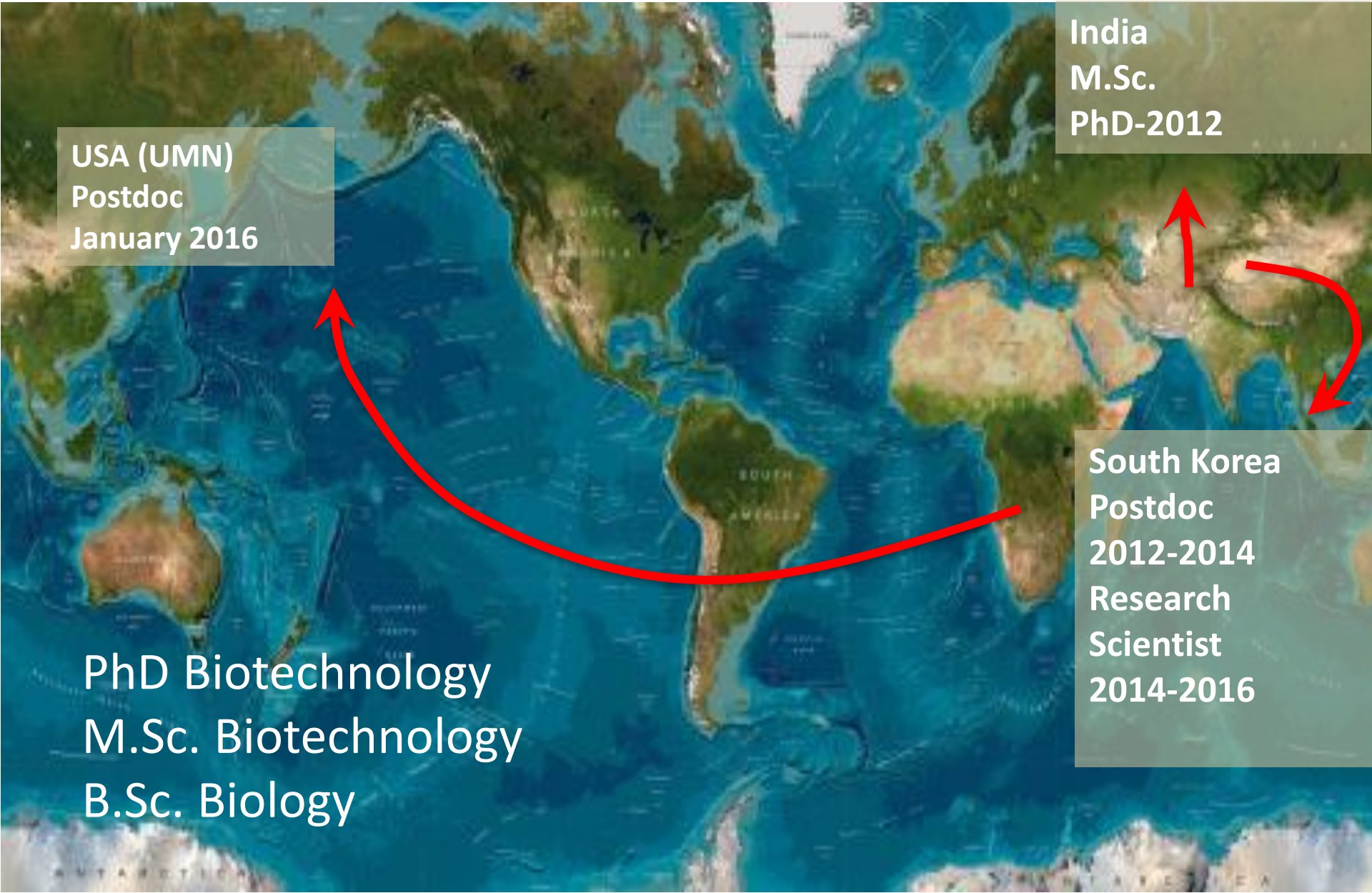
Outlook

1- Personal Background

2- Our Research Group

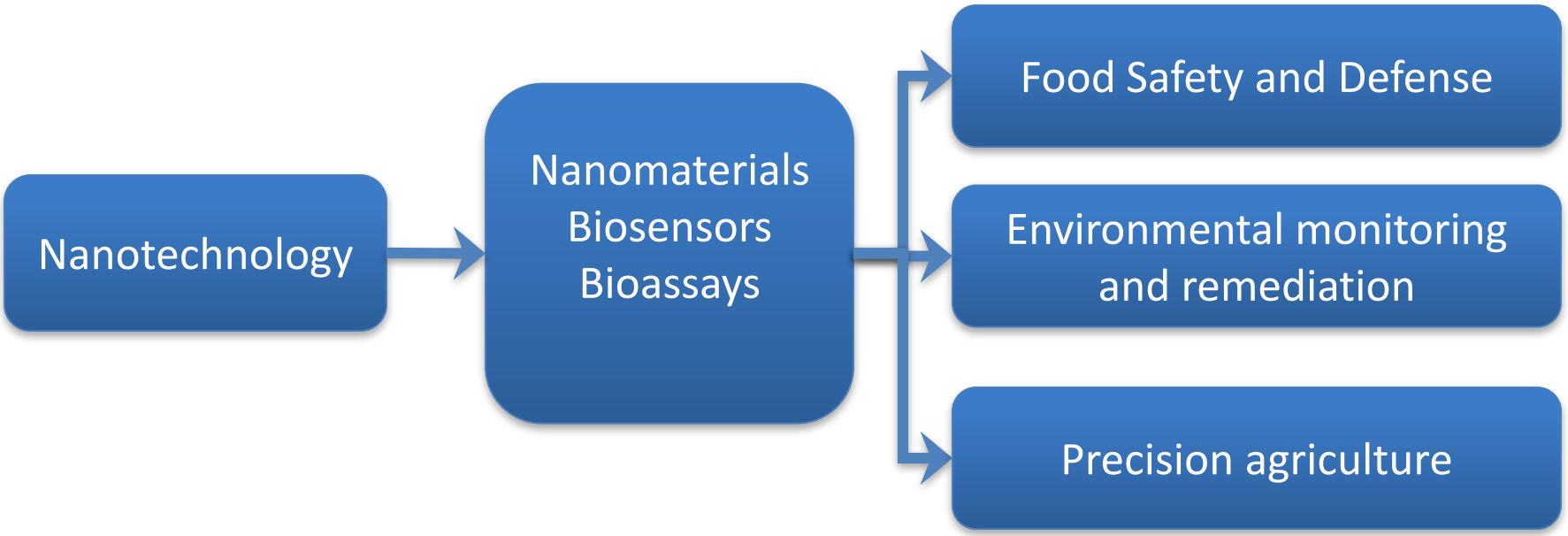
3- Oak wilt: Problem, Significance, Diagnosis, Technology

