Fall 2014 MI SAF Meeting

Update on Breeding for BBD Resistant American beech And Selection & Characterization of "Lingering Ash"

HOST RESISTANCE AS A TOOL TO COMBAT INVASIVE THREATS

Jennifer Koch & Dave Carey
Northern Research Station
US Forest Service

Genetic Variation is the Basis for Tree Improvement

Natural stands have LOTS of genetic diversity

- Rare alleles may confer resistance (or tolerance) even when there is no co-evolution
- Rare because no selective advantage prior to invasive threat

Careful selection and breeding are required to maintain genetic diversity

Breeding for Resistance:

Select: Identify Resistant Germplasm

↓

Accession: Preserve & propagate resistant germplasm

↓

Phenotype: Verify resistance (test plantings and/or bioassays)

↓

Validate: Genetic studies (controlled crosses) to demonstrate heritability and mode of inheritance

↓

Breed & Deploy: Develop operational program to breed resistance & maintain genetic diversity at population level

Beech Bark Disease: The Causal Complex

Cryptococcus fagisuga:
the beech scale insect

(fruiting bodies photograph from David R. Houston and James T. O'Brien FID Leaflet #75)

Sexual fruiting bodies, or perithecia
Neonectria faginata **–possibly derived from introduced species**
Neonectria ditissima-**native species**

***Resistance is to the scale insect!!!**

Genetic Studies

Full- and Half-Sib Families

Family	Source
1505(R) x 1504(R)	Controlled cross
ME(R) OP	Managed stand No S
1506(S) x 1504(R)	Controlled cross
1506(S) OP	Open Pollinated
1504(R) OP	Open Pollinated
1510(S) OP	Open Pollinated

Using the Artificial Inoculation Technique to Screen Seedlings for Beech Scale Resistance

Collect eggs from an infested tree (look up!)

Beech scale adults & eggs.

Scale eggs are placed on foam and tied to the stems of seedlings.

Eggs hatch & colonize susceptible trees.

(Derived from D Houston 1982 Res. Paper NE-506)

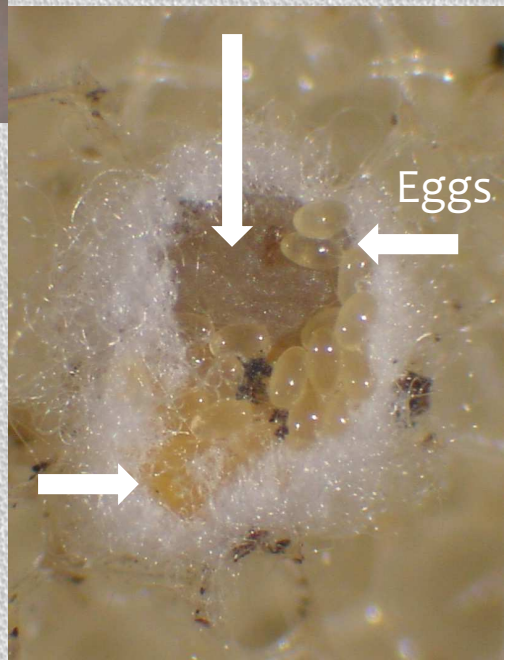
Artificial Inoculation Technique-One Year Later:

