IMPROVING OAK WILT DIAGNOSTICS IN MINNESOTA

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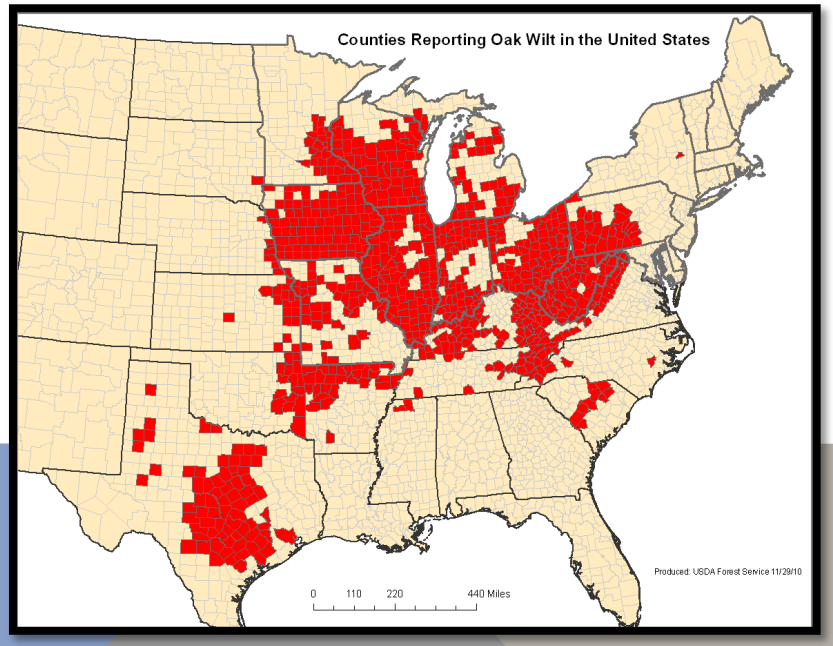
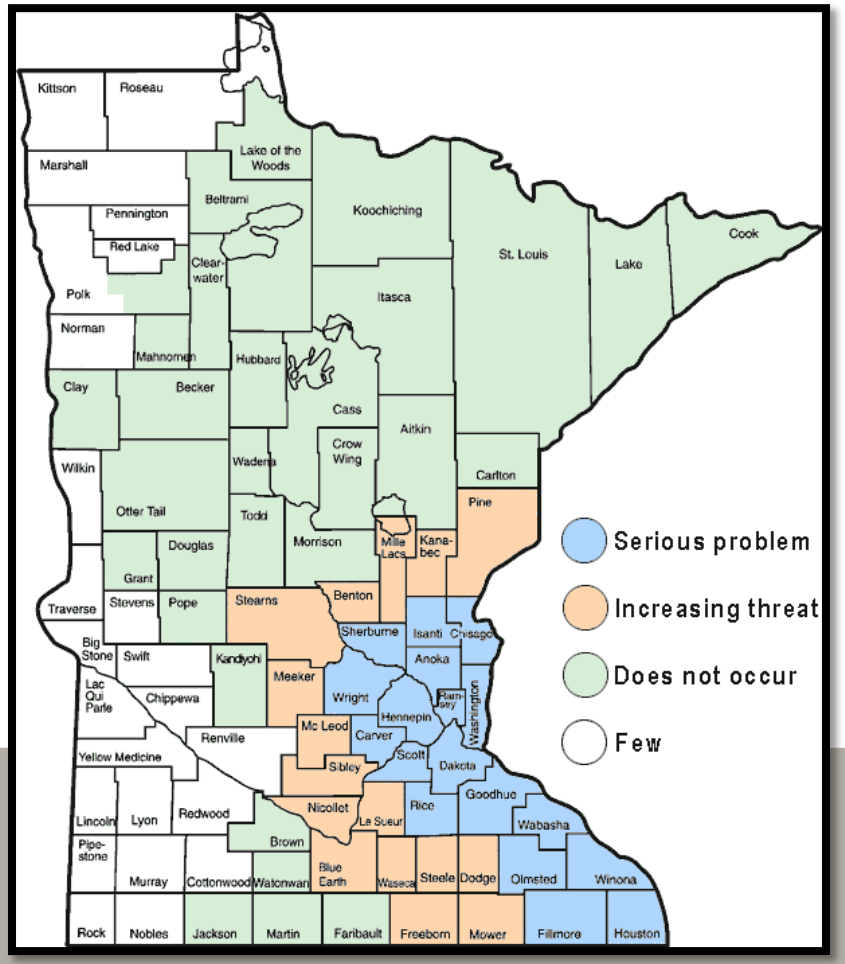
OAK WILT

- Significant disease of oak species
- Caused by *Ceratocystis fagacearum*



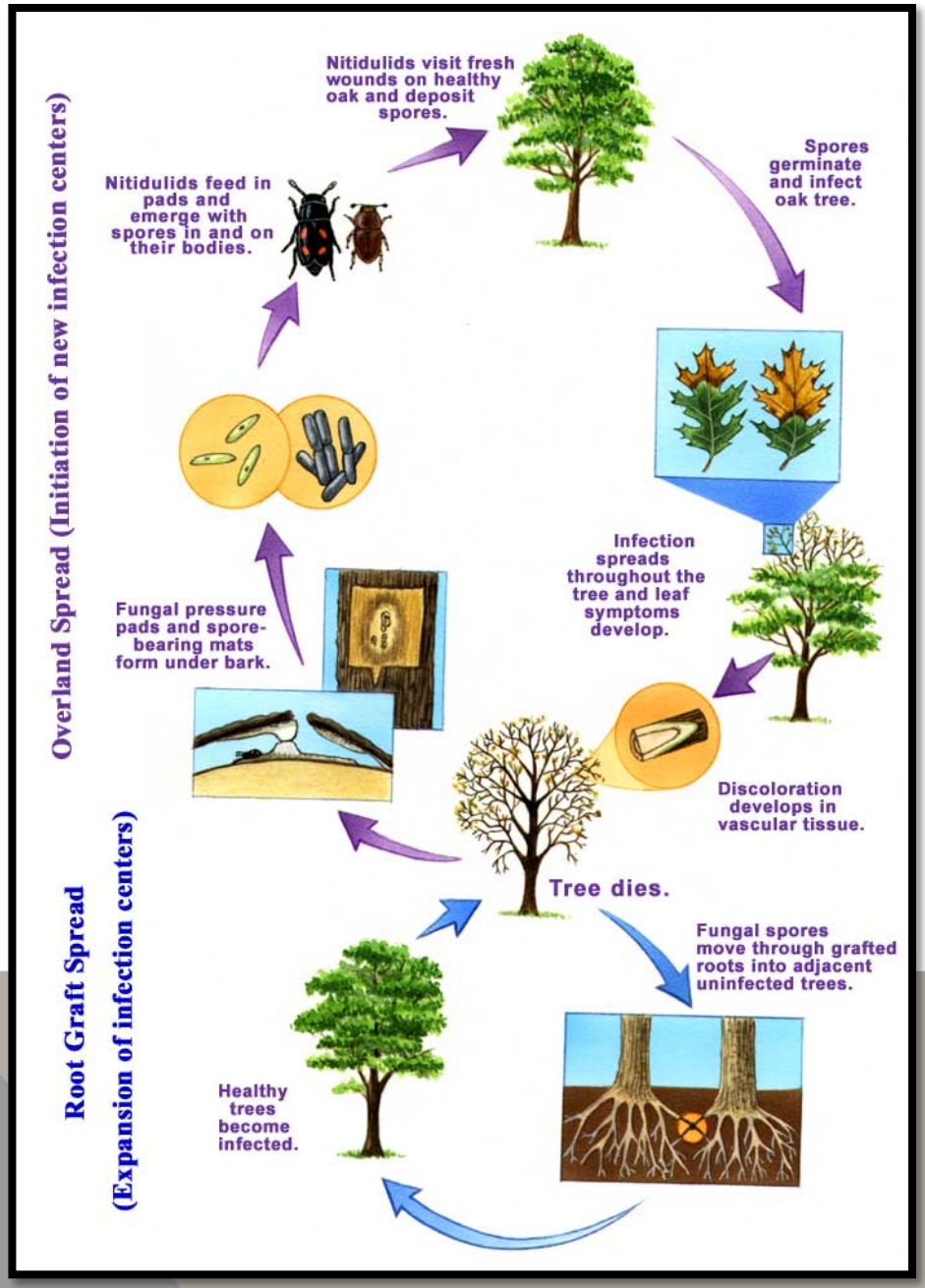
U.S. Distribution in 2010

Source: Q. Chavez and J. Pokorny. Northeastern Area State and Private Forestry, U.S. Forest Service.