Personal Background



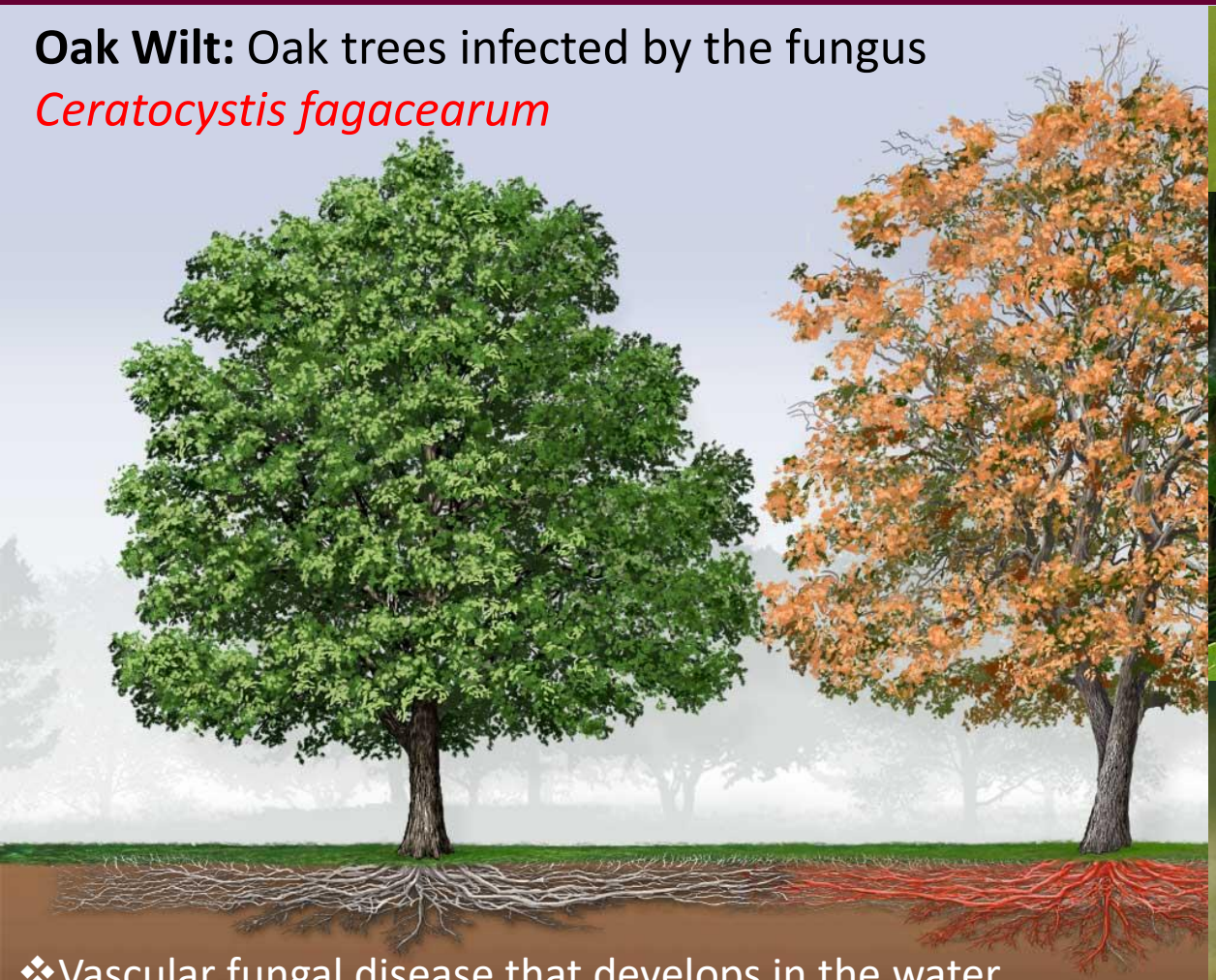
Our Research Group

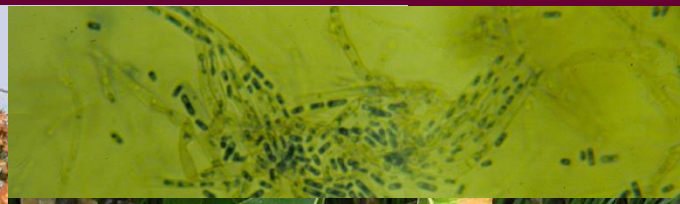
Biosensors and Bionanotechnology lab



The Problem

Oak Wilt: Oak trees infected by the fungus *Ceratocystis fagacearum*