Resistant

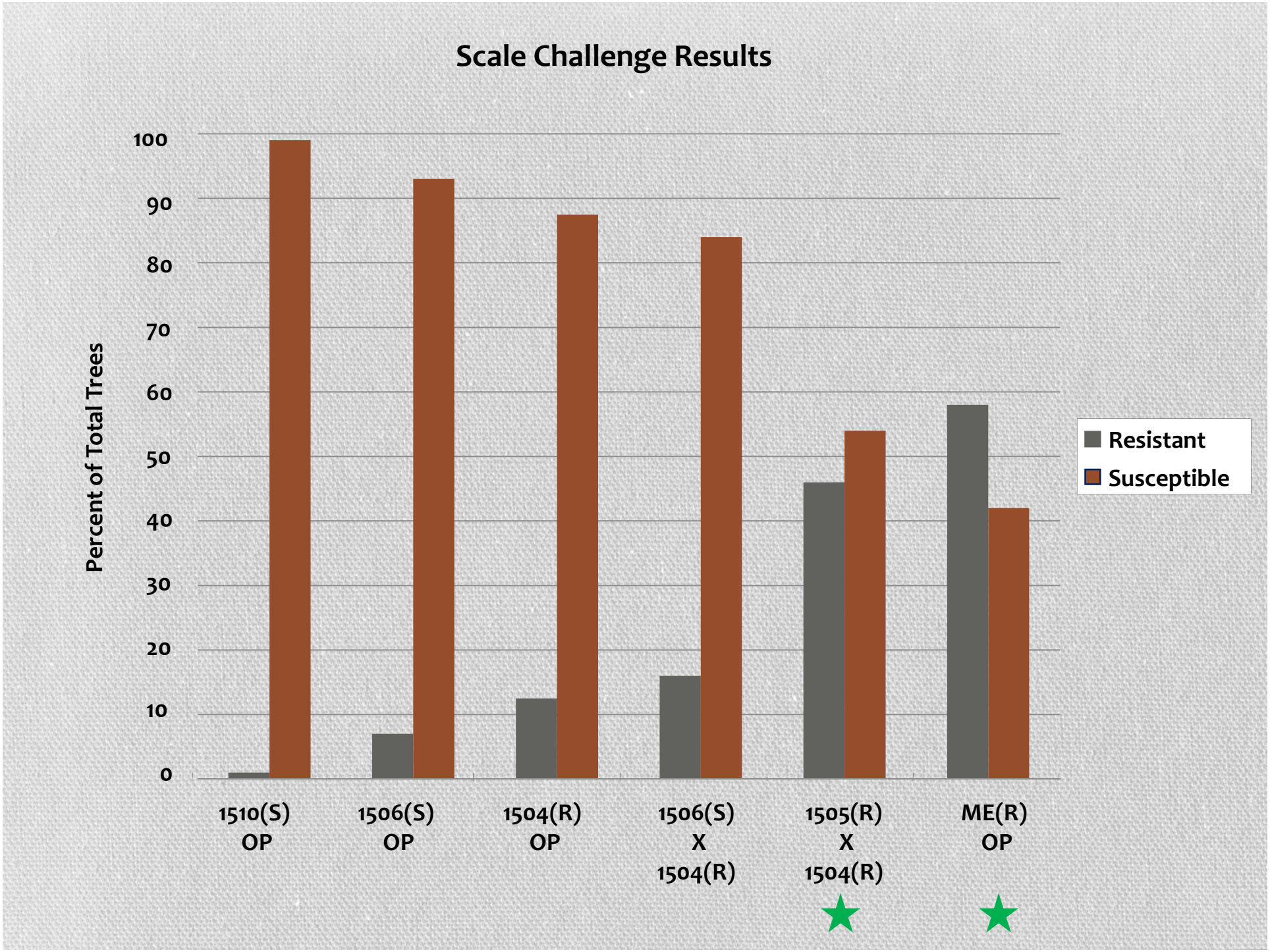
Susceptible

Highly Susceptible

Adult Scale Insect



Juvenile nymphs


Scale Challenge Results
Percent of Total Trees
100
90
80
70
60
50
40
30
20
10
0
1510(S) OP
1506(S) OP
1504(R) OP
1506(S) X 1504(R)
1505(R) X 1504(R)
ME(R) OP
Resistant
Susceptible

What we have learned about beech scale resistance:
(in collaboration with Dana Nelson, SRS)

1. Confirmed that scale resistance is inherited
2. Resistance can be increased through breeding
3. Approx. 50 % resistant progeny when both parents are resistant
4. Basis for development of American beech seed orchards

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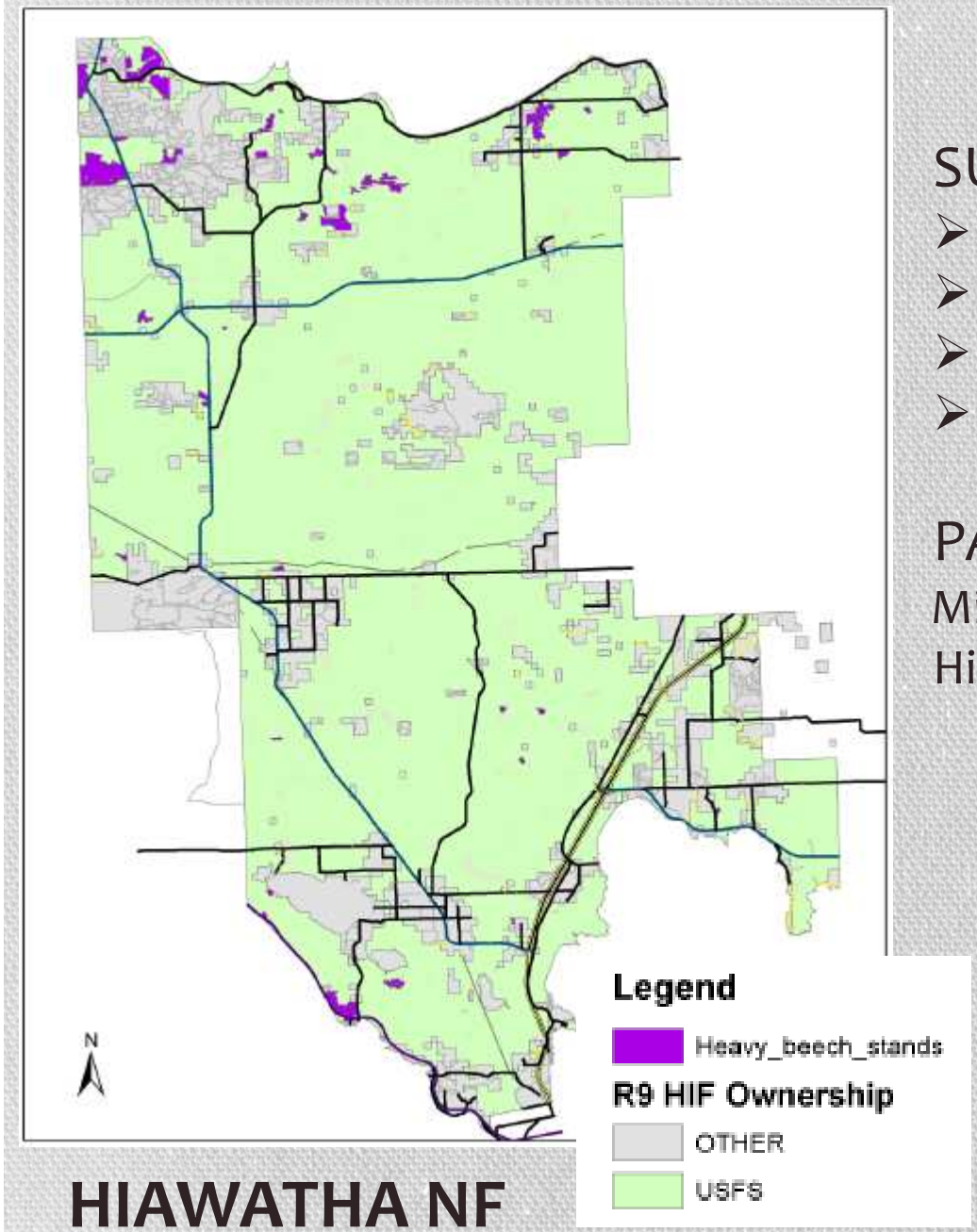
MI BEECH TREE IMPROVEMENT PROGRAM: SELECTION