MN Distribution

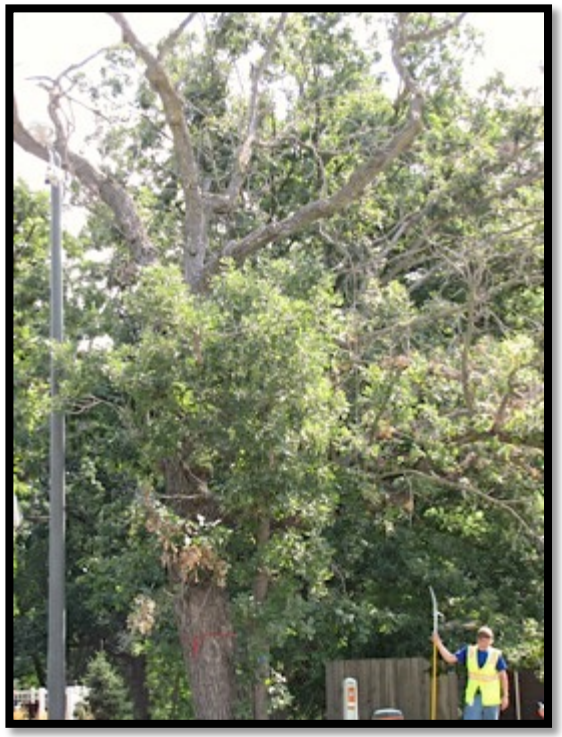
Source: J. Juzwik and D. French, 2002.


Overland Spread (Initiation of new infection centers)
Nitidulids visit fresh wounds on healthy oak and deposit spores.
Spores germinate and infect oak tree.
Infection spreads throughout the tree and leaf symptoms develop.
Discoloration develops in vascular tissue.
Tree dies.
Fungal spores move through grafted roots into adjacent uninfected trees.
Healthy trees become infected.
Root Graft Spread
(Expansion of infection centers)
Fungal pressure pads and spore-bearing mats form under bark.
Nitidulids feed in pads and emerge with spores in and on their bodies.

SYMPTOMS

Red Oak

Bur Oak



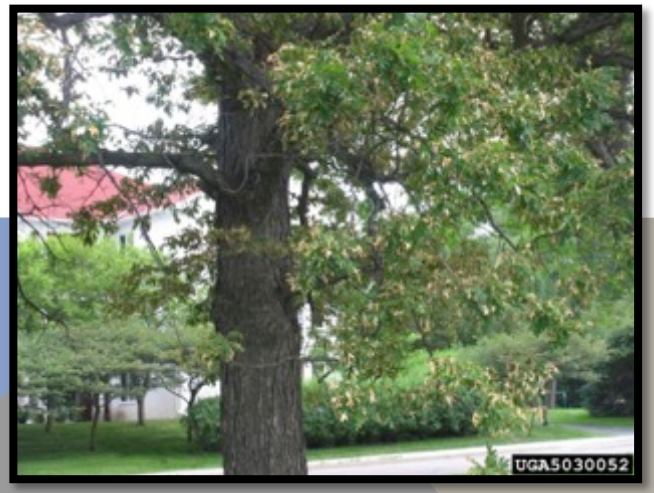
White Oak

Other insects and disease may mimic symptoms

COPY CATS

Bur Oak Blight

Source: T. Harrington, Iowa State University



Anthracnose

Source: J. O'Brien, U.S. Forest Service

Twolined Chestnut Borer

Source: S. Katovich, U.S. Forest Service

And many more!

DISEASE MANAGEMENT

- Effective management options available
- Dependent on ***accurate*** and ***timely*** diagnosis

Vibratory Plow Lines

Source: B. Cook, Michigan State University

CURRENT DIAGNOSTICS

Approach

- Visual assessment
- Sampling for diagnostic clinic

Lab Diagnosis

- Isolation from wood chips
- Long incubation
- Dependent on sample quality
- Occurrence of false negatives

Management / Treatment

CURRENT DIAGNOSTICS

Approach

- Visual assessment
- Sampling for diagnostic clinic

Sampling Steps:

1. Select partially wilted branch
2. Look for discoloration in sapwood
3. Sample from up to three branches
4. If branches are too high, sample main stem
5. Keep samples cool during transport

CURRENT DIAGNOSTICS

Lab Diagnosis

- Isolation from wood chips
- Long incubation
- Dependent on sample quality
- Occurrence of false negatives

Poor Sample Quality:

- Dead branches
- Dry samples
- Other fungi present

Total Time: 6-14 days

CURRENT DIAGNOSTICS

Approach

- Visual assessment
- Sampling for diagnostic clinic

Lab Diagnosis

- Isolation from wood chips
- Long incubation
- Dependent on sample quality
- Occurrence of false negatives

Effective treatment depends on early and accurate diagnosis

NEW METHODS

Rapid and accurate detection of *Ceratocystis fagacearum* from stained wood and soil by nested and real-time PCR

By C. P. Wu[1,2], G. Y. Chen[1], B. Li[2], H. Su[2], Y. L. An[2], S. Z. Zhen[2] and J. R. Ye[1,3]

[1]Jiangsu Province Key Laboratory for Prevention and Management of Invasive Species, Nanjing Forest University, Nanjing 210037, China; [2]Plant Quarantine Laboratory, Jiangsu Entry-Exit Inspection and Quarantine Bureau, Nanjing, China; [3]E-mail: jrye@njfu.com.cn (for correspondence)

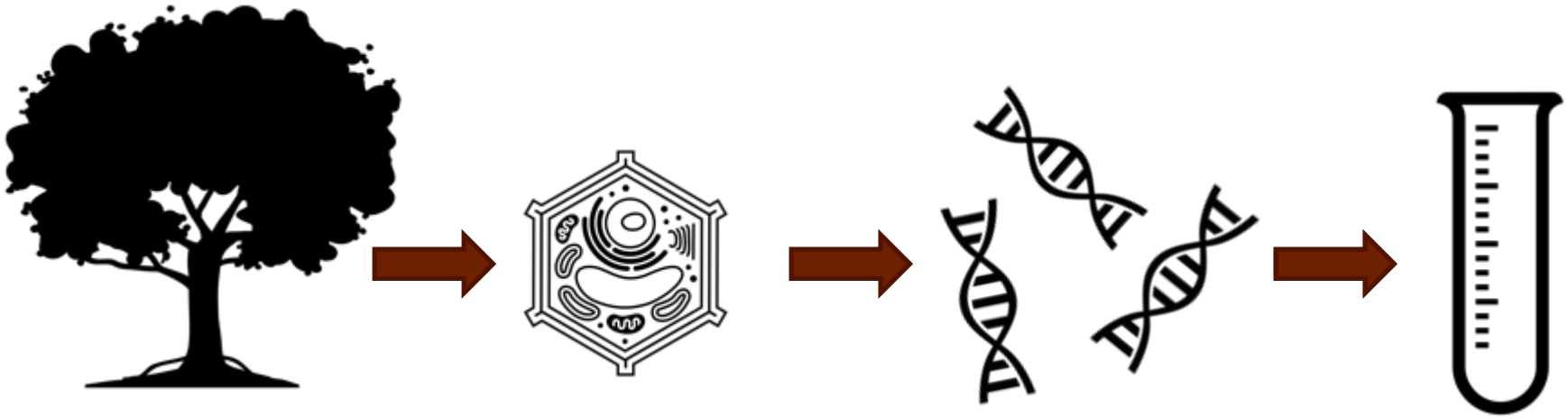
The detection of *Ceratocystis fagacearum* in Texas live oak using real-time polymerase chain reaction

T. KURDYLA (1), D. Appel (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 101:S95

Developed, but not fully useful yet.

