Tree Improvement 101:
Resistance breeding
MODULE 4

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https://rngr.net/

Resistance breeding is a type of tree improvement

The same principles of selection and breeding for wood quality traits apply for resistance in forest trees

What's the difference between resistance and tolerance?

Resistance	Tolerance
…reduces the harm caused by disease by preventing infection or limiting subsequent pathogen growth and development within the host through avoidance or clearance of infection (Horns and Hood 2012).	

What's the difference between resistance and tolerance?

Resistance

…refers to the ability of a system to actively oppose a threat, leading to minimized damage. (Conrad et al. *in preparation*)

From a fitness standpoint:
Tree = winner. Pest = loser.

Tolerance

Host-resistance doesn't impact the pathogen directly, but instead influences traits that can limit the health and fitness costs of being infected (Martins et al. 2019).

From a fitness standpoint:
Tree = winner. Pest = winner.

Notable pests of forests in North America


Beech bark disease


Hemlock woolly adelgid


Emerald ash borer


Dutch elm disease


Phytophthora lateralis


Fusiform and blister rust


Butternut canker

Notable pests of forests in North America: Beech bark disease

Host: *Fagus grandifolia*, American beech

Insect: Non-native beech scale *Cryptococcus fasiuga was* introduced from Europe late 1890s. Scale vectors a native *Nectria* fungus.

Status: Great progress until recent introduction of a nematode, that leads to beech leaf disease, has stalled resistance breeding work.

Notable pests of forests in North America: Dutch elm disease

Host: *Ulmus americana*, American elm

Pathogen: native insect vectors non-native vascular wilt, *Ophiostoma ulmi* and *O. novo-ulmi*

Introduced ~1930 and wiped out many large urban and rural trees.

Status: Elm remains a component of many forests in the eastern US; great progress on resistance.

Notable pests of forests in North America: Hemlock woolly adelgid

Host: Eastern and Carolina hemlock, *Tsuga canadensis, T. caroliniana*

Insect: *Adelges tsuga,* introduced from Asia.

Status: insect spread is very slow but persistent and occurs across most of the range of eastern hemlock.

Notable pests of forests in North America: Emerald ash borer

Photo: N. Seigert, USDA Forest Service

Host: *Fraxinus* spp (brown/black, green, white, pumpkin, Oregon ash, blue ash)

Insect: *Agrilus planipennis*

Introduced 1990s in Michigan on wood pallets from Asia.

Status: spreading very quickly. Billions of trees are threatened in riparian and upland forests.

Notable pests of forests in North America: Butternut canker

Host: *Juglans cinerea*

Pathogen: *Ophiognomonia clavigignenti-juglandacearum*, ***Oc-j***

Introduction date unknown but likely in the 1960s.

Status: Listed on Canada's Species at Risk Act (SARA). Endangered on some state listings but not on federal Endangered Species Act.

Hybrids with Japanese walnut tend to survive while native germplasm is quickly disappearing.

Notable pests of forests in North America: Port-Orford-cedar root rot

Host: *Chamaecyparis lawsoniana*

Pathogen: *Phytophthora lateralis*, oomycete root rot.

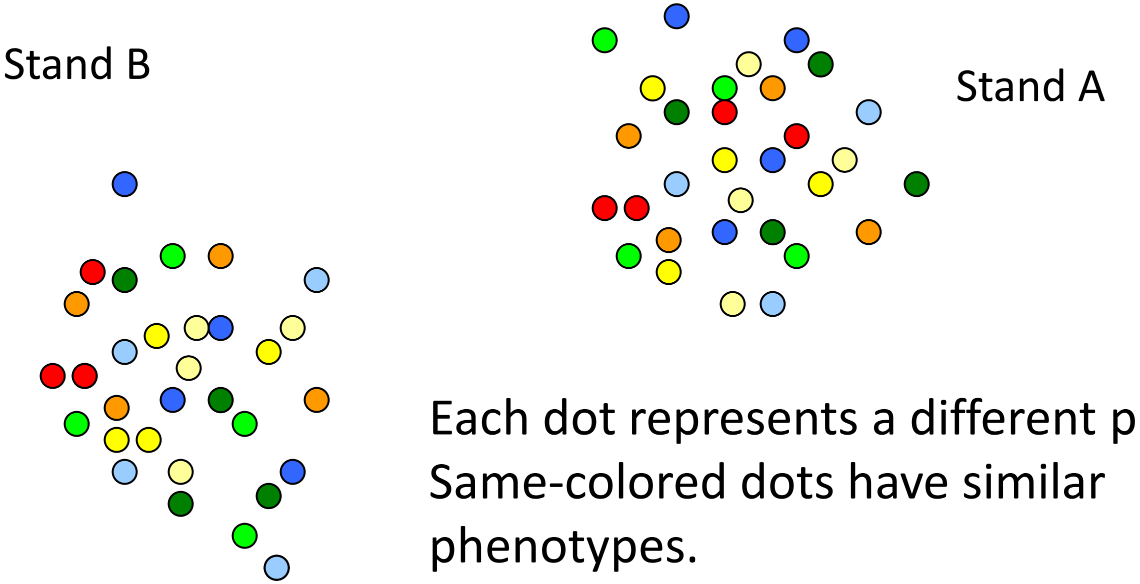
Status: Some hard-hit forests are being restored with seedlings that have demonstrated improved resistance.

Native: Fusiform rust *Cronartium quercuum* f.sp. *fusiforme*). Affects southern hard pines. *Quercus* spp. is alternate host

Photo: Forestry Ima

Non-native: white pine blister rust *Cronartium ribicola*. Affects all 5-needled pines. *Ribes* spp. (currants or gooseberry) is alternate host

Module 1: Most tree species have "low" population structure because of excessive gene flow



Each dot represents a different phenotype. Same-colored dots have similar phenotypes.

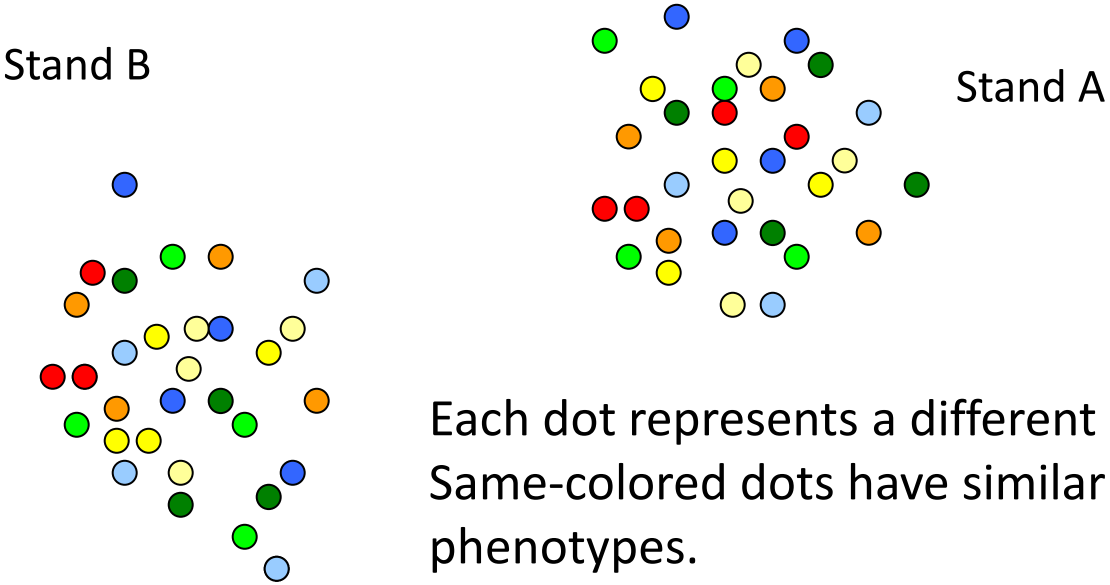
Very often (but not always) in forest trees we find:

Large differences within a population or stand (high within-stand variation)

Few differences among populations, or stands (low among-stand variation)

But this may not hold for all trees in all forests!!!