HIAWATHA NF

SURVEYING

- Focus on areas with high % beech
- Focus on heavily BBD impacted areas
- Look for trees with healthy crowns
- Lack of any insect/disease symptoms

PARTNERS

Michigan DNR
Hiawatha NF

Mention Mi DNR resistant beech
Reporting form & bring copies

BEECH TREE IMPROVEMENT PROGRAM: SELECTION

Field Screen for Scale-Resistance

Collect scale eggs

Foam pad with eggs placed against bark and covered with Tyvek

Test pad removed after a year, reveals susceptible tree

Koch, Jennifer L.; Carey, David W. 2014. A technique to screen American beech for resistance to the beech scale insect (*Cryptococcus fagisuga* Lind). Journal of Visualized Experiments. e51515.
http://www.fs.fed.us/nrs/video/46347/

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BEECH TREE IMPROVEMENT PROGRAM: PROPAGATION

1. **SCION COLLECTION** (Partners: MI DNR, Region 9, ORSO)
2. **SEED COLLECTION & ROOTSTOCK GERMINATION** (Partners: NRS, Region 9, ORSO, MI DNR, MSU/MI Tree Cooperative)
3. **GRAFTING** (Partners: NRS, ORSO)

Photo: T.Hall

Photo: P.Berrang

Beech rootstock germinating

Hot-callus beech grafting

Carey, David W.; Mason, Mary E.; Bloese, Paul; Koch, Jennifer L. 2013. Hot callusing for propagation of American beech by grafting. HortScience. 48(5): 620-624.

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BEECH TREE IMPROVEMENT:BREEDING & TESTING

POTTED SEEDLING/GRAFTED RAMET SCREEN (Northern Research Station, The Holden Arboretum)

Containerized crosses on grafted ramets

Beech scale screening facility
The Holden Arboretum

Resistant Susceptible

Containerized Seed Orchard: More Families!

Maternal Parent	Paternal Parent	Family Size	Number of Resistant Progeny	Number of Susceptible Progeny	Proportion of Resistant Progeny
1201	1208	24	12	12	**50 %**
1202	1208	13	7	6	**54 %**
1209	1219	21	8	13	**38 %**
1211	1228	44	23	21	**52 %**
1228	1211	43	25	18	**58 %**
1505	ME-85	11	5	11	**45 %**
1505	ME-23	22	18	4	**81 %**

- Confirms initial results
- Possibly different combining abilities
- Pilot restoration plantings

BEECH TREE IMPROVEMENT PROGRAM: PROPAGATION

SEED ORCHARD ESTBLISHMENT (Partners: NRS, MI DNR, MSU,HTRIC)

22 BBD Resistant Genotypes

- 16 from UP State Land
- 5 from UP Hiawatha NF
- 1 from LP Ludington SP
- 8 ramets per genotype per site = 176

Two Replicate Planting Sites

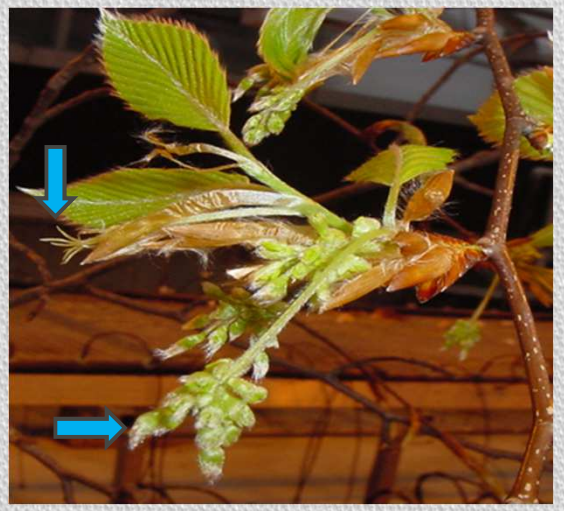
- Hardwood Tree Regeneration & Improvement Center
  Purdue University & USFS Northern Research Station
  West Lafayette, IN
  Installation fall 2015 (97% of propagation complete)

- Kellogg Experimental Forest
  Michigan State Univerity
  Augusta, MI
  Installation fall 2016 (57 % of propagation complete)

We've continued to perform controlled crosses as opportunities arise.