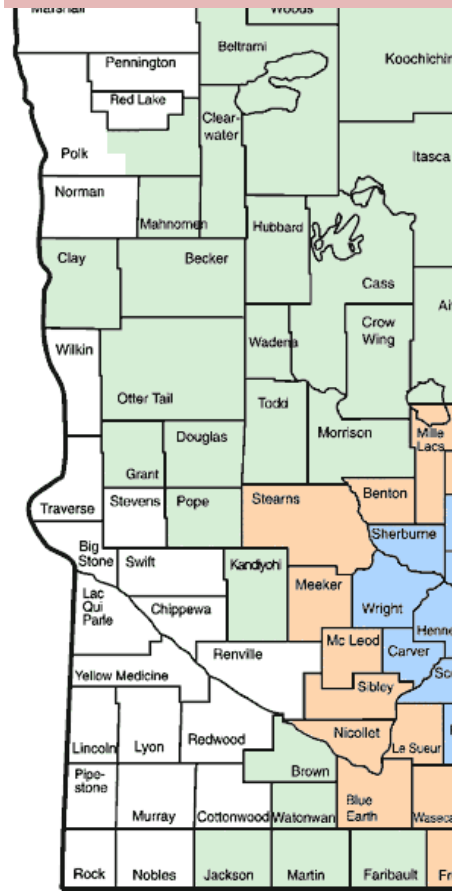


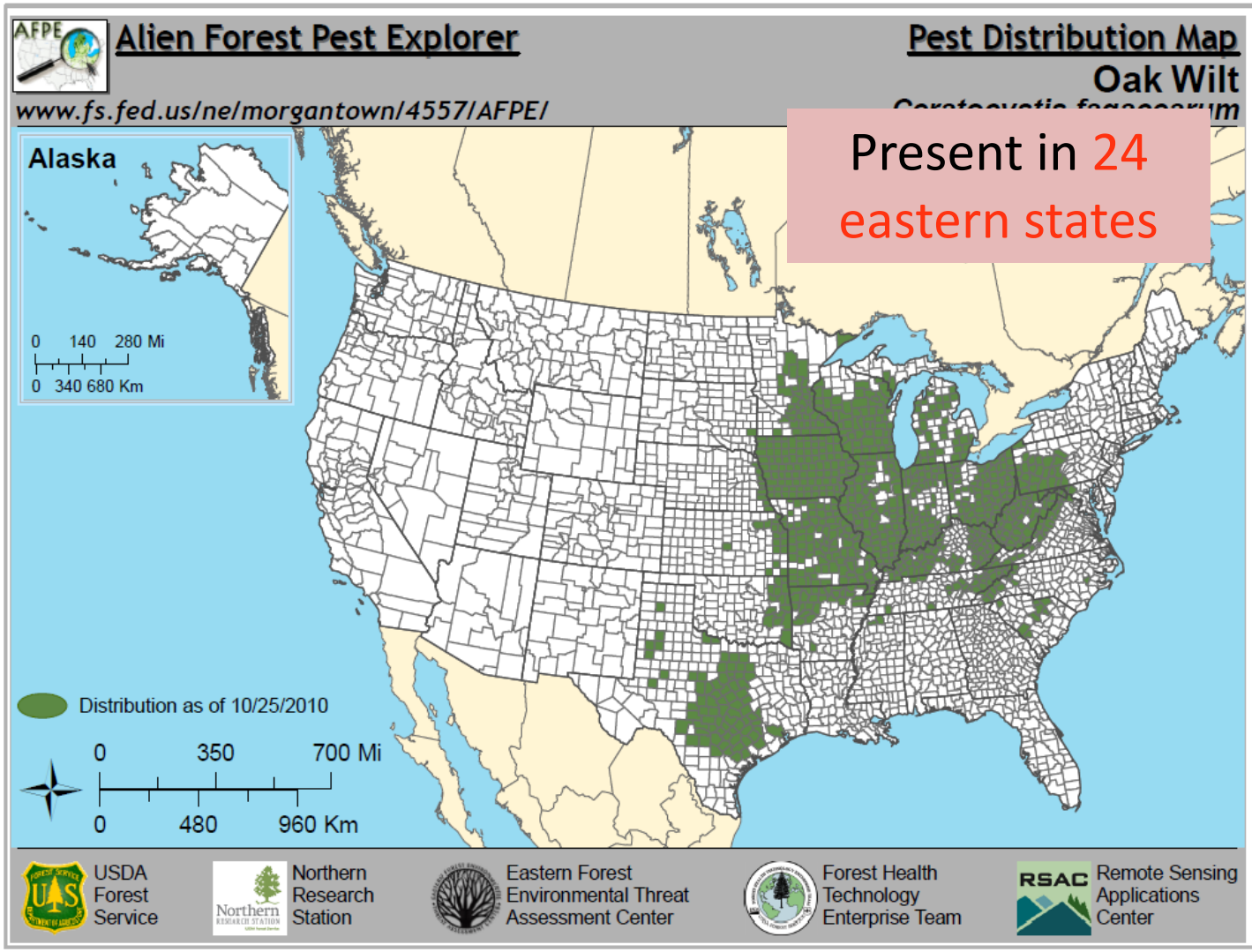
- Vascular fungal disease that develops in the water conducting vessels (xylem)

- Can quickly kill an oak tree by plugging up the vessels and reduced water flow in trees.