Module 1: Most tree species have "low" population structure because of excessive gene flow



Each dot represents a different phenotype. Same-colored dots have similar phenotypes.

Genes for resistance traits to novel pests may also be scattered across the landscape! Or not….

Module 1. Most native trees in North America have high genetic variation that is well distributed across the landscape. Some genetic structure may still be evident for some species!

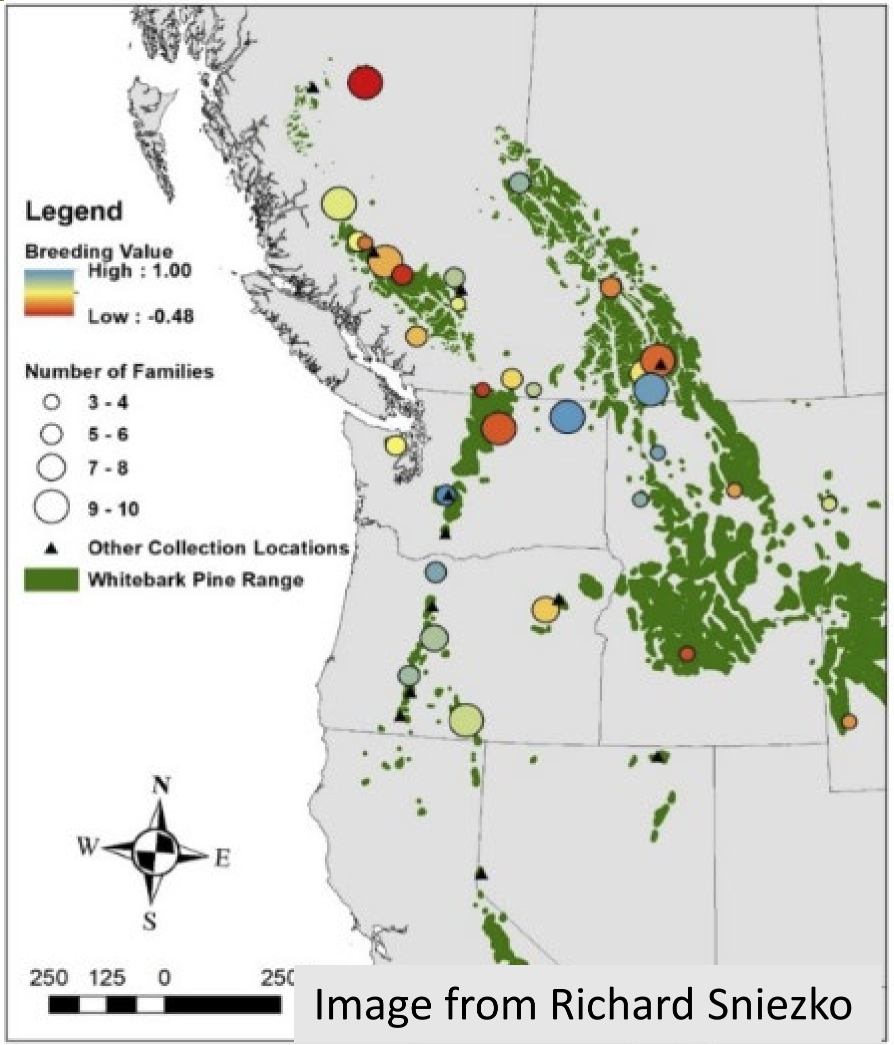


Image from Richard Sniezko

…some populations might have a higher frequency of resistance traits than others.

It won’t be known until you sample a large geographic area.

Module 1. Most native trees in North America have high genetic variation that is well distributed across the landscape. Some genetic structure may still be evident for some species!

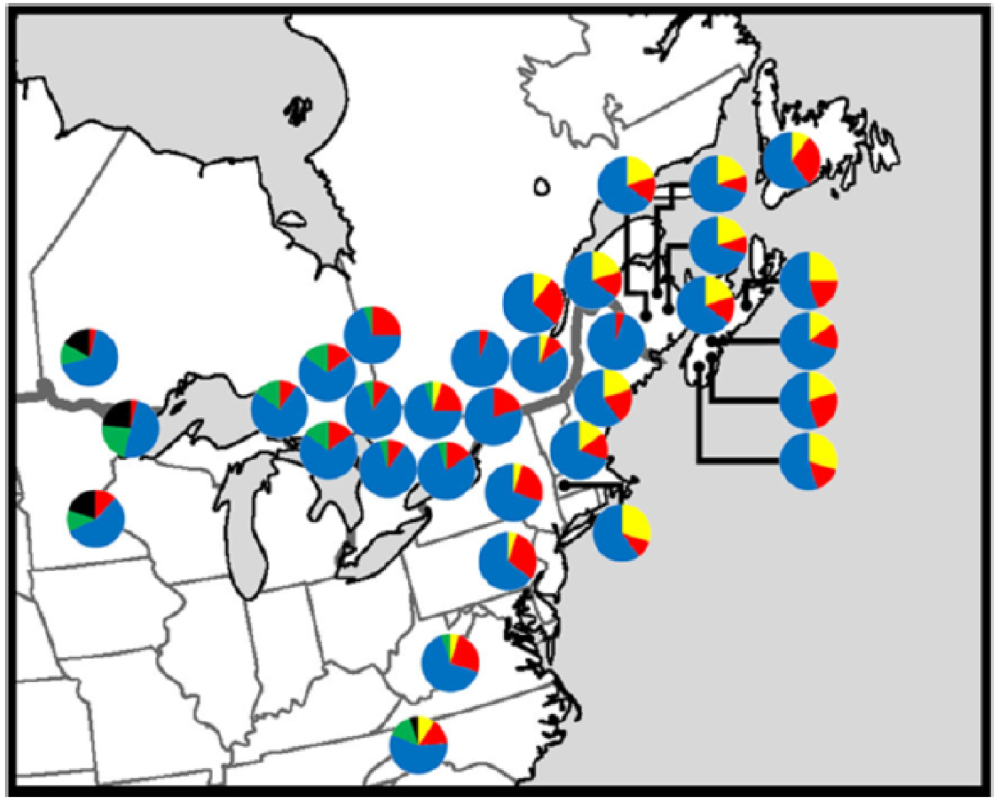


Fig. 4 Geographic distribution of the most abundant chloroplast haplotypes in eastern white pine populations. Colours correspond to individual haplotype. Yellow, Haplotype S; Red, Haplotype V; Green, Haplotype AG; Black, Haplotype AJ; Blue, Haplotype AP. The allelic composition of the haplotype is provided in Table 4

Zinck and Rajora BMC Evolutionary Biology (2016) 16:56

Resistance breeding:

Rare alleles may confer resistance (or tolerance).

They are rare because there was no selective advantage to having these genes prior to invasive threat.

Module 3: Genetic Variation is the Basis for Tree Improvement

Genetic gain: breeders' equation

$$\Delta g = i^* h^2 {}^* V_p$$

"i" = selection differential, h^2=heritability and V_P = phenotypic variation

Module 2:
Heritability (between 0 and 1) is the proportion of variation attributable to genetic differences among families.