American beech is monoecious and self sterile.

The goal is to have each parent in at least 2 crosses (daisy chain strategy).



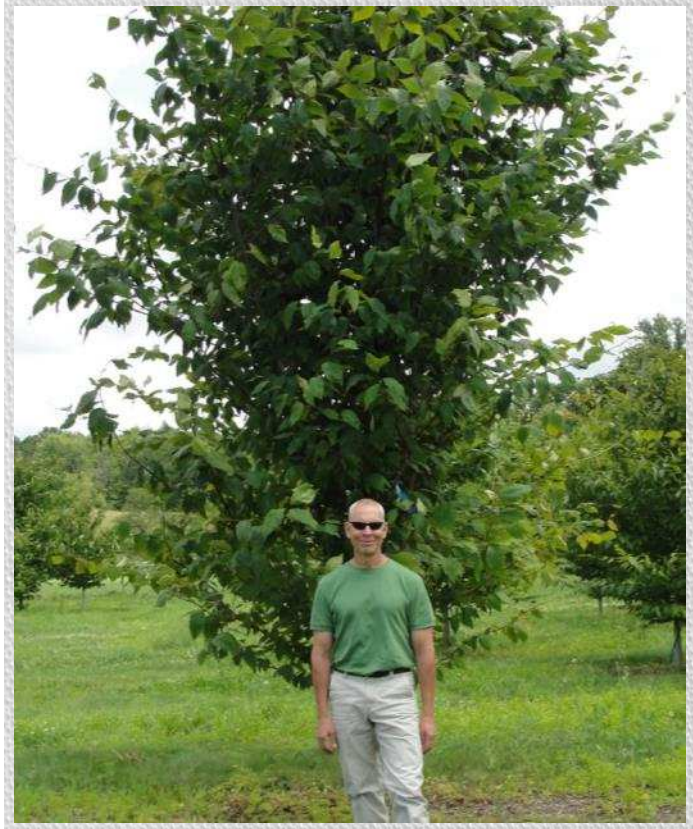
To estimate orchard output we're looking at the proportion of resistant progeny as well as the distribution of the "S" progeny.

Past present and on deck from MI

25 parents
24 mating combinations
470 progeny tested
900 progeny (pending funding)
new parents and pairs

Families are a resource (mechanisms?)

BEECH TREE IMPROVEMENT:BREEDING & TESTING



Beech seedling evaluation research planting at The Holden Arboretum

The HOLDEN Arboretum

- Evaluate age-age correlation of scale resistance
- Evaluate durability of resistance
- Holden committed to culling susceptible trees and maintaining as a seed orchard

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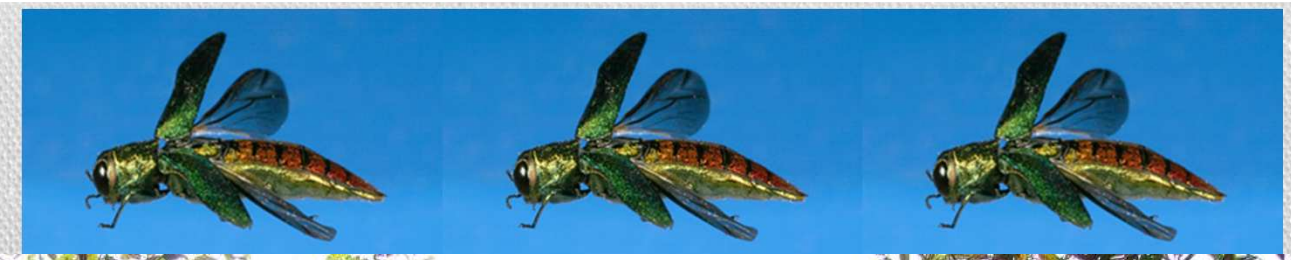
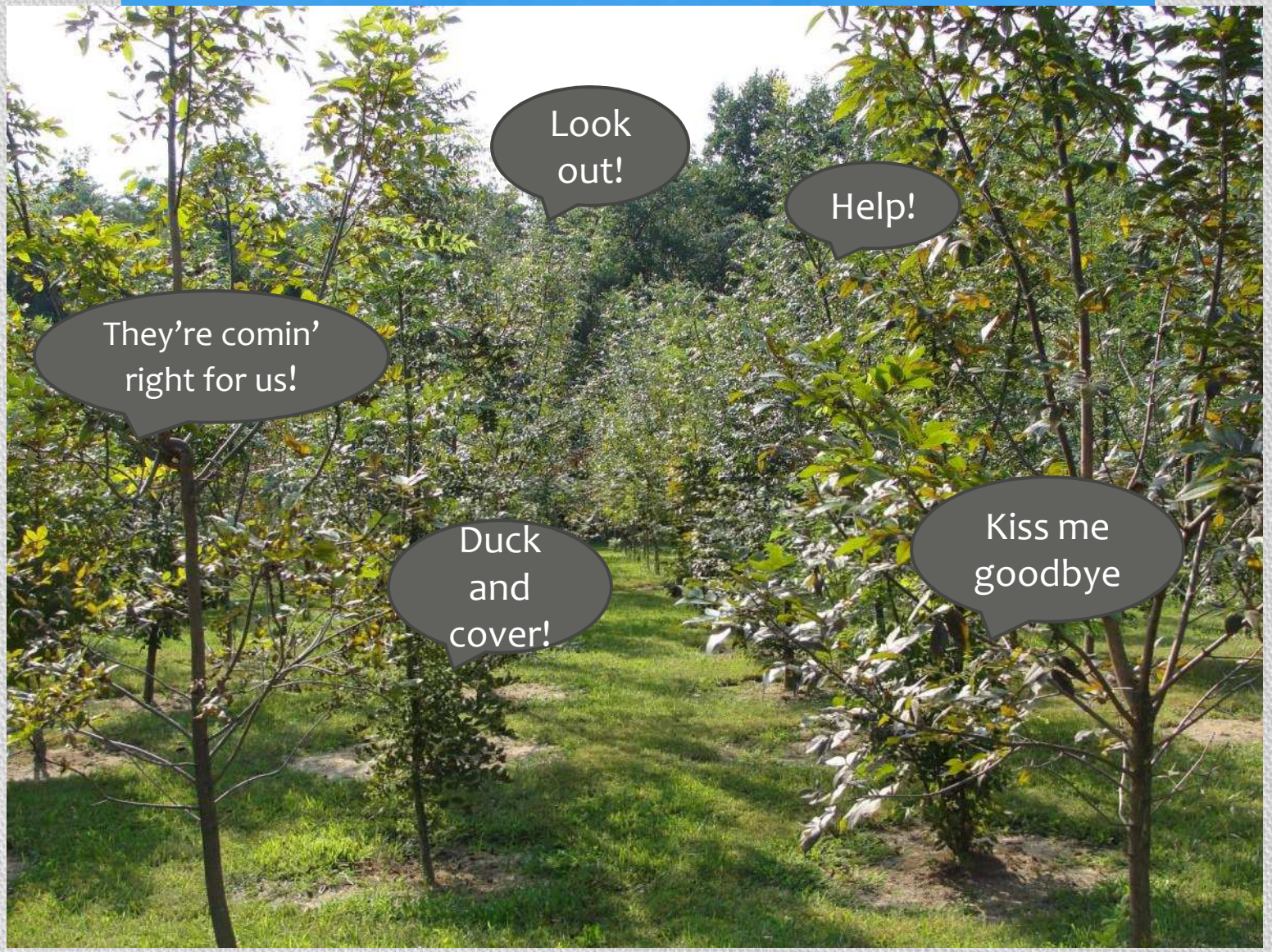
BEECH TREE IMPROVEMENT: OUTPLANTING