OBTAINING DNA

DNA is extracted directly from wood

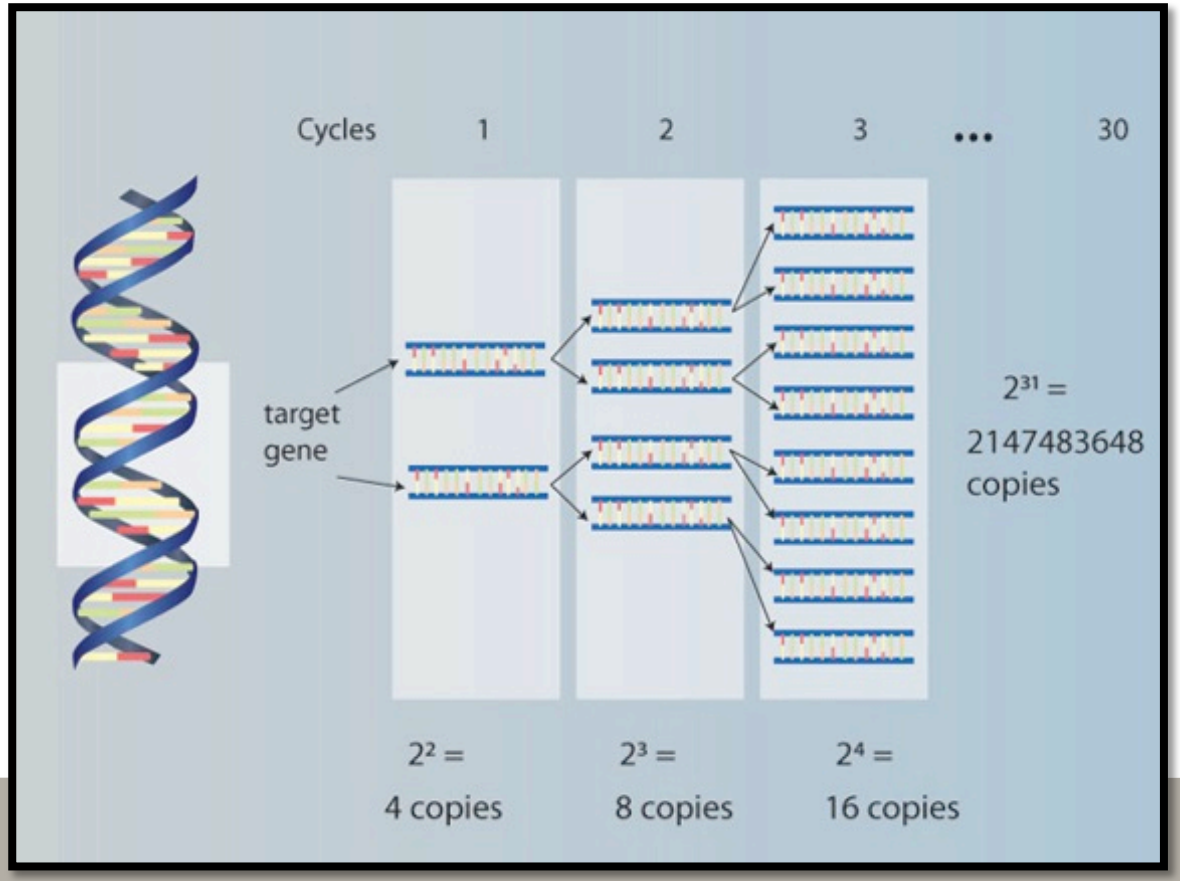


Tree by Humberto Pornaro from The Noun Project
DNA by Gilad Fried from The Noun Project
Test Tube by Olivier Guin from The Noun Project
Icon by Ben Didier from The Noun Project

WHAT IS PCR?

Polymerase
Chain
Reaction

Making millions of copies of a portion of DNA (think CSI).



Nested PCR

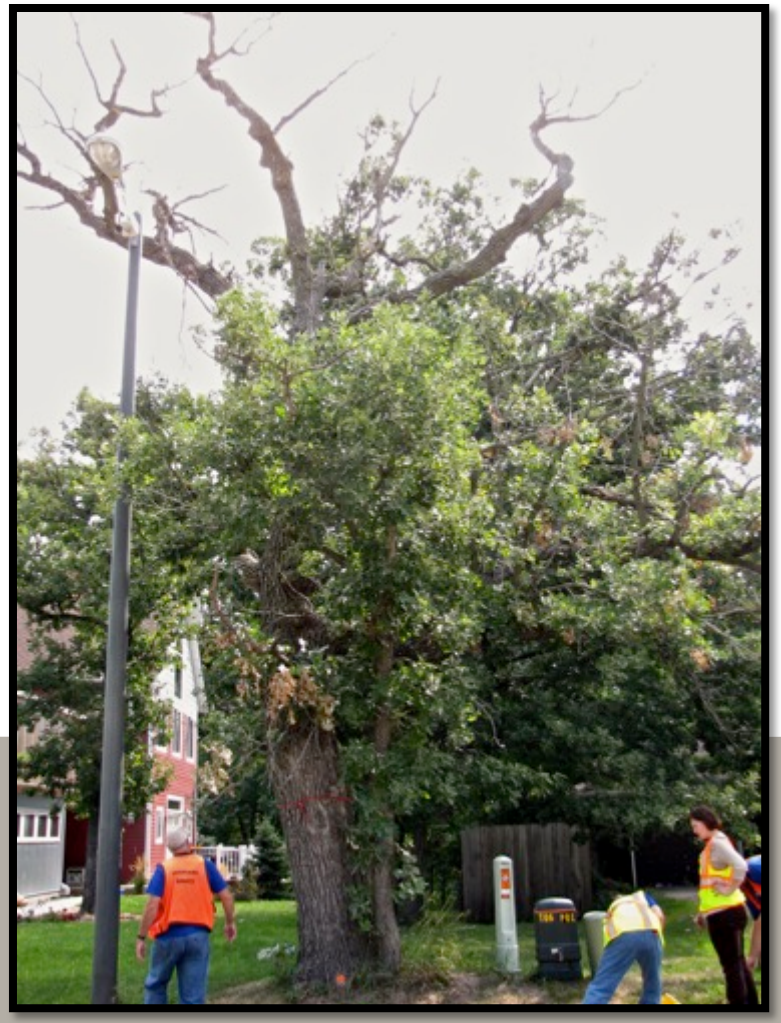
Real-Time PCR

RESEARCH OBJECTIVES

Test and modify nested and real-time PCR protocols for detection of *C. fagacearum* in sapwood