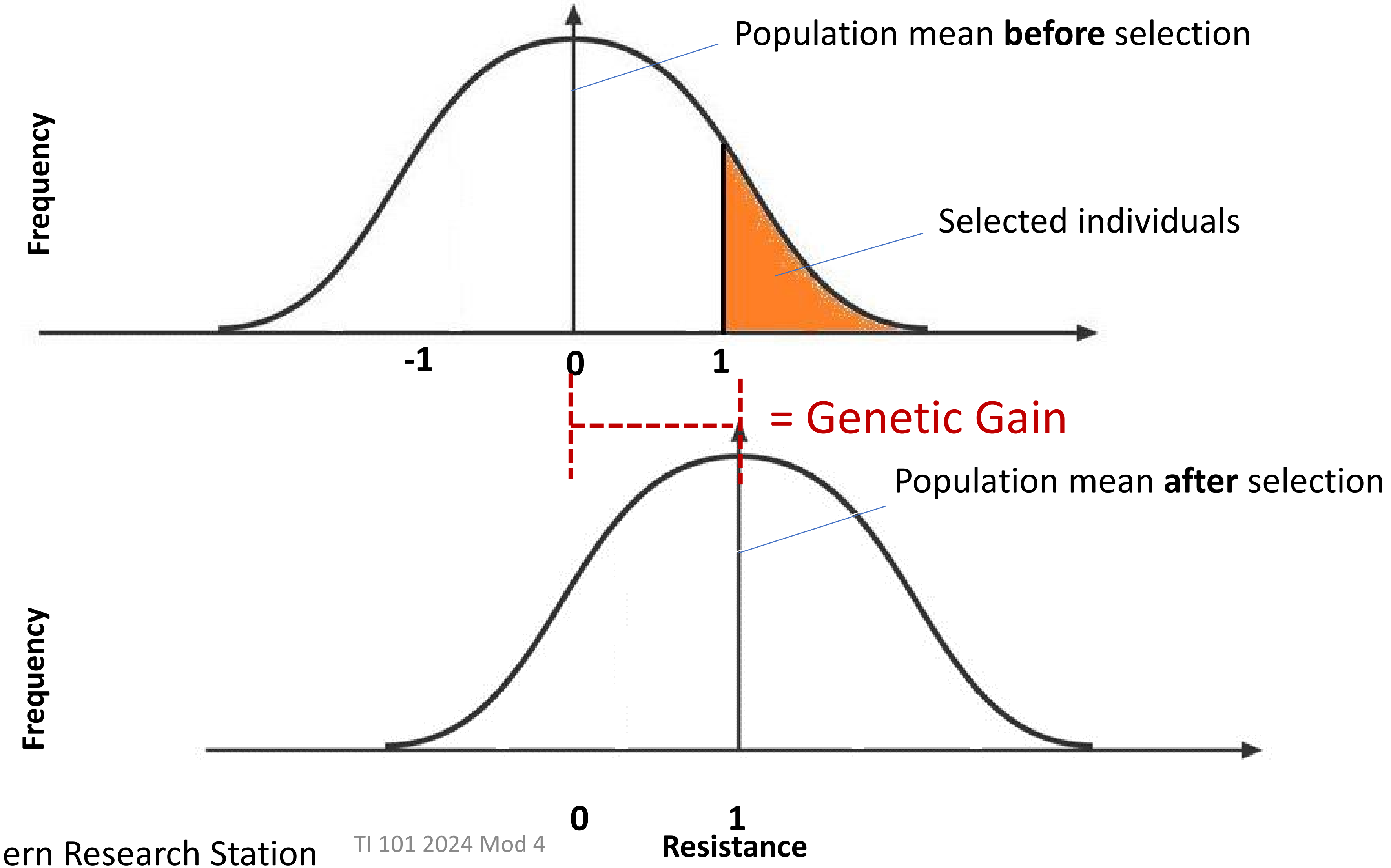
It must exceed zero for any improvement to take place through selection!

If heritability is high then the gains for each generation will be relatively high.

Dead and lingering hemlocks in Pennsylvania. From Kinahan et al. University of Rhode Island

Genetic gain: breeders’ equation

$$\Delta\, g = i^{*}\, h^{2}\, {}^{*}V_{p}$$



Population mean **before** selection

Selected individuals

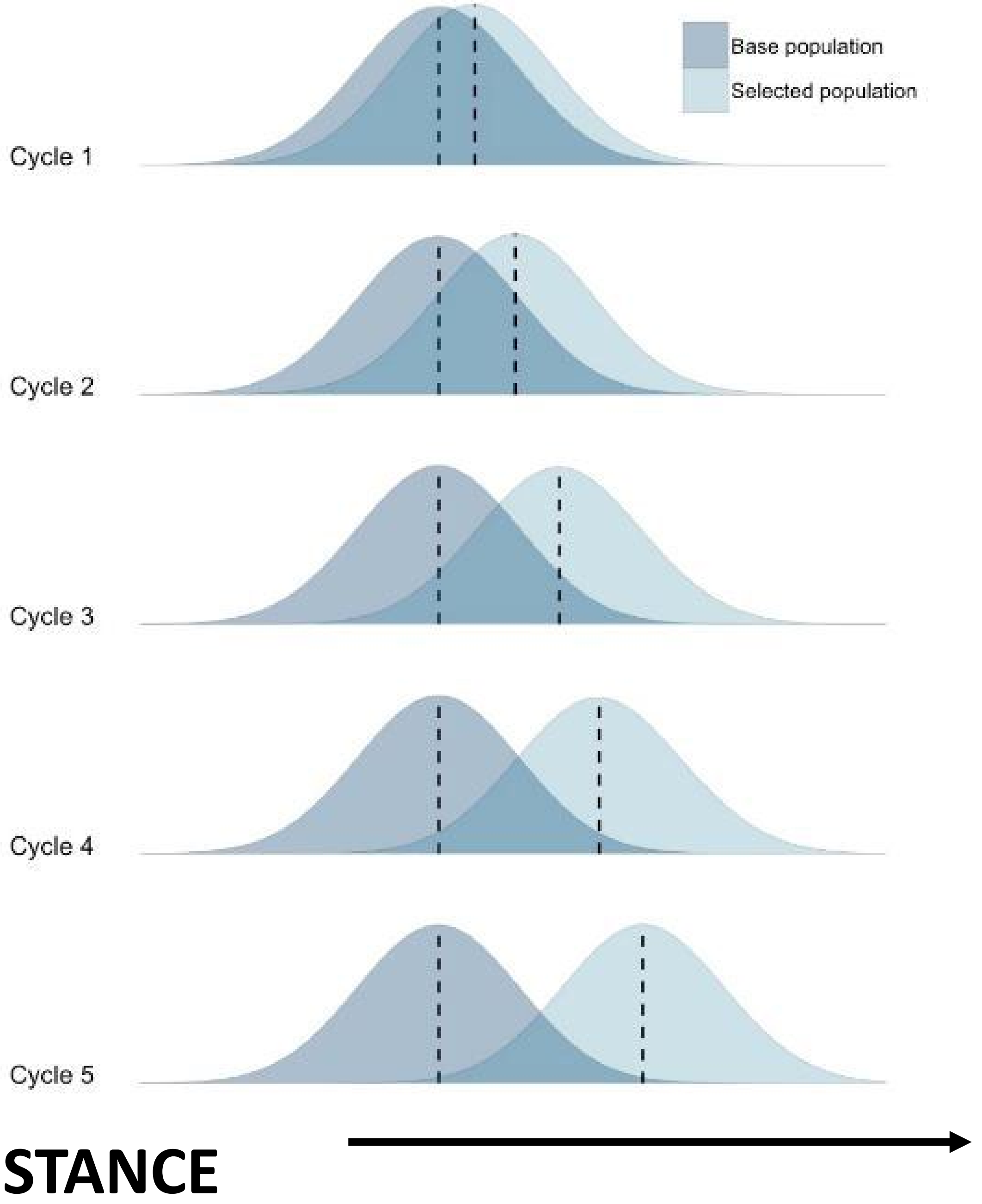
= Genetic Gain

Population mean **after** selection

Slide from Dave Carey, USFS Northern Research Station

Tree improvement in a nutshell

Resistance of the selected (breeding) population will increase with each generation.