JUST BEGINNING

- MI DNR, NRS
- Planted 138 seedling in 2011
- Over half are BBD-resistant
- Survival summer 2013 = 80 %

American Beech Seedling Underplanting, Naubinway, MI




Look out!
Help!
They're comin' right for us!
Duck and cover!
Kiss me goodbye

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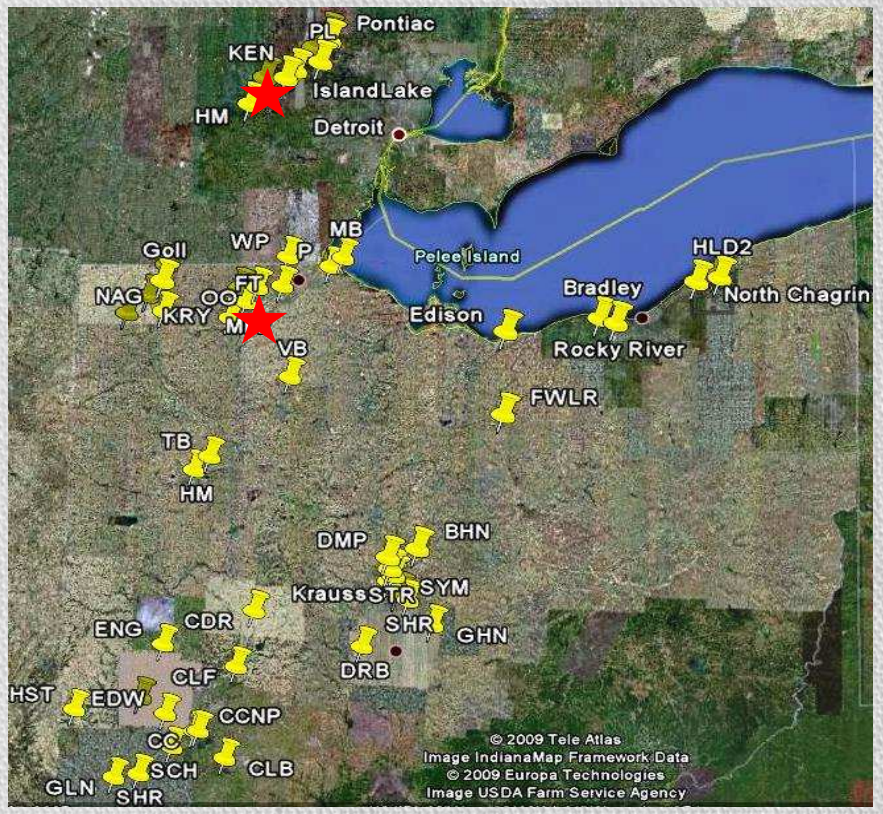
Selecting Lingering Ash in Natural Forest Areas