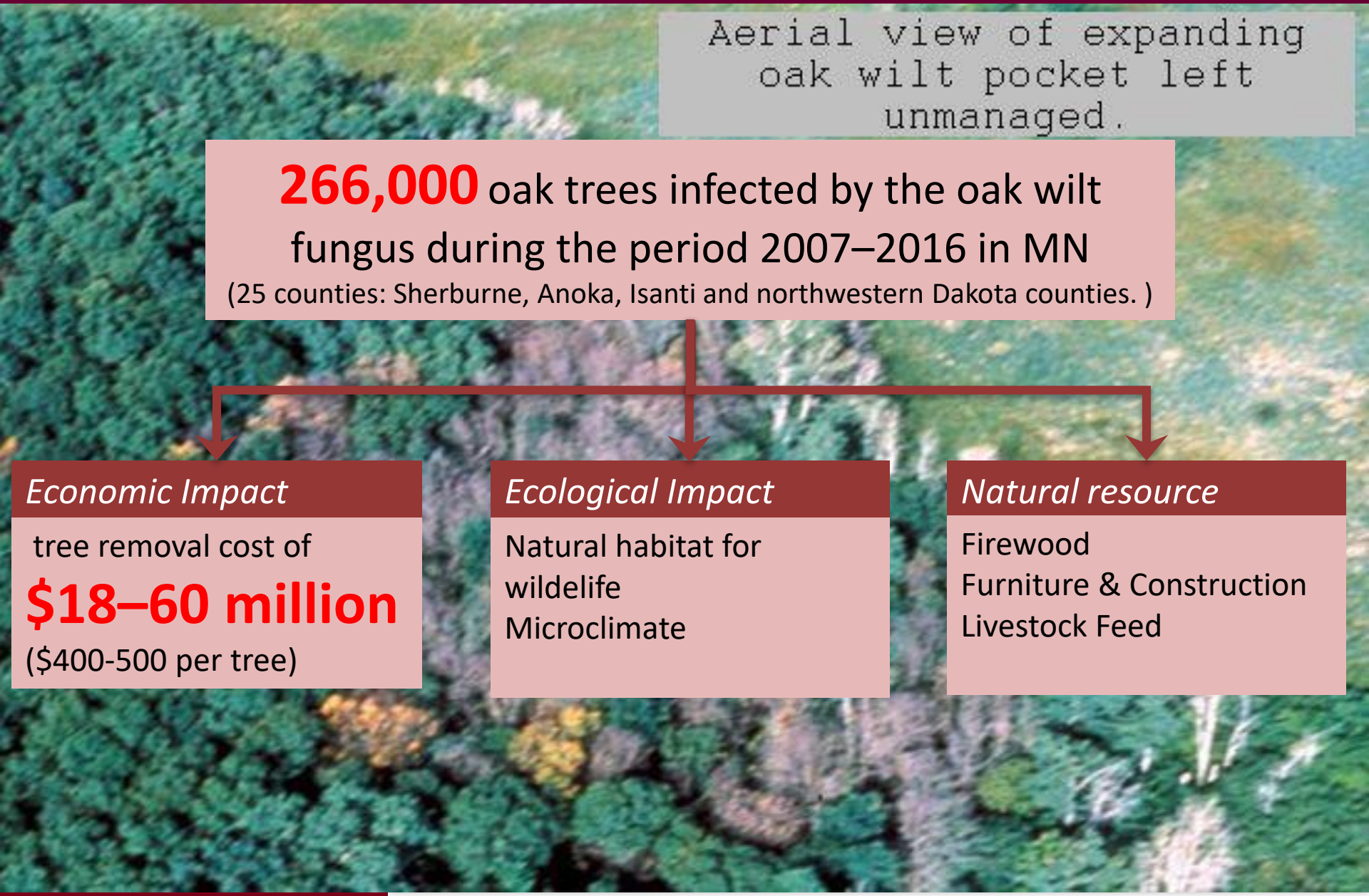
The Problem

Present in 25 Counties in MN





Why Does it Matter?



266,000 oak trees infected by the oak wilt fungus during the period 2007–2016 in MN

(25 counties: Sherburne, Anoka, Isanti and northwestern Dakota counties.)

Economic Impact

tree removal cost of

$18–60 million

($400-500 per tree)

Ecological Impact

Natural habitat for wildelife

Microclimate

Natural resource

Firewood

Furniture & Construction

Livestock Feed

Diagnosis of Oak Wilt

Visual diagnosis

Veinal necrosis

The fungus can be in the tree **2-3 weeks** without leaf symptoms appearing.

Oak trees infected die within **4-6 weeks**

Current Lab Testing

Fungal isolation and growth

- ✓ 1-2 weeks for cell growth
- ✓ $59 per sample
- ✓ Not in Dormant season

DNA tests

- ✓ Labor-intensive protocols (up to 6 hours)
- ✓ $120 per sample
- ✓ Not on dead tissues

This Project

- ✓ Detection in less than **2 hours**
- ✓ Less than **$5** per sample

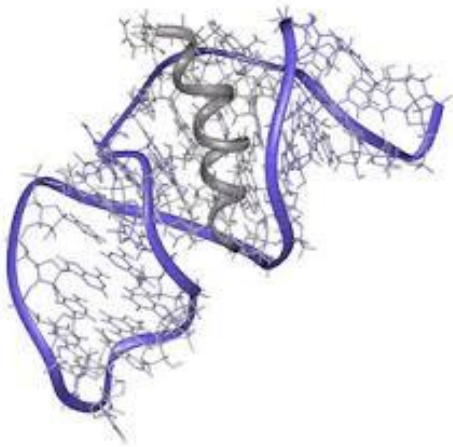
Why Aptamers ?