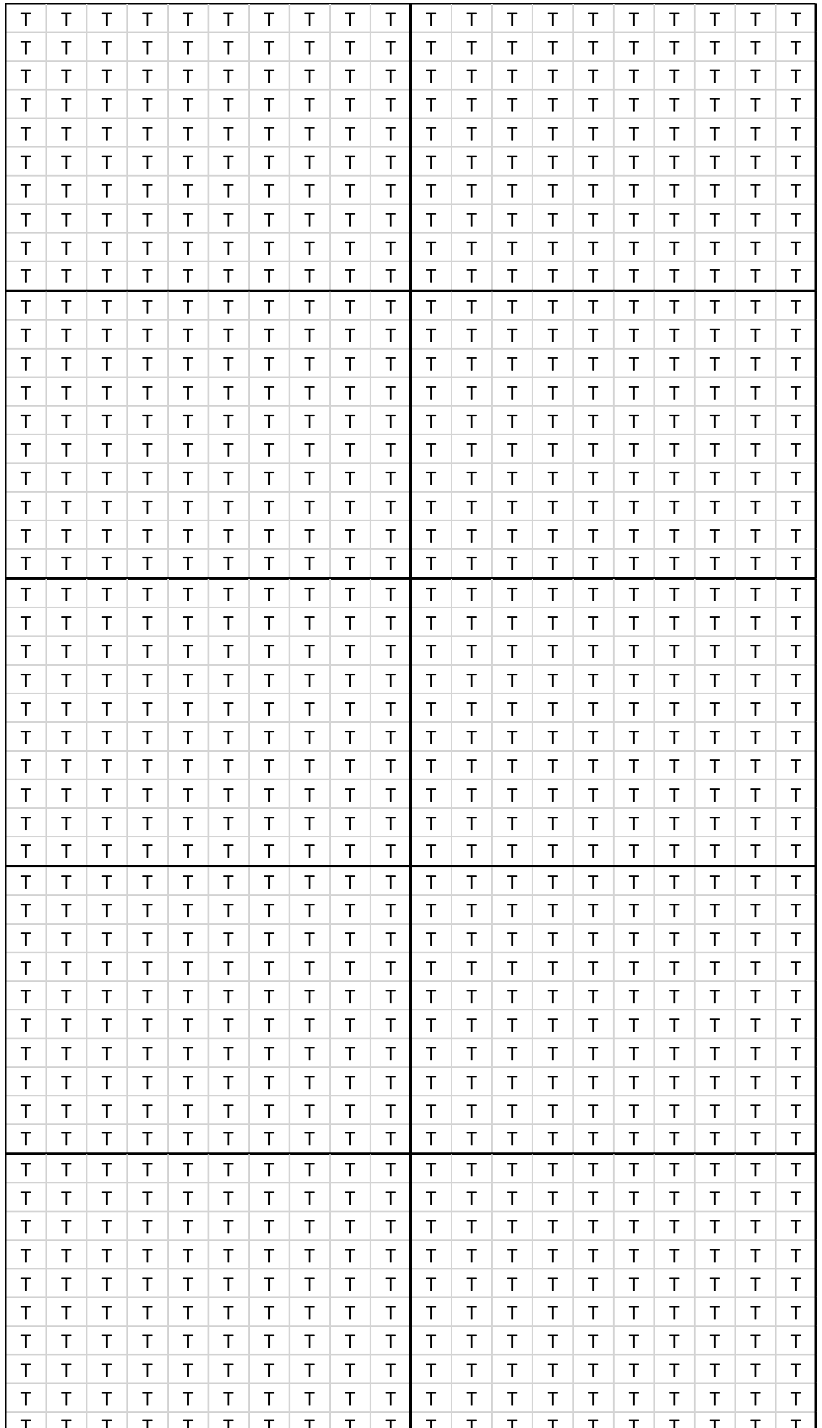
Slide from Katie McKeever, USFS Forest Health Protection

Rutkoski 2019. In: Advances in Agronomy Vol. 157 pp. 217-219

Module 1: Tree improvement starts with natural populations in the woods!

Resistance breeding programs often start with lingering trees: trees that survive early waves of pests (insects or disease)

Lingering (left) and dead ash (right). Kathleen Knight, USFS - Northern Research Station



Module 1: progeny tests are the primary means for assessing genetic ‘worth’ of families and individual trees within families

We often use randomized complete or incomplete block designs.

Module 1: progeny tests are the primary means for assessing genetic ‘worth’ of families and individual trees within families

For resistance breeding, we employ common gardens in the field, a greenhouse, growth chamber – the principals are the same.

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Photo by Scott Rogers, USDA Forest Service – Oconto River seed orchard

Module 1: Most traits we rely upon in improvement programs are "quantitative traits"

These are traits that you measure on a continuous scale.

Example: height, diameter

These traits tend to be controlled by many genes and different combinations of alleles.

Some traits also closely interact with the environment.

Module 1: Most traits we rely upon in improvement programs are "quantitative traits"

Crown dieback on American elm inoculated with Dutch elm disease.

Resistance breeding: we need to develop traits (screen) that represent resistance!

These may include counts of insect eggs, weight of larvae, larval instar, host survival, insect survival, percent crown dieback, etc.

Artificial Inoculation Technique to Screen Seedlings for Beech Scale Resistance

Collect eggs from an infested tree

Beech scale adults & eggs.

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

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

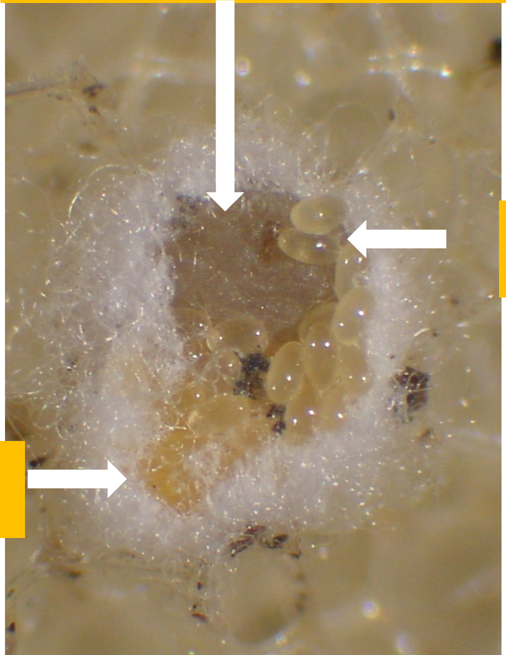
Eggs hatch & colonize susceptible trees.

Artificial inoculation technique for beech bark disease - one year later:

Resistant

Susceptible

Highly Susceptible



Adult Scale Insect

Eggs

Juvenile nymphs

Slides/photos from Dave Carey and Mary Mason, USFS Northern Research Station

Phenotyping protocol: egg bioassay for emerald ash borer

EAB Egg Bioassay
(assess resistance phenotype)

Coffee filter with eggs affixed to bark

Three grafted replicates of each genotype

Slide: J. Koch, USFS Northern Research Station

Phenotyping protocol: egg bioassay for emerald ash borer


EAB larva being excised from a bioassay.