Develop and test field sampling and laboratory processing guidelines

Evaluate reliability and practicality for routine use by diagnostic laboratories



1. SAMPLING METHODS
2. SAMPLE PROCESSING
3. NESTED PCR + RESULTS
4. REAL-TIME PCR + RESULTS
5. CURRENT STATUS

SAMPLING METHODS – YEAR 1

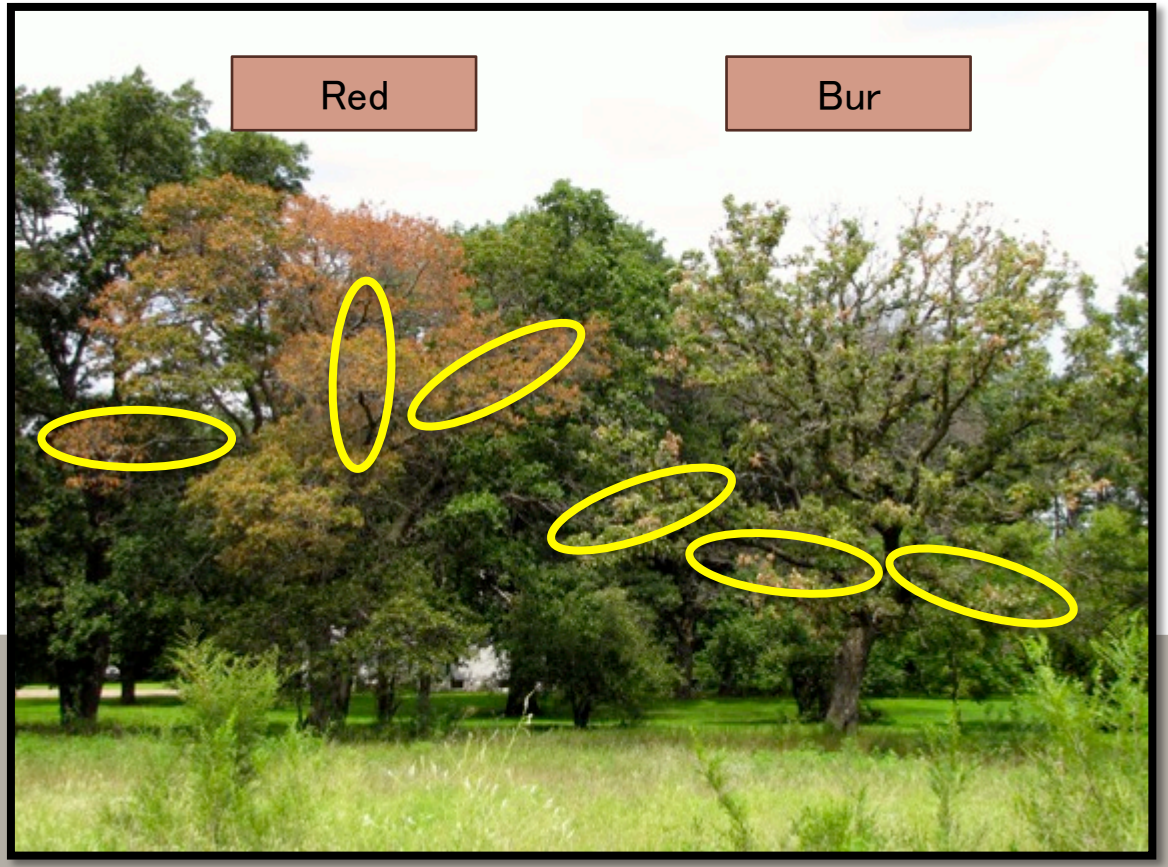
Actively Wilting Crowns

Three branches selected from each tree:

- Nine red oak
- Eight bur oak
- Eight white oak

Three healthy control trees of each species

Seven communities sampled



SAMPLING METHODS – YEAR 2

≥1 Year Dead Branches

Three branches selected from each tree:

- Three bur oak
- Four white oak

Two non-oak wilt affected control trees for each species

Seven communities sampled

SAMPLING METHODS – YEAR 2

≥1 Year Dead Red Oak

Three "windows" removed from each red oak tree:

- Six streaking
- Seven mat scar

Two non-oak wilt killed control trees

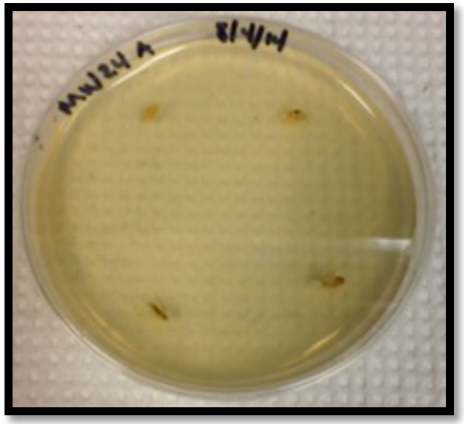
Two communities sampled

LABORATORY PROCESSING

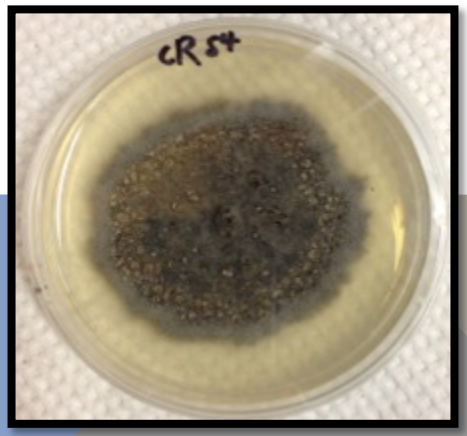
Isolation

Molecular Detection

Isolation



Plating Wood Chips



14 day incubation

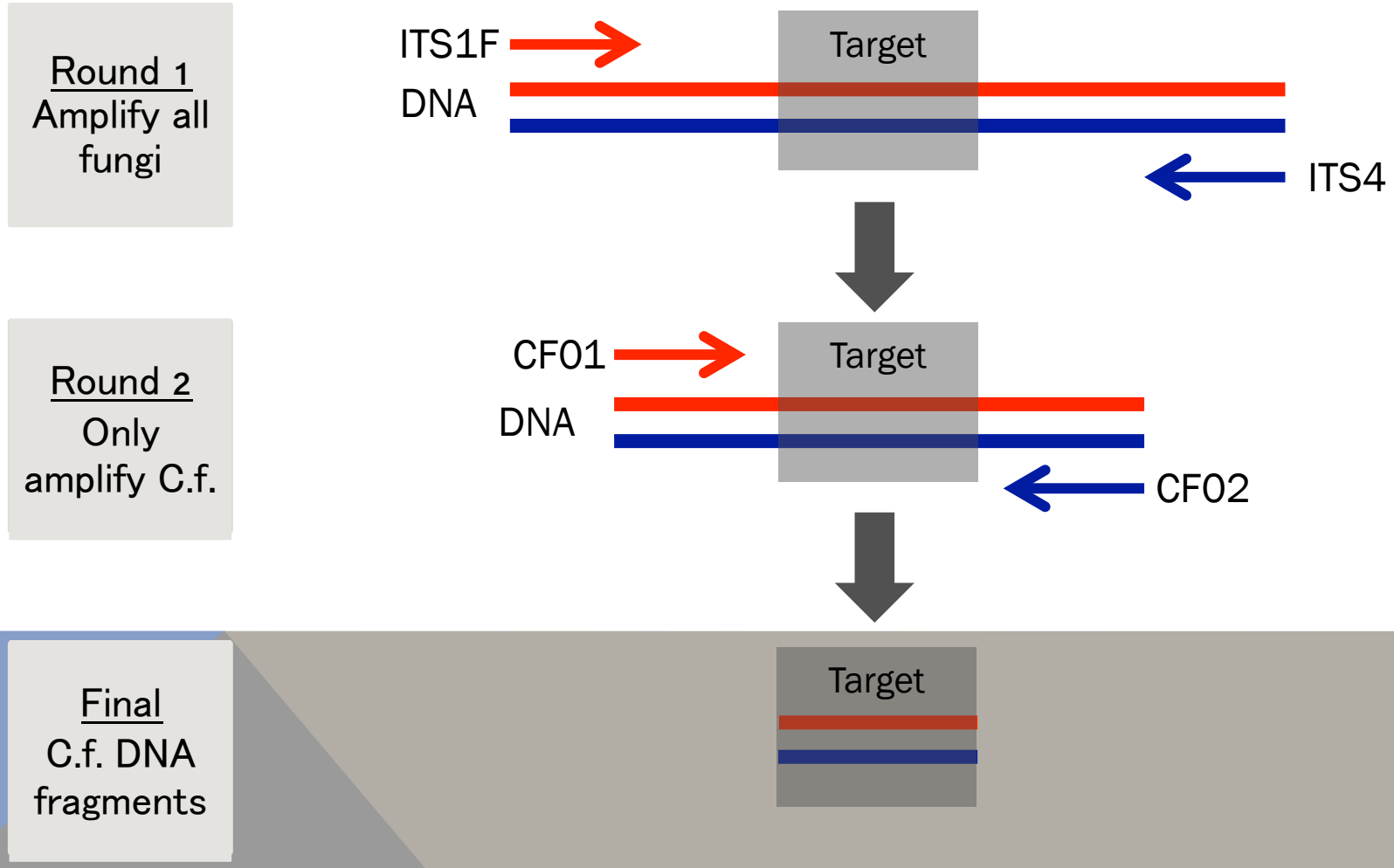
Molecular Detection

Sapwood Drilling

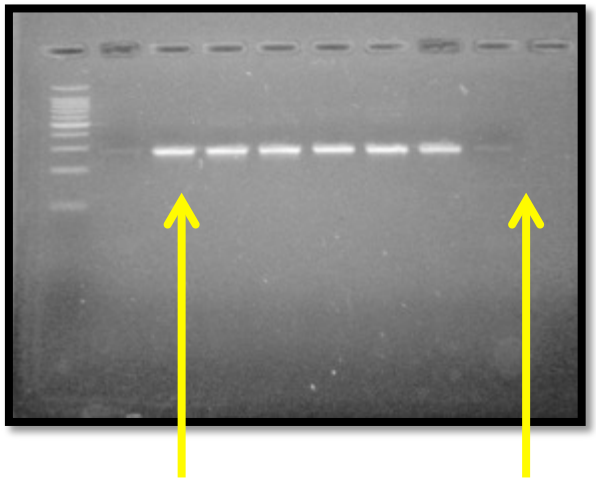
DNA Extraction

Nested PCR & Real-Time PCR

NESTED PCR


Round 1
Amplify all fungi
ITS1F
DNA
Target
ITS4
Round 2
Only amplify C.f.
CF01
DNA
Target
CF02
Final
C.f. DNA fragments
Target

NESTED PCR



Positive Negative

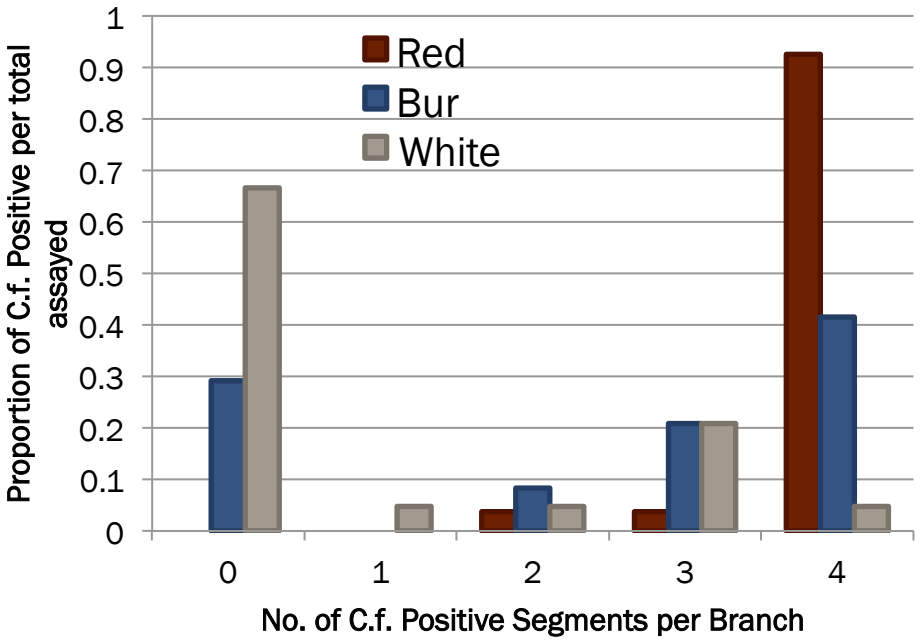
Description	Max score	Total score	Query cover	E value	Ident	Accession
Ceratocystis fagacearum strain C 660 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	379	379	100%	7e-102	100%	KC305153.1
Ceratocystis fagacearum strain C 520 18S ribosomal RNA gene, partial sequence; internal transcribed spacer	379	379	100%	7e-102	100%	KC305152.1