- Use PCR as we do not have antibodies
- Receptors

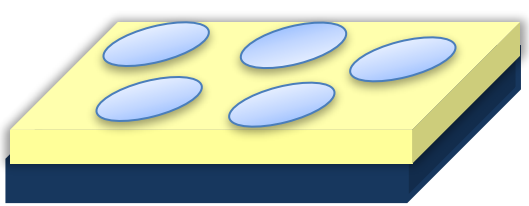
DNA Aptamers:

"Chemical Antibody" for Theranostics (Therapy & Diagnostics)

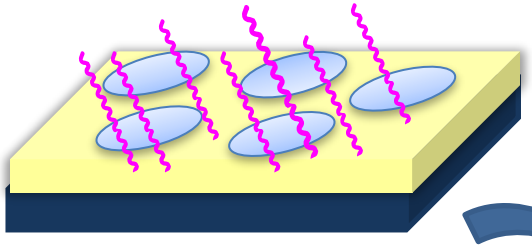
- Ligand binding against unknown and undiscovered biomarkers
- Manufacturing (pennies on the dollar)
- Stability (long shelf-life; heat denature/refold)



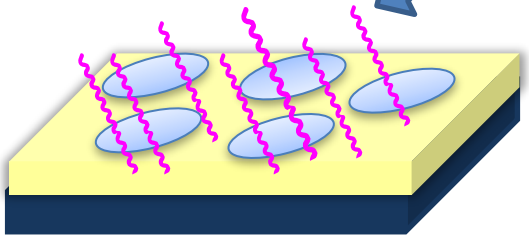
Aptamer Selection Protocol



Step 1. Attachment



Step 2. Addition of library



Step 3. Salt elution protocol

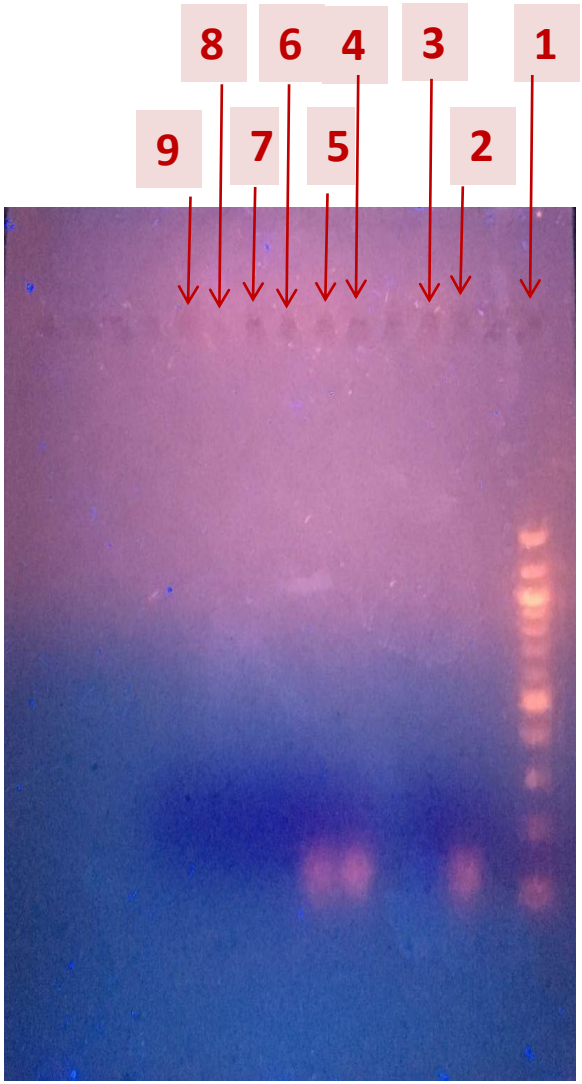
Step 4. PCR amplification

Step 5. Gel electrophoresis

Step 6. Dot-BLOT, Cloning, sequencing

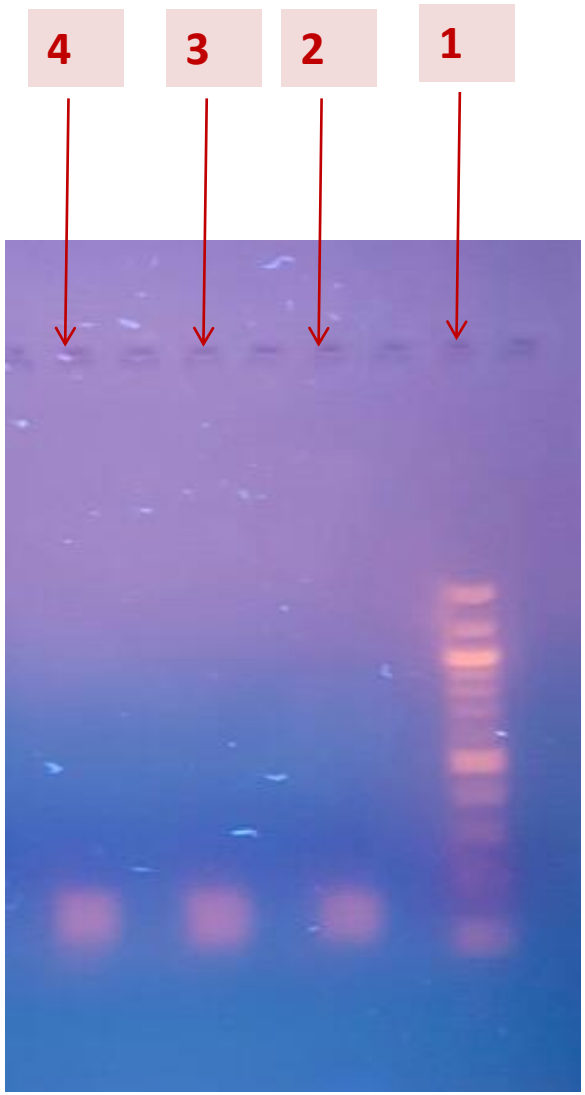
Negative selection with other fungal strains