Metrics for egg bioassay used for emerald ash borer in *Fraxinus* spp:
- Egg hatched Y/N
- Larval outcome: Larval instar (L)1, L2, L3, L4, host-killed
- Larval weight (quantitative)

Slide: J. Koch, USFS Northern Research Station

Phenotyping protocol: Dutch elm disease

Screening: after 5-6 years of growth, the elms are inoculated with DED. Crown dieback is assessed 8 weeks, 1 year, and 2 years post-inoculation

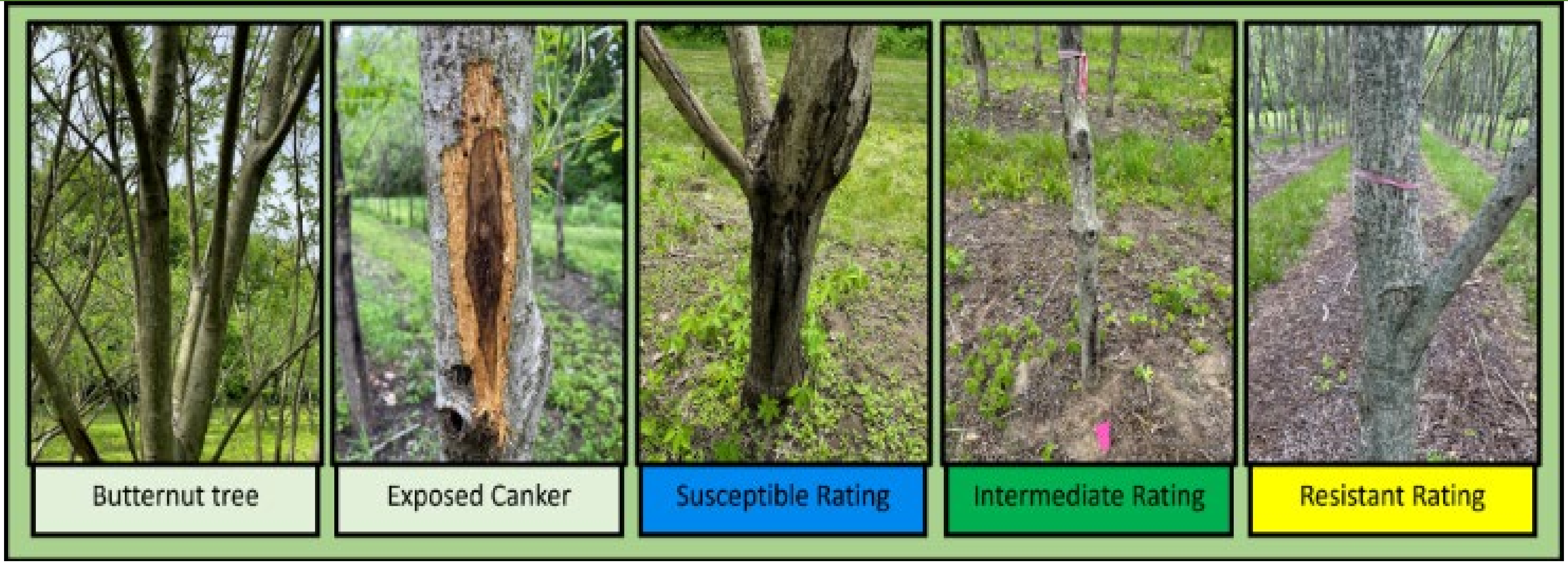
Slide from Northern Research Station Delaware, OH

Phenotyping protocol: Dutch elm disease

New screen on small potted seedlings is in development.

Slide from Northern Research Station Delaware, OH

Phenotypes: Butternut canker



- Trees are naturally infected
- Collect ratings at ~10-years post-planting
- Assess visually with 5-point rating system (1: very resistant; 5: very susceptible)

Slide from Anna Conrad, USFS Northern Research Station
Photo credit: Jonathan Shimizu and Michael Holt

Phenotypes: white pine blister rust disease on 5-needled white pines

Scott Rogers, USDA Forest Service

Inoculation chamber at Oconto River Seed Orchard in Wisconsin

Phenotypes: white pine blister rust disease on 5-needled white pines

Inoculation chamber at Dorena Genetics Resource Center in Oregon

White pine species in the blister rust resistance breeding program at Dorena Genetics Resource center in Oregon: western white (*P. monticola*), sugar (*P. lambertiana*), limber (*P. flexilis*), and whitebark pine (*P. albicaulis*).

Phenotypes: white pine blister rust disease on eastern white pine

6-point scale of infection for eastern white pine to white pine blister rust disease
Survival was also assessed.

Photos by C. Pike at Oconto River Seed Orchard in WI.

Phenotypes: white pine blister rust disease on *Pinus strobiformis*

Resistance traits in southwestern white pine:

(1) early stem symptoms
(2) frequencies of bark reactions, and
(3) severity of infections over the duration of the trial.

Conklin, D.A. et al. 2009. USDA Forest Service, Southwestern Region, R3-FH-09-01.

Johnson and Sniezko. 2021.

Phenotypes: fusiform rust resistance

Select “winning” families

Screen out “losing” families

Gall < 25 mm

Symptomatic no gall

Rough Gall

Gall (smooth)

Slide from Katie McKeever, USFS Forest Health Protection

Genetic underpinnings of resistance in plants

"Disease resistance in plants is mostly quantitative, with both major and minor genes controlling resistance." Merrick et al. 2021.

Qualitative resistance

- Traits controlled by a few genes, each with large effects on the phenotype.
- Also called "Major Gene Resistance" or MGR.

Quantitative resistance

- Traits controlled by many genes, each with a small effect on the phenotype.
- Leads to more durable resistance than MGR.

Which is better for forest trees?

Phenotype = Genotype + Environment + G x E

Genetic

- Additive genetic variation
- Non-additive

Dominance = heterozygotes are masked

Epistasis = genes interact with other genes in unpredictable ways.

?

Qualitative resistance

Quantitative resistance

Environment

- ✓Soils
- ✓Slope
- ✓Aspect
- ✓Spacing
- ✓Silviculture, etc.

G x E Interactions

Phenotype = Genotype + Environment + G x E
From Module 2

Genetic effects

Additive → Quantitative resistance (polygenic = many genes)

Most of the time*

Non-additive → Qualitative resistance (one or two genes)

Major gene resistance (MGR)

* some dominant genes may be associated with quantitative resistance.

Why is MGR potentially problematic? Insect or disease pests can evolve virulence (fungi) or tolerance (insects) to the gene(s).

Module 2. Verify that the trait (phenotype) used for resistance breeding is heritable!

"The most important function of the heritability in metric characters is its predictive role expressing the reliability of the phenotype value as a guide to the breeding value."
-Falconer and Mackay, 1996, chapter 10

A low or zero heritability might mean that the screen used for phenotyping isn't effective – continue refining the screen until you see separation among the families.