Extraction: 2 hours
Nested PCR : 6 hours
Total time: 1-2 days

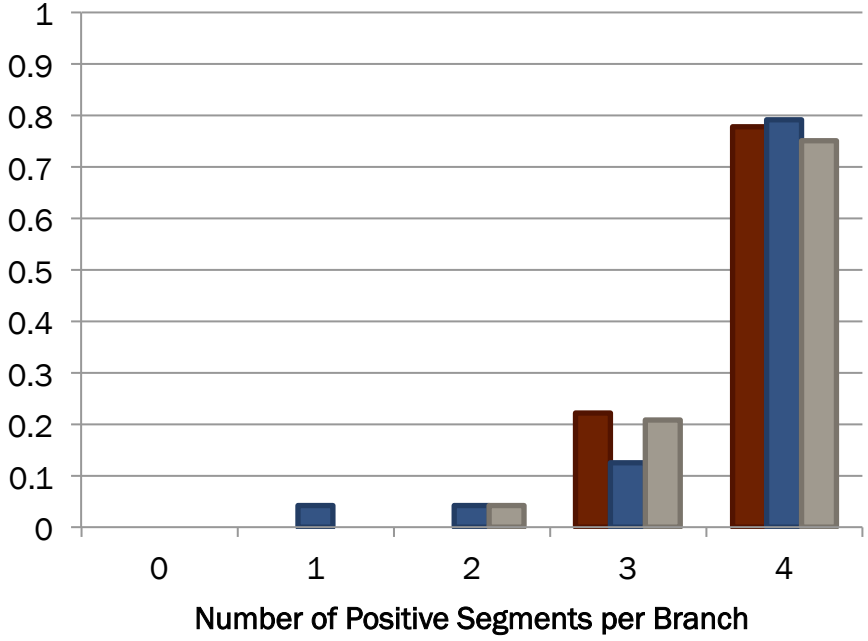
NESTED PCR RESULTS

ACTIVE WILT

Traditional Isolation



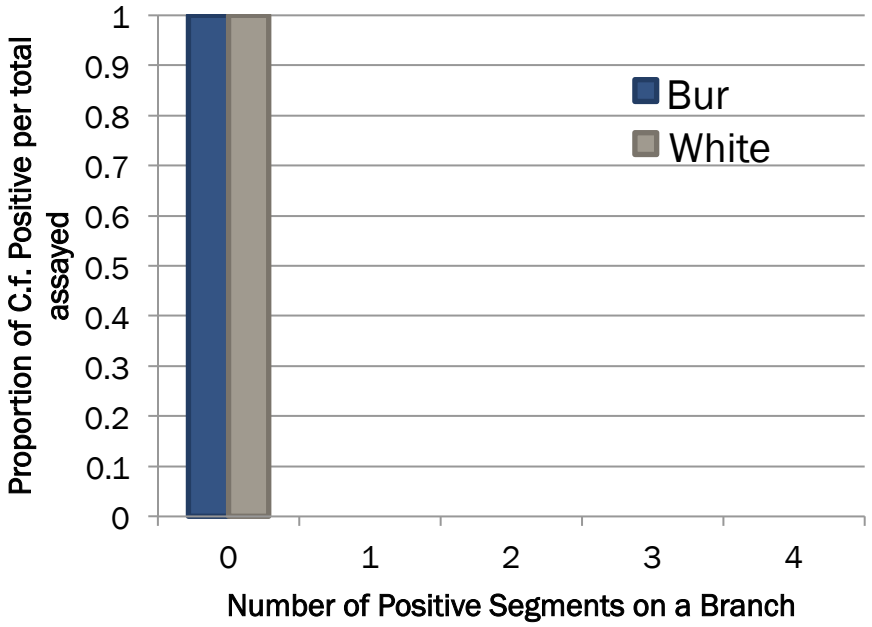
Nested PCR



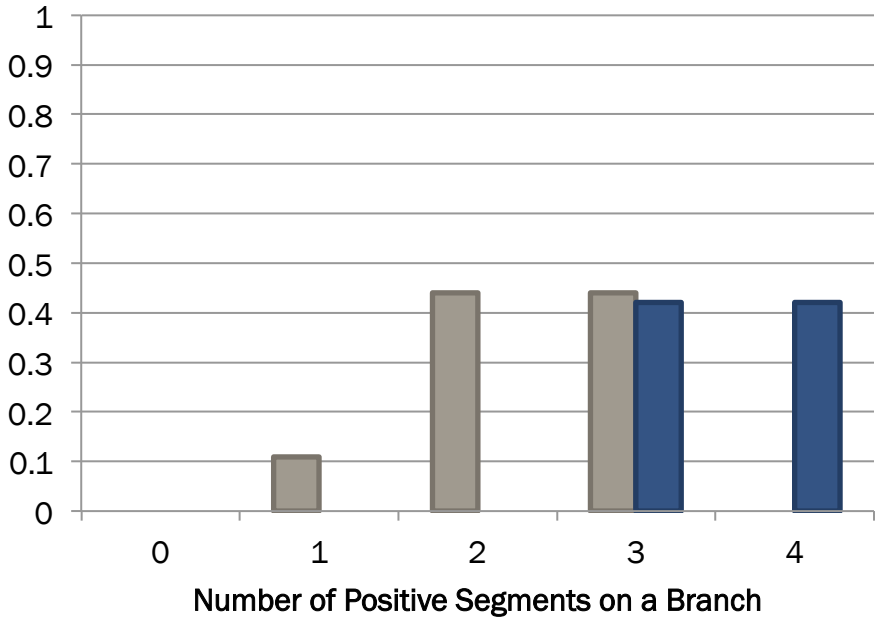
- Red Oak - little difference in detection between methods
- Bur and White Oak - nested PCR superior

≥1 YEAR DEAD BRANCHES

Traditional Isolation

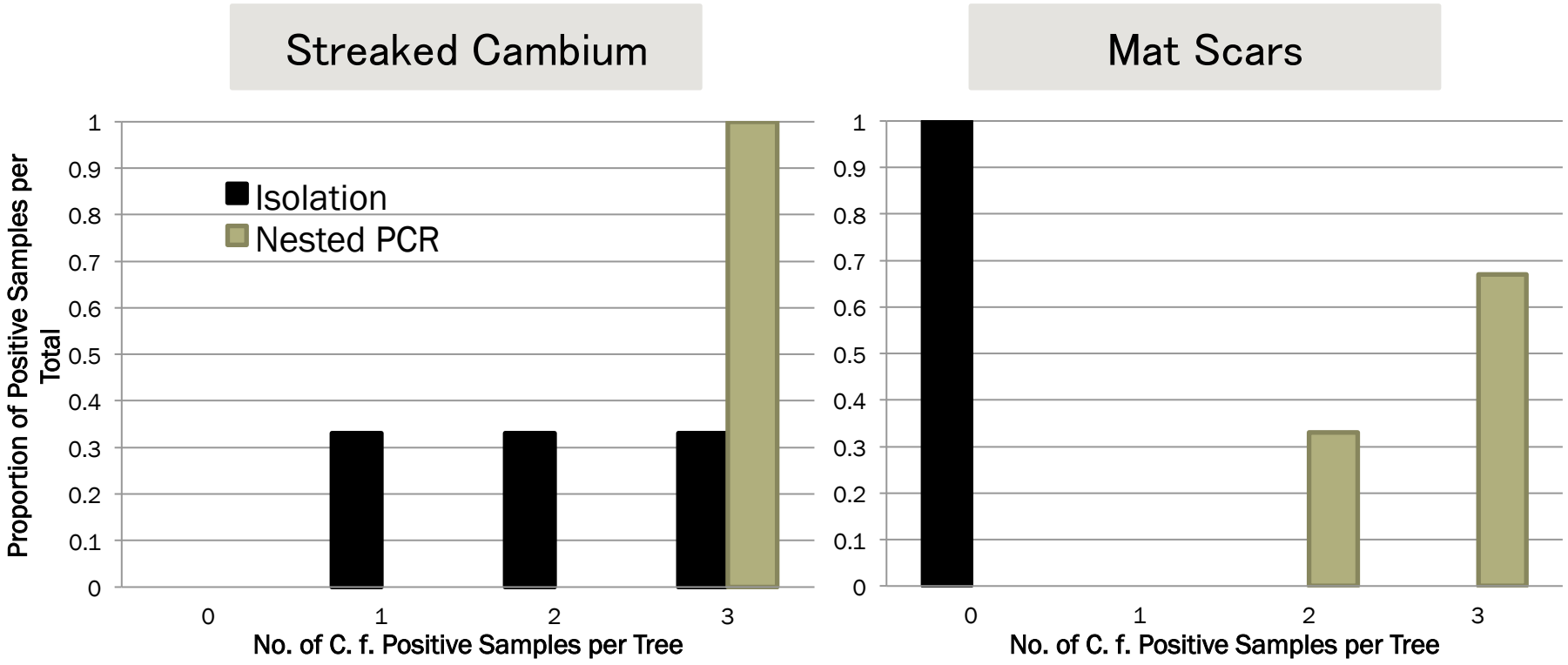


Nested PCR



- Bur and White Oak - only detected through nested PCR

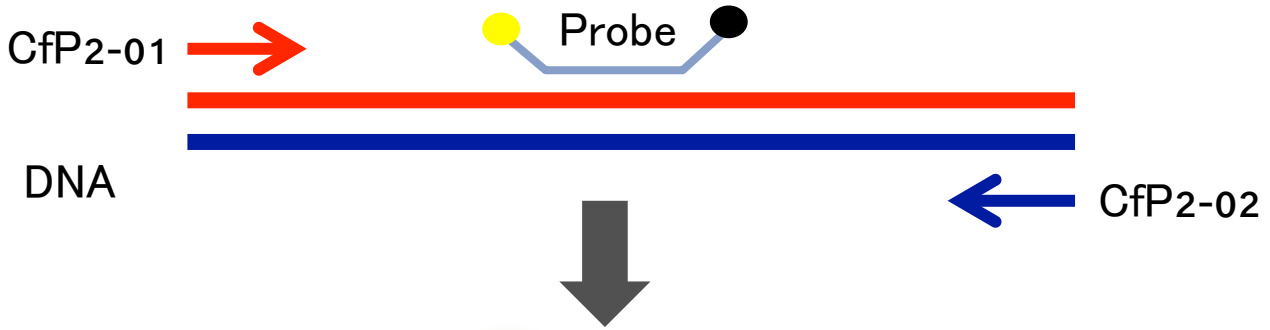
RED OAK - MAIN STEM SAMPLES



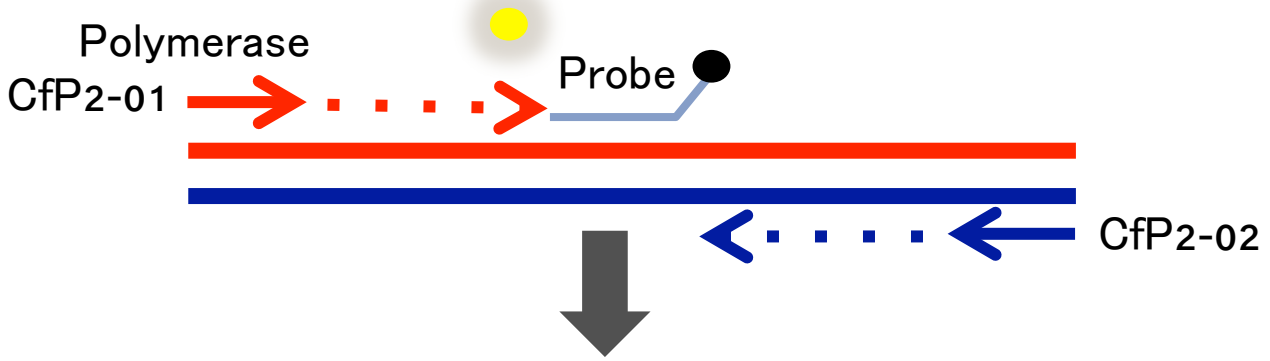
- Streaked Cambium - slightly higher detection through nested PCR
- Mat Scars - only detection through nested PCR

REAL-TIME PCR

Step 1:
Primer + Probe to amplify C.f.