PCR Amplification of Salt Elutes



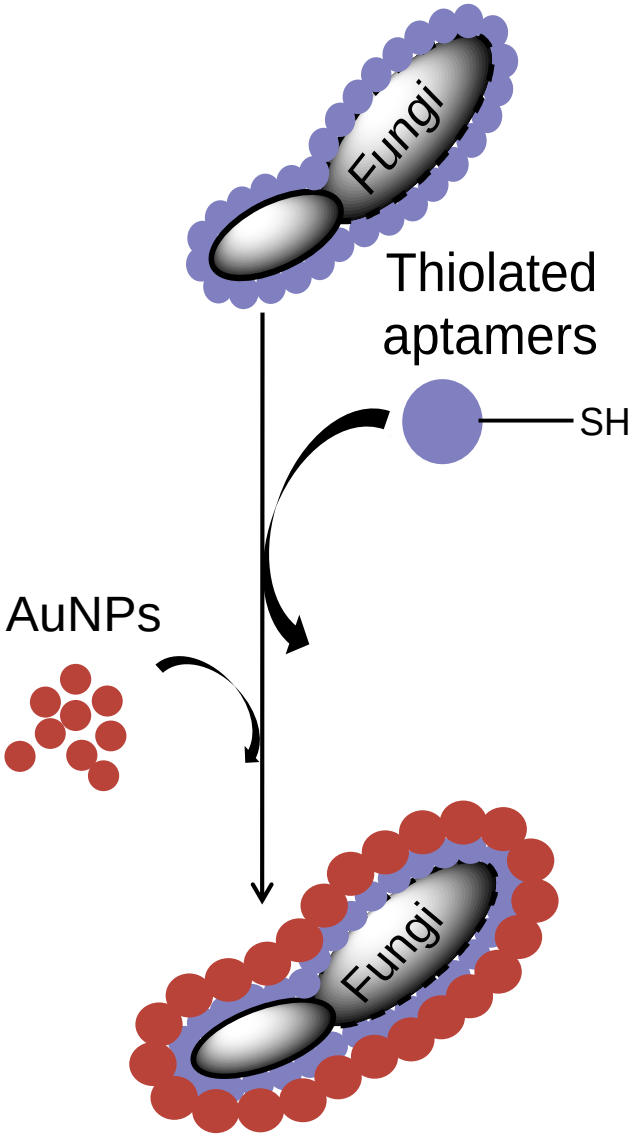
1	100 bp ladder
2	10 nm Library
3	Control
4	0.15 M NaCl
5	0.5 M NaCl
6	1.0 M NaCl
7	1.2 M NaCl
8	1.4 M NaCl
9	1.5 M NaCl