Heritability of resistance in lingering x lingering crosses of green ash (*Fraxinus pennsylvanica*)

Second generation population of green ash at Holden Forests and Gardens. Photo: Rachel Kappler

2018-2020

- 27 full-sib families produced
- 773 total 2-3 year old progeny screened
- Heritability = 0.63 ± 0.065 *

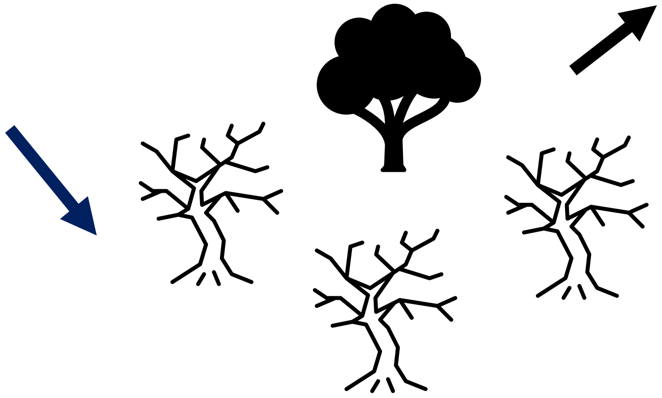
63% of the expression of resistance is under genetic control.

*Koch, J.L. and Mason, M.E. personal communication

Module 2:
Forward selection

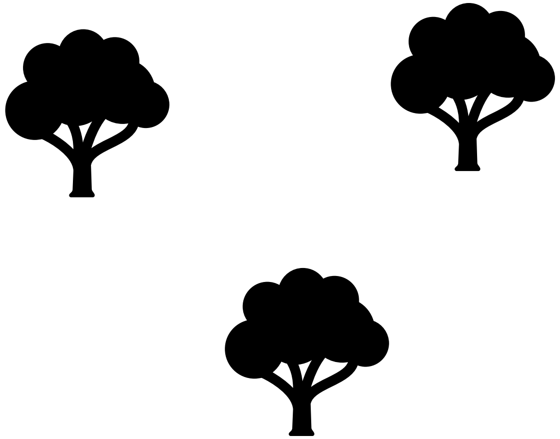
1. Select plus trees, collect open-pollinated seed

2. Progeny from hundreds of mother trees planted in common garden



3. Nature does the thinning to ("rogue out") worst trees from worst families

4. Use remaining trees as a seed orchard.



Backward (family) selection

1. Select plus trees

2. Progeny from hundreds of mother trees planted in a multi-site progeny test

3. Measure progeny test over time

4. Create a separate grafted orchard to use as a seed source.

Why bother creating a grafted seed orchard?

If a progeny test reveals that a tree typically produces excellent offspring, then we want an exact copy of that “mother” tree in our orchard.

Top 30 clones from screening eastern white pine for white pine blister rust disease at Minnesota DNR, Willow River MN.

Grafted American beech at grafted seed orchard in Michigan.

USDA Forest Service: Hot-Callus Grafting Resistant American Beech

DW Carey, ME Mason, P Bloese, JL Koch. 2013. HortScience 48(5):620-624.

Previously published success rates grafting American beech: 12-30%
Success rate of hot-callus grafting: 67 % average over 5 yrs; 90-100% max

Breeding is a key next step to stack the genes for resistance!

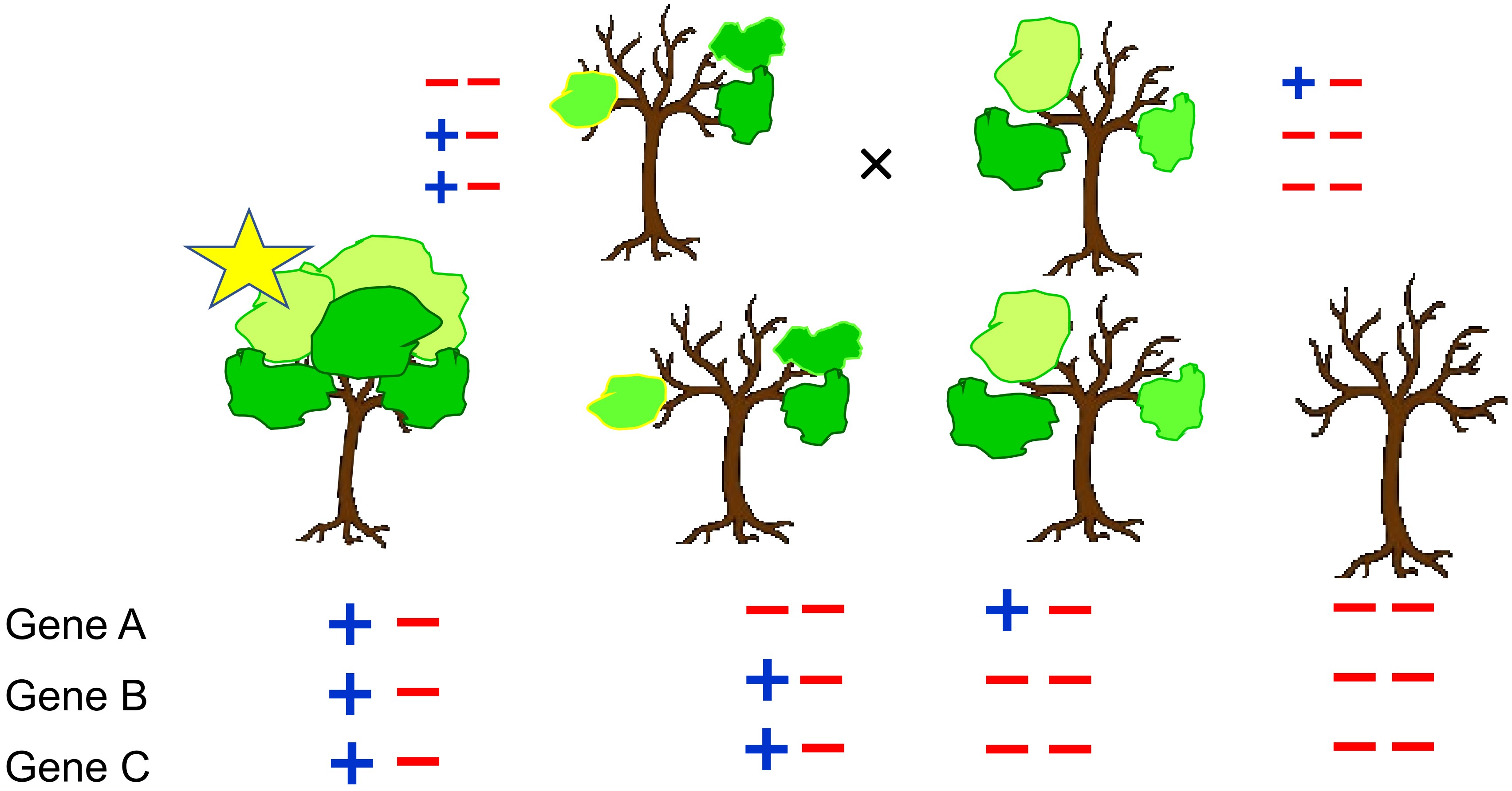
Breeding Port-Orford-cedar. Richard Sniezko, USFS

Breeding American beech. Dave Carey, USFS

Breeding allows combining best genes from each parent!