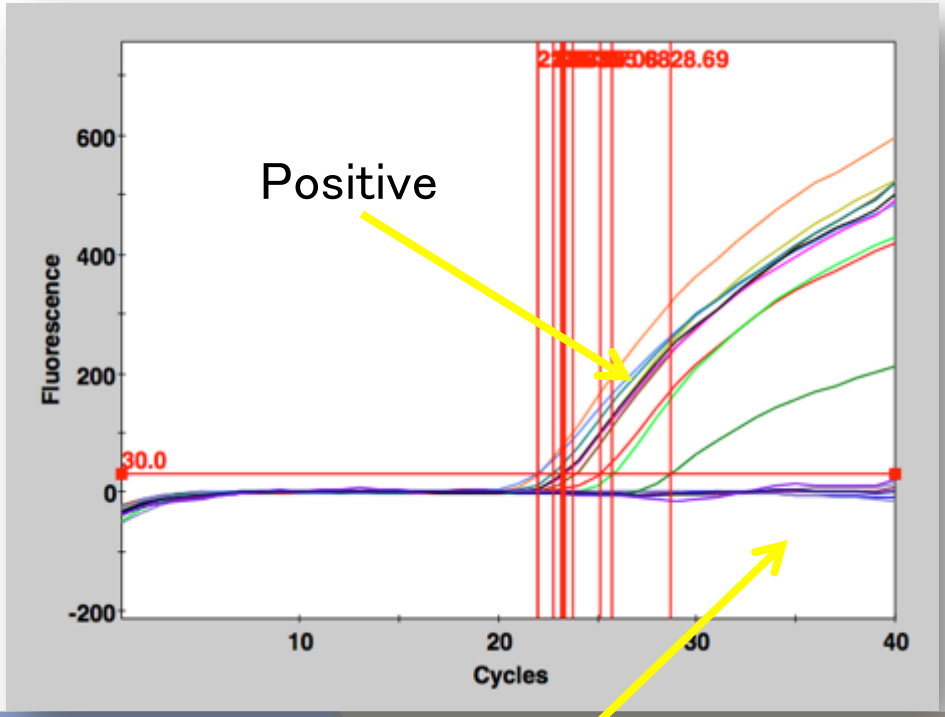


Step 2:
"Light up" every time DNA is doubled



Final:
C.f. DNA and "light up" molecules

REAL-TIME PCR



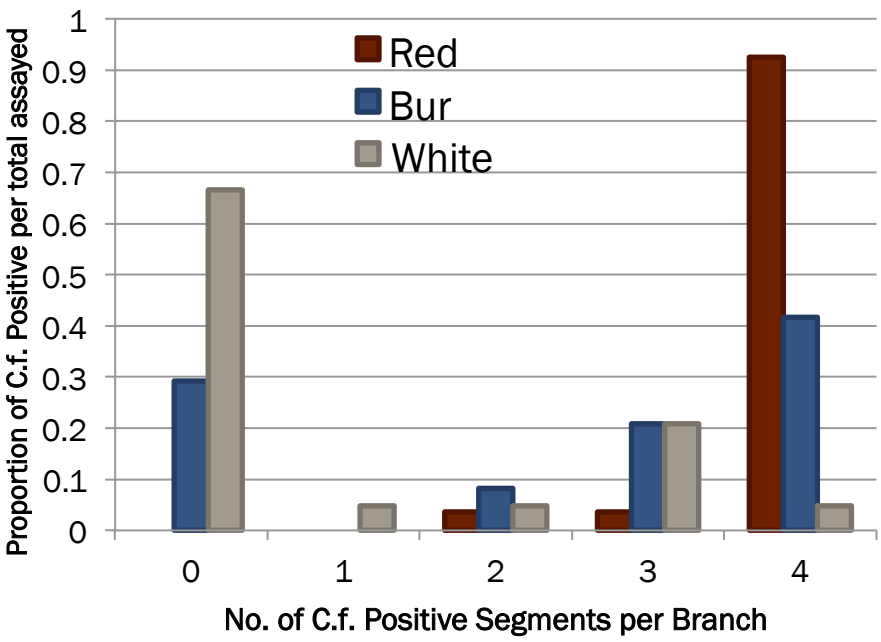
Site ID	Sample ID	FAM Ct	Protocol
A1	NTC	0.00	OW test
A2	NTC	0.00	OW test
A3	CF-0	22.03	OW test
A4	CF-2	28.69	OW test
A5	501	23.33	OW test
A6	501	23.77	OW test
A7	502	25.08	OW test
A8	502	25.68	OW test
A9	503	21.99	OW test
A10	503	22.75	OW test
A11	504	23.30	OW test
A12	504	23.18	OW test
A13	505	0.00	OW test