Biotinylation of Salt Elutes

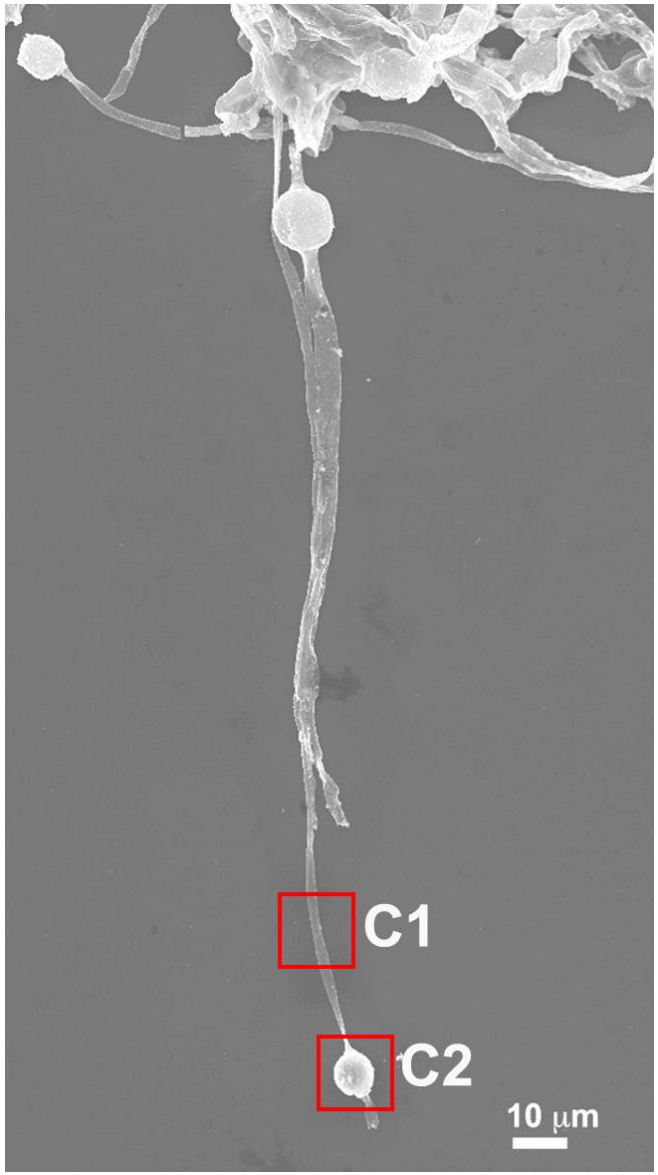


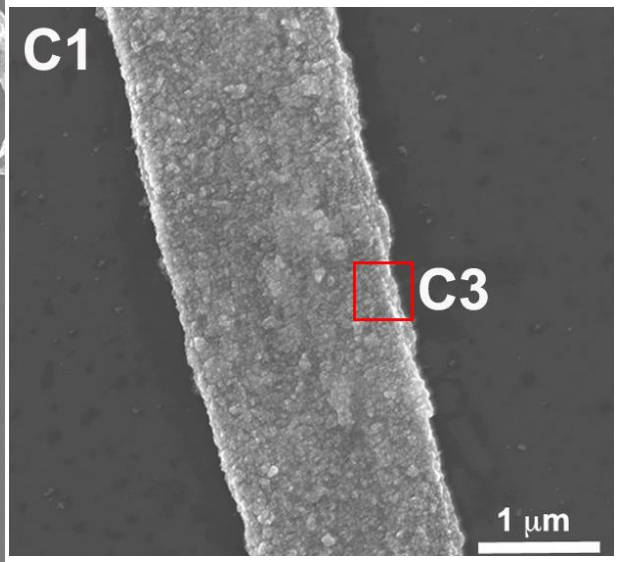
1	100 bp ladder
2	10 nm Library
3	0.15 M NaCl_Salt elutes
4	0.5 M NaCl_Salt elutes