Best growth inhibition

Highest larval kill

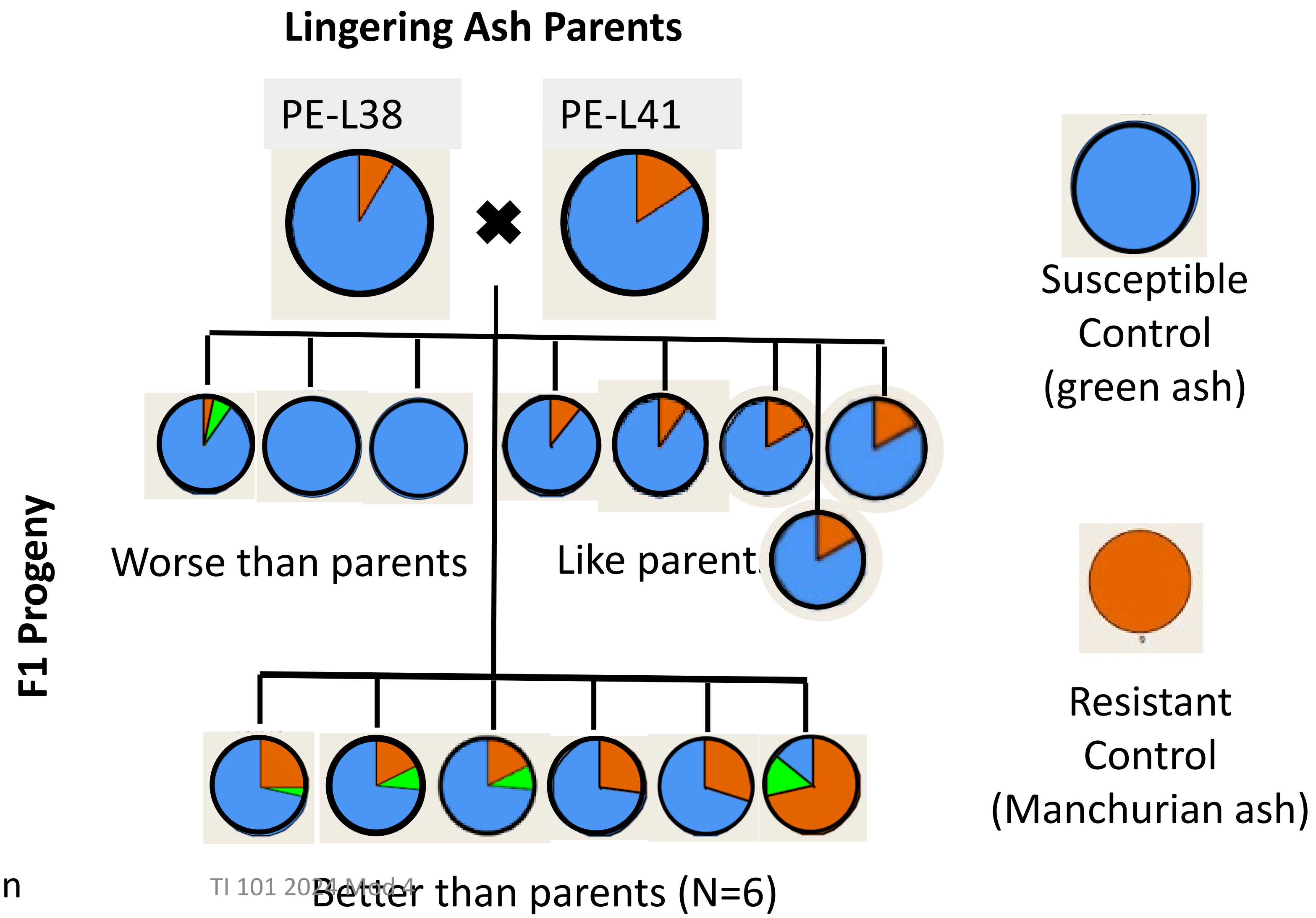


Further improvement with each generation!

Slide: J. Koch, USFS Northern Research Station

When we make controlled crosses between lingering trees resistance in the progeny may range from low to high.

Category
- 🟧 host-killed EAB
- 🟩 L1-L2
- 🟦 L3-L4



Lingering Ash Parents

PE-L38 ✖ PE-L41

F1 Progeny

Worse than parents

Like parent

Better than parents (N=6)

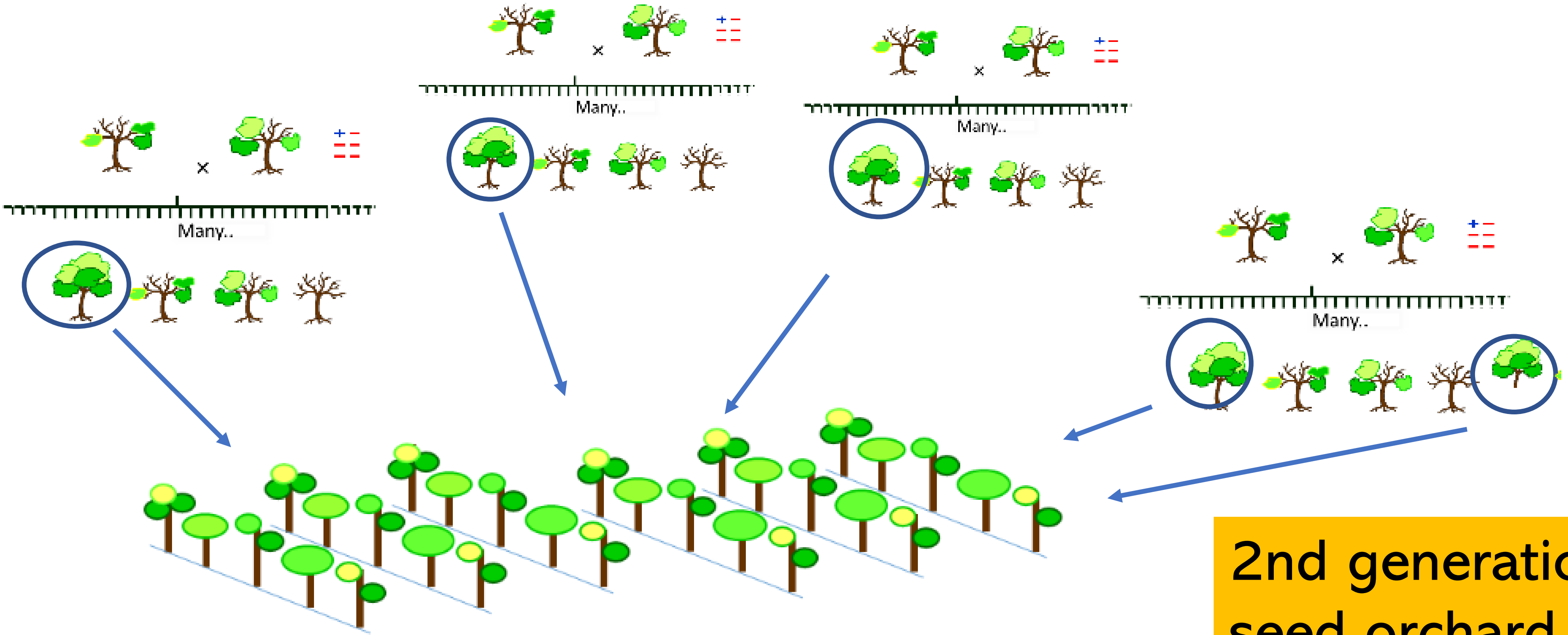
Susceptible Control (green ash)

Resistant Control (Manchurian ash)

Select the progeny whose resistance exceeds the parents.