Extraction: 2 hours
Real-Time PCR : 2 hours
Total time: approx. 1 day

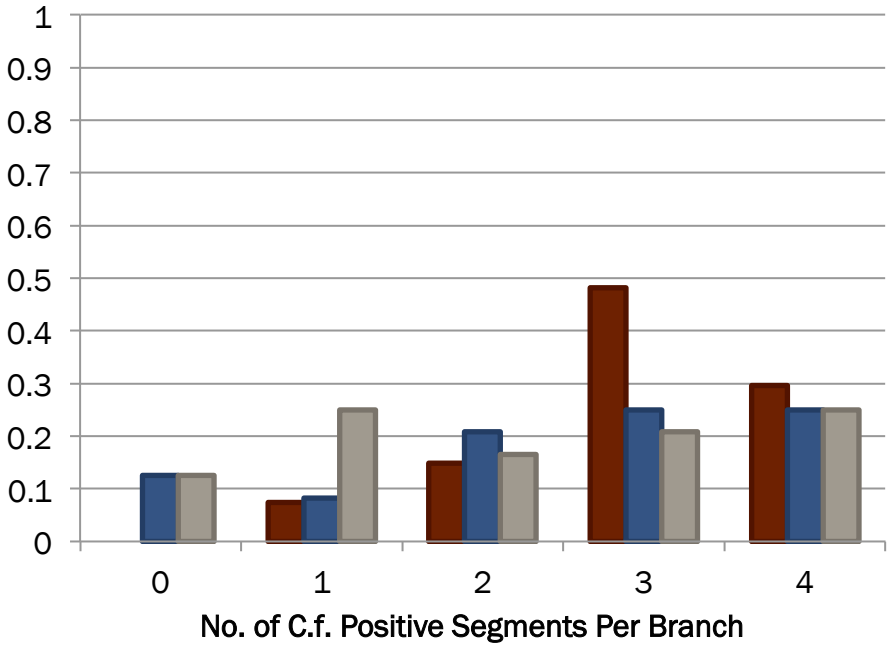
REAL-TIME PCR RESULTS

ACTIVE WILT

Traditional Isolation



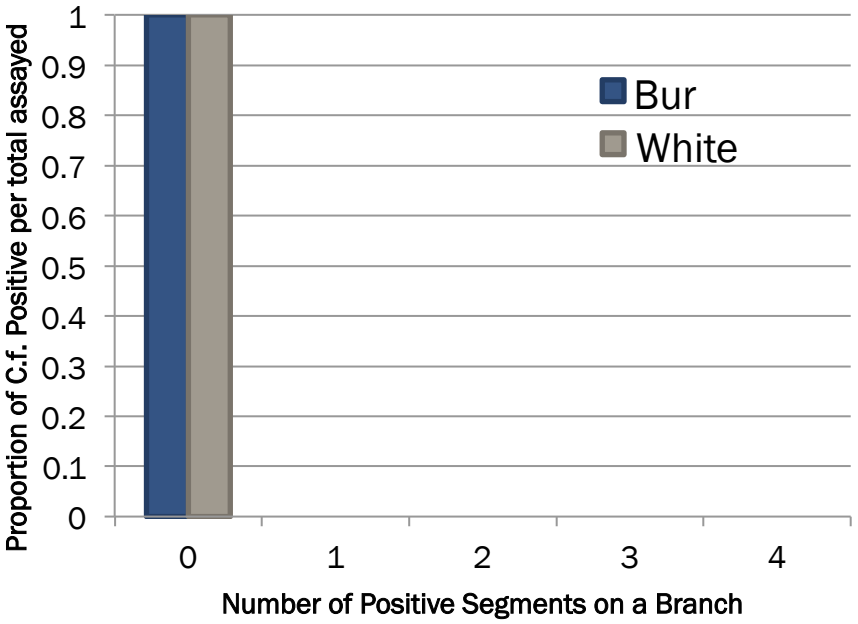
Real-Time PCR



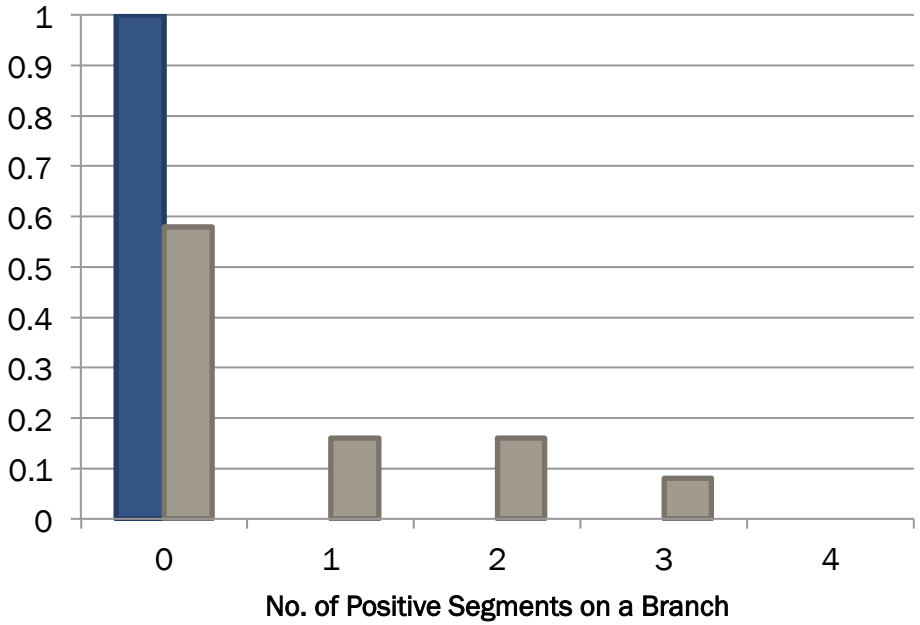
- Red Oak - isolation resulted in more frequent detection
- Bur and White Oak - hard to tell

≥1 YEAR DEAD BRANCHES

Traditional Isolation



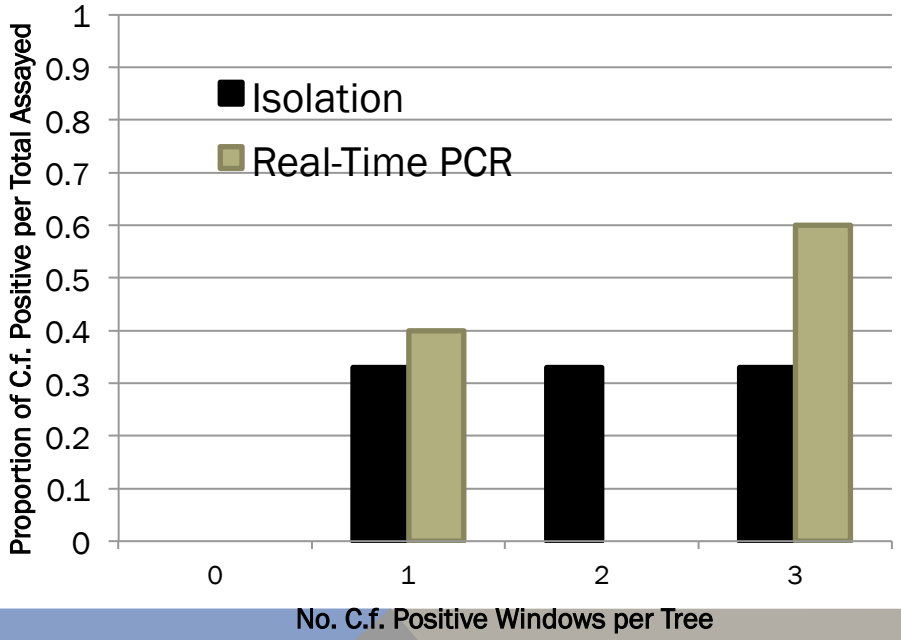
Real-Time PCR



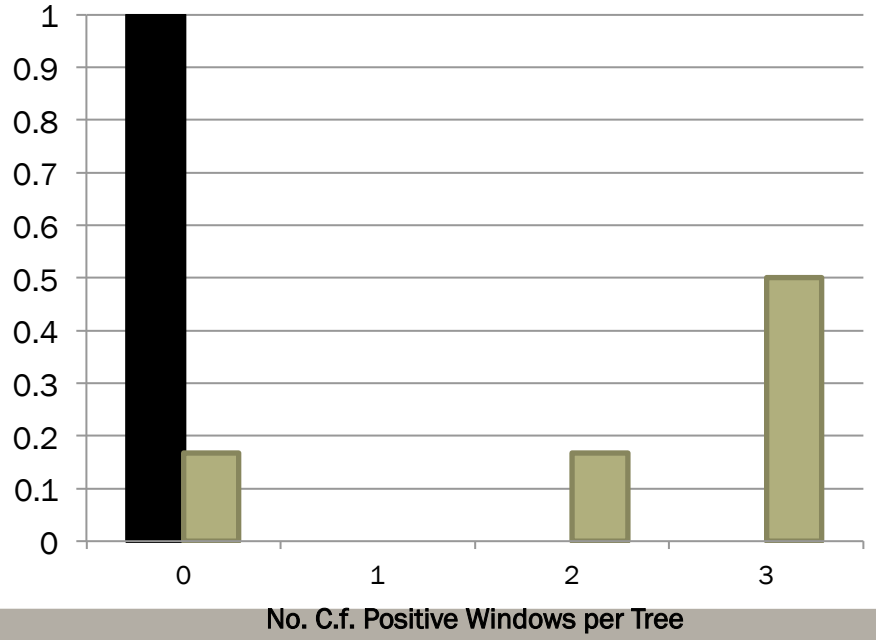
- Bur Oak - Not detected with either
- White Oak - only detected through real-time PCR

RED OAK - MAIN STEM SAMPLES

Streaked Cambium



Mat Scars



- Streaked Cambium - little difference between methods
- Mat Scars - better detection through real-time PCR

NEXT STEPS

Compiling data to compare all three methods to answer questions

Assess usability of new protocols

BETA-TESTING NEW PROTOCOLS

University of Minnesota

Nested PCR

University of Wisconsin

Real-Time PCR

- Cost Efficiency
- Reliability
- Practicality for routine use
- Differences between sample types/qualities

TECHNOLOGY TRANSFER

- Work with UMN and UW-Madison to publish in NPDN newsletter
- Other interested diagnostic clinics

ACKNOWLEDGEMENTS

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- City of Eagan
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- Three Rivers Park District
- City of Oakdale
- Boston Scientific
- City of Minnetonka
- Whispering Pines Realty
- G. Feasky, Private Land Owner
- City of Apple Valley

THANK YOU!