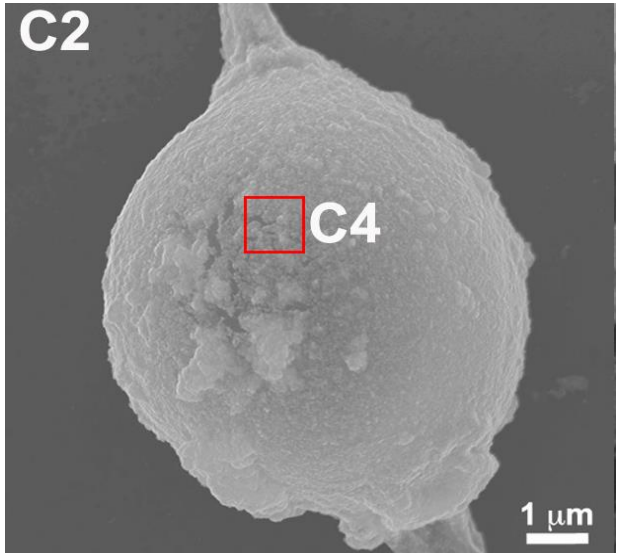
Our Technology

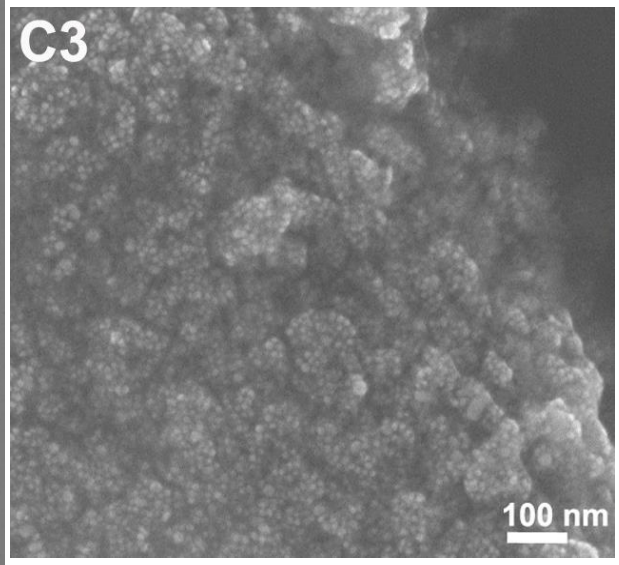


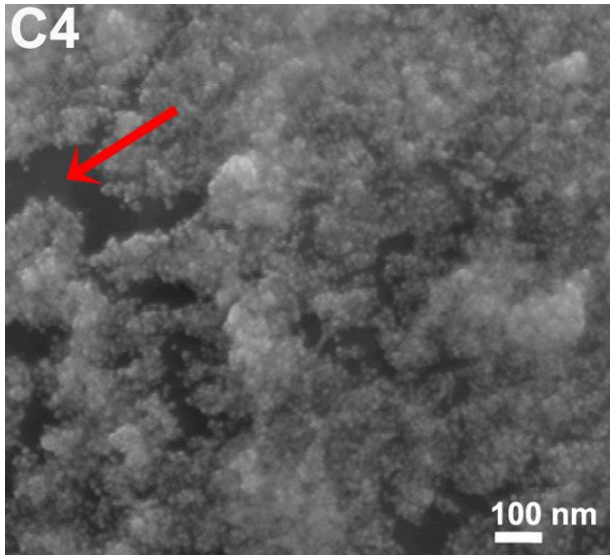
Our Technology





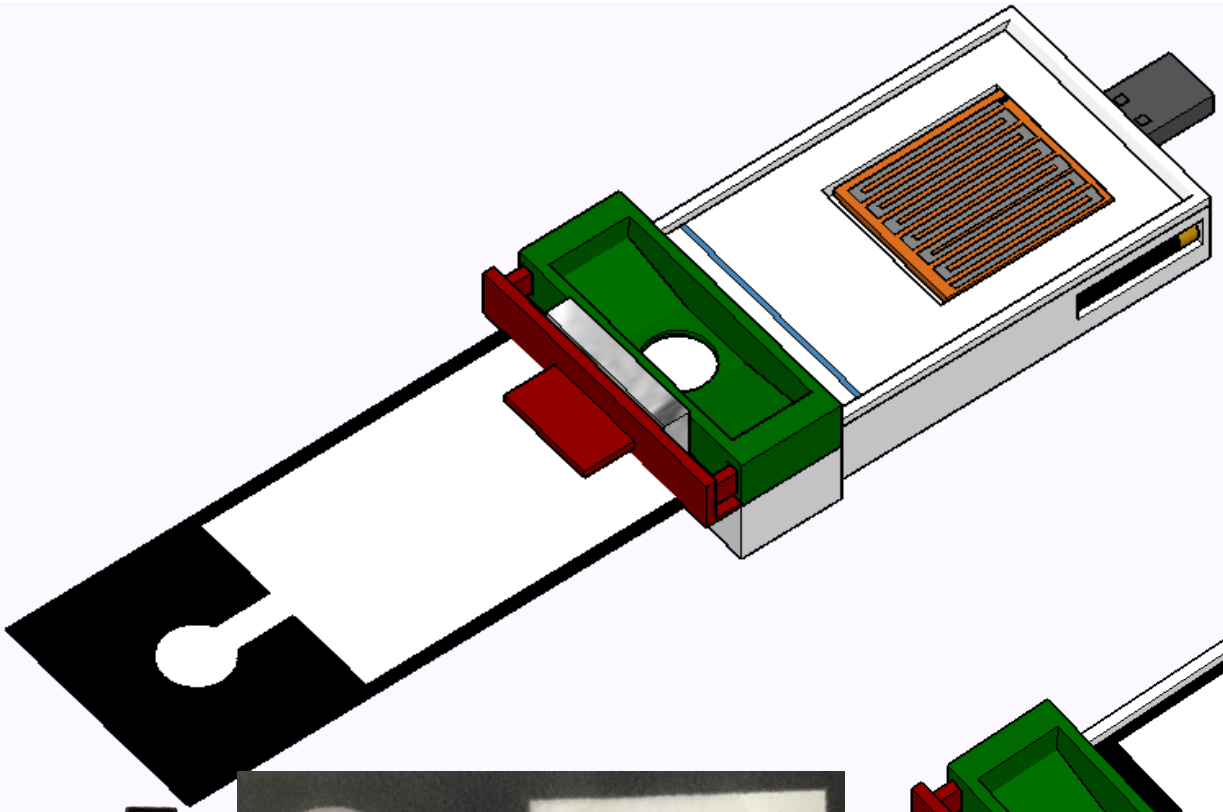


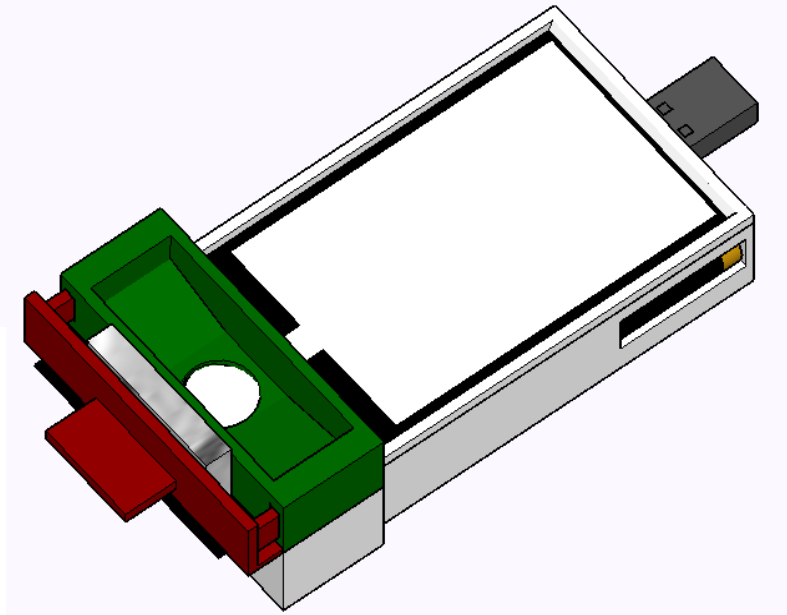


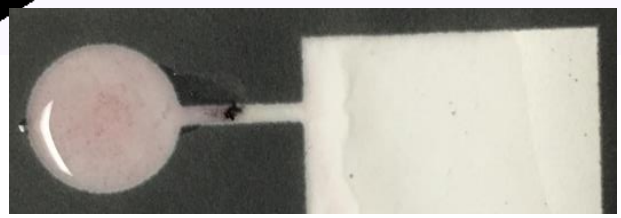


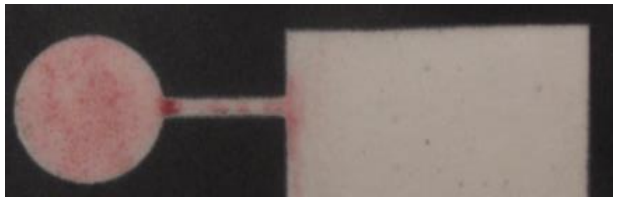
US Patent Pending, 2016

Our Technology









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Project Sponsors

Minnesota Invasive Terrestrial Plants and Pest Center (MITPPC)

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