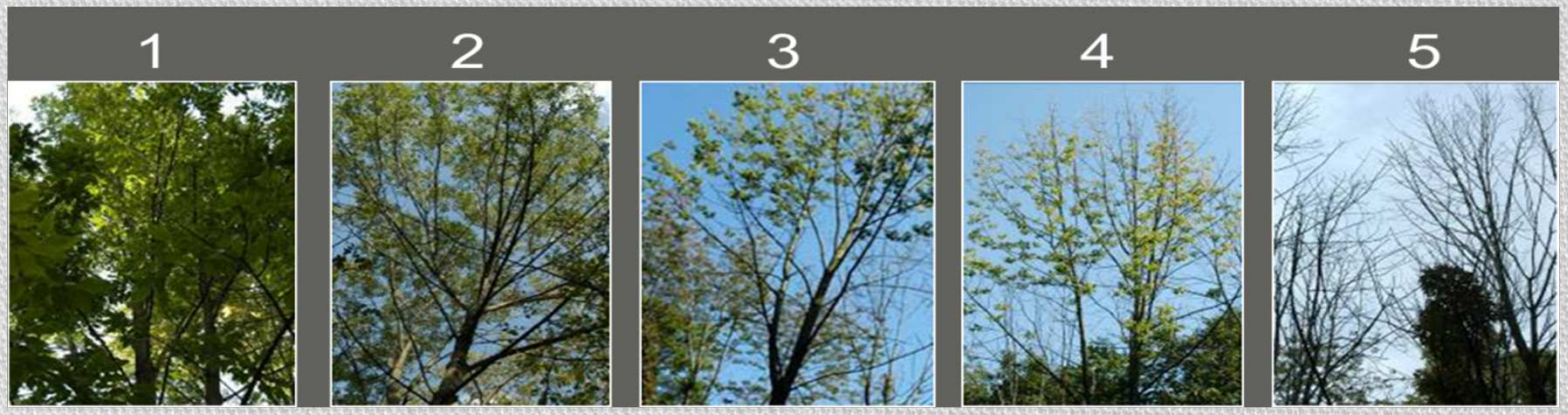
Kathleen Knight, US Forest Service
Dan Herms, The Ohio State Univ.

Three main areas:

1. Long term monitoring plots:
   - Michigan: 33 sites w/ 99 plots
   - Ohio: 50 sites, 165 plots
2. FHP sponsored fly-over (MTU study)
1. Two Clusters:
   - Oak Openings ★ Metropark
   - Indian Springs Metropark

SELECTION CRITERIA FOR "LINGERING" ASH

1. >95% mortality of mature ash trees has occurred at least 2 years ago
2. Tree was large enough(4" dbh) to be infested during the peak EAB infestation
3. Tree currently has a healthy canopy (1 or 2) and is at least 10" dbh.



Submit a survivor ash: www.nrs.fs.fed.us/SurvivorAsh

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Scion Collection

Lingering Ash Grafting

Bud Grafting

Hot-Callus Top Grafting

Grafted clones of a lingering ash selection

Controlled cross on newly grafted lingering ash

Identification of Resistant Germplasm: “Lingering Ash”

Multiple grafts of each selection will be installed in research plantings

Species	Number of genotypes
Green Ash	40
White Ash	8
Black Ash	3
Unknown (green or white?)	6
TOTAL	**57*** (21 sites)

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Bioassay Development

- Many points of interaction
- One point may break cycle
- Or better at several points may mean tolerance
- Multiple bioassays may be needed to find and measure resistance

EAB Life Cycle*

Entomology.osu.edu

Photo by David Cappaert. Reprinted with permission.

Photo by David Cappaert. Reprinted with permission.

Adult Landing and Feeding Preference

Collaborator Therese Poland, NRS, East Lansing, MI

experimental cage with leaves and EAB

Development of egg bioassay to confirm resistance:

Destructive sampling 8 weeks
After estimated hatch date

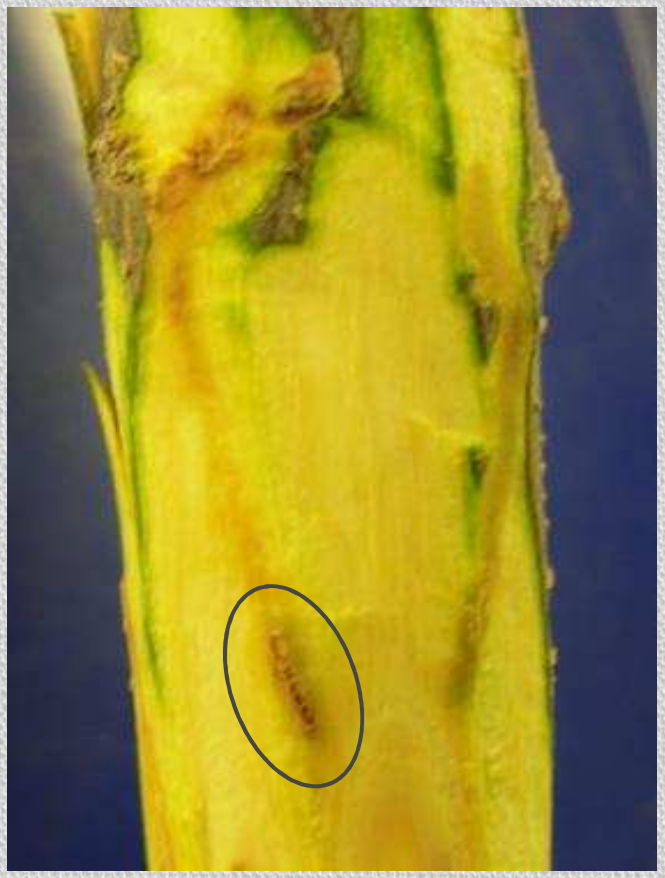
Coffee filter with eggs affixed to bark

Healthy larva

Host-killed larva

Development of Egg Bioassay to Confirm Resistance:

Healthy larva



Host-killed larva

Data Collected:

-Egg hatched

-Larval outcome
L1, L2, L3, L4, host-killed dead-other

-Larval weight

Variation in "Host-Killed" Larvae Phenotypes: EAB killed at entry point

Manchurian Ash

White Ash

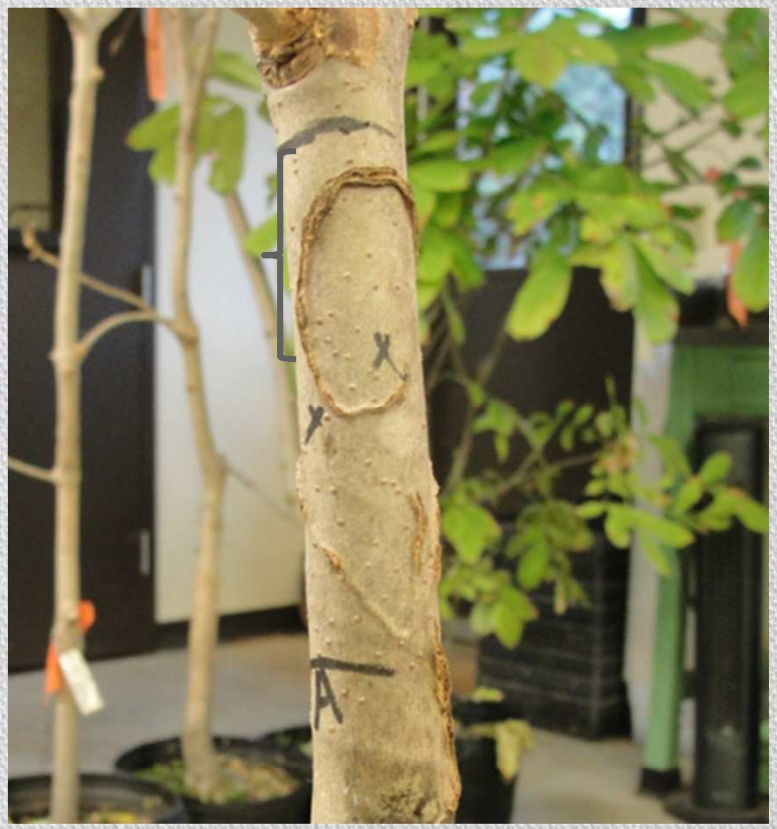
Calloused dead-end gallery

Chinese Ash

Green Ash

“Host-Killed” Larvae Phenotypes: Raised surface galleries

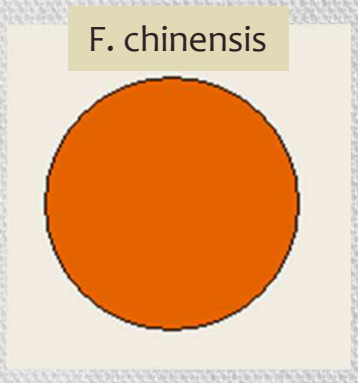
Asian species

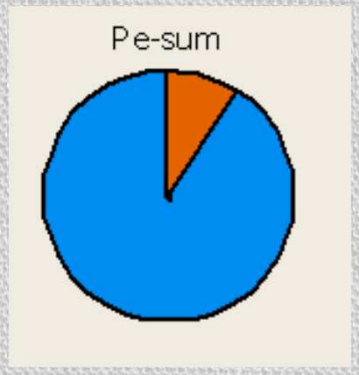


North American species

Green ash

2012 Egg Bioassay Results Summary

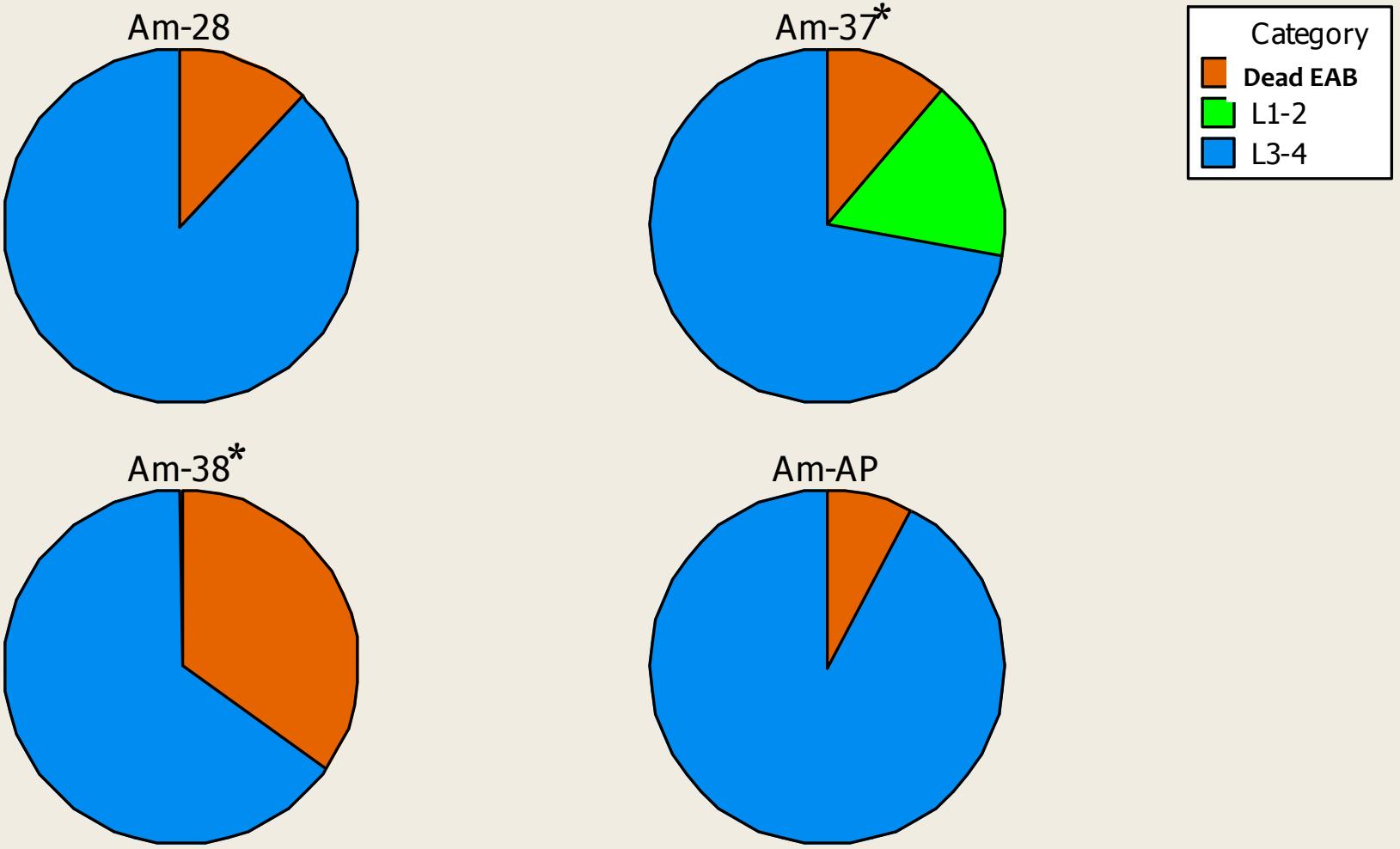




'Summit'
EAB susceptible control

2012 Egg Bioassay Results Summary

Lingering White Ash



Autumn Purple
EAB-susceptible control

Lingering Ash: Multiple Phenotypes/Mechanisms?

2011 Data Statistical Analysis

Genotype	Adult Feeding Preference	Egg Bioassay: Larval Outcome (Dead, L1-4)	Egg Bioassay: Larval Weight	% Stand/Plot Mortality 2012
PE-15	**SIG***	NA	NA	100 %
PE-19	NS†	**SIG***	NS†	99 %
PE-20	NS	NA	NA	100 %
PE-21	NS	NS	NS	100 %
PE-22	**SIG***	NS†	**SIG***	100 %
PE-24	**SIG***	NS†	NS	96 %

NA=not available NS=not significant

*significantly different relative to EAB-susceptible control

†p-value low but not significant – additional replicates may push toward significance

Field Plantings

- 40 Green + 8 White "Lingering Ash" + controls
  - *Dawes Arboretum, Newark OH*
  - *Holden Arboretum, Kirtland, OH*
- Validate initial selection phenotype
- Validate bioassay rankings
- Integrates performance over life cycle
- Analyze genetics x environment interaction

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Green & White Ash Genetic Families

- **Mapping families for both green & white ash**
  - Genetic Linkage Maps
    John Carlson, Penn State University
  - Genetic Markers
    Jeanne Romero-Severson, University of Notre Dame
  - NSF Hardwood Genomics Project (hardwoodgenomics.org)
- **Lingering x Lingering families**
  - Determine if lingering trait is heritable
  - Stack multiple tolerance mechanisms

Koch Group

Ryan Reynolds | Mark Miller | Jennifer Koch | Mary Mason | Dave Carey

Funding

USDA APHIS

USDA NRI Competitive Grants

US Forest Service

American Recovery and Reinvestment Act (ARRA)

Forest Health Management

Special Technology Development Program

Thank You!

BBD RESISTANCE WORK:

Region 9, ORSO *Paul Berrang, Scott Rogers*

HNF *Majorie Allmaras, Sam Barnes, Tim Baker*

MI DNR *Bob Heyd, Rich Mergerner, Ron Murray, Roger Mech*

MSU *Paul Bloese*

LINGERING ASH WORK:

Toledo Metroparks

Huron-Clinton Metroparks

MI DNR

Kathleen Knight Lab (USFS, NRS)
Britton Flash, Rachel Kappler

Dan Herms Lab (OSU) *Diane Hartzler*

Therese Poland Lab (USFS, NRS)

Andrew Storer Lab (MTU)
Jordan Marshall (Purdue, Fort Wayne)