Slide: J. Koch, USFS Northern Research Station

Select best trees from many different Lingering x Lingering crosses



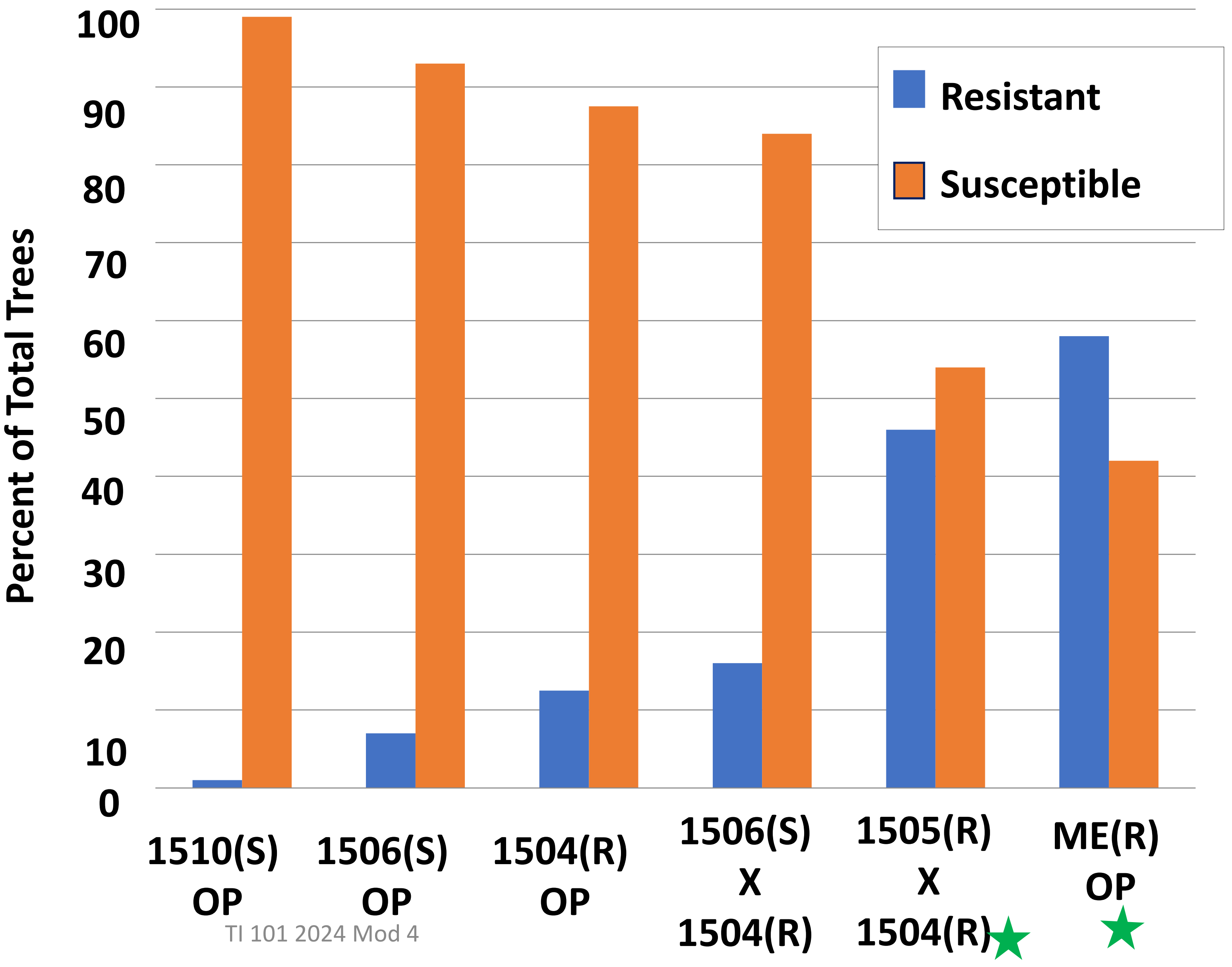
2nd generation seed orchard

Produce seed with increased defenses against EAB, retain genetic diversity and adaptive capacity

Slide: J. Koch, USFS Northern Research Station

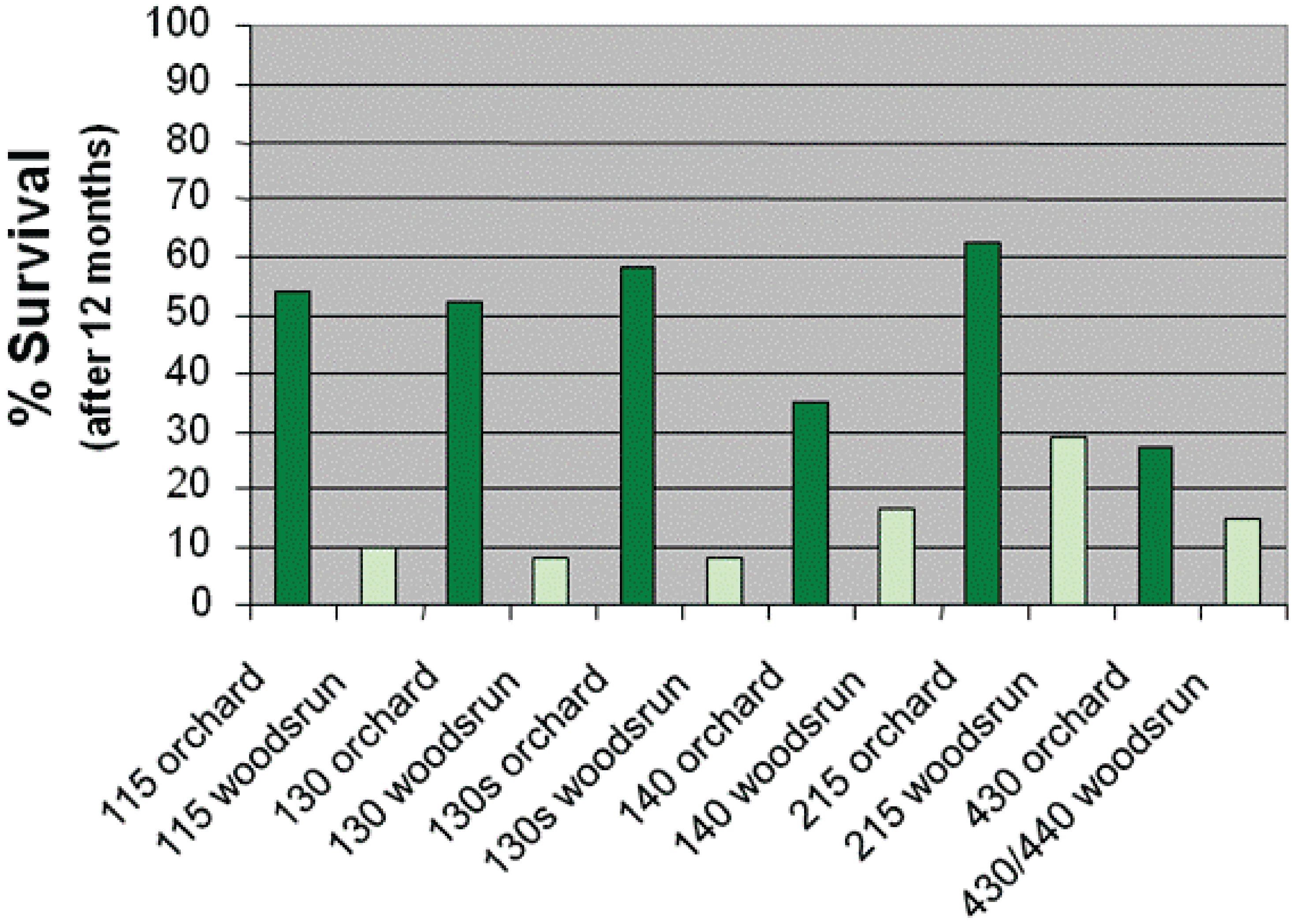
Resistance in American beech to beech bark disease

Survival of resistant x resistant crosses is 40-50% improved over susceptible trees.



Slide by D. Carey, J. Koch and M. Mason, USFS Northern Research Station

Seed orchards and resistance levels in Port-Orford-cedar



Survival improvement of > 40+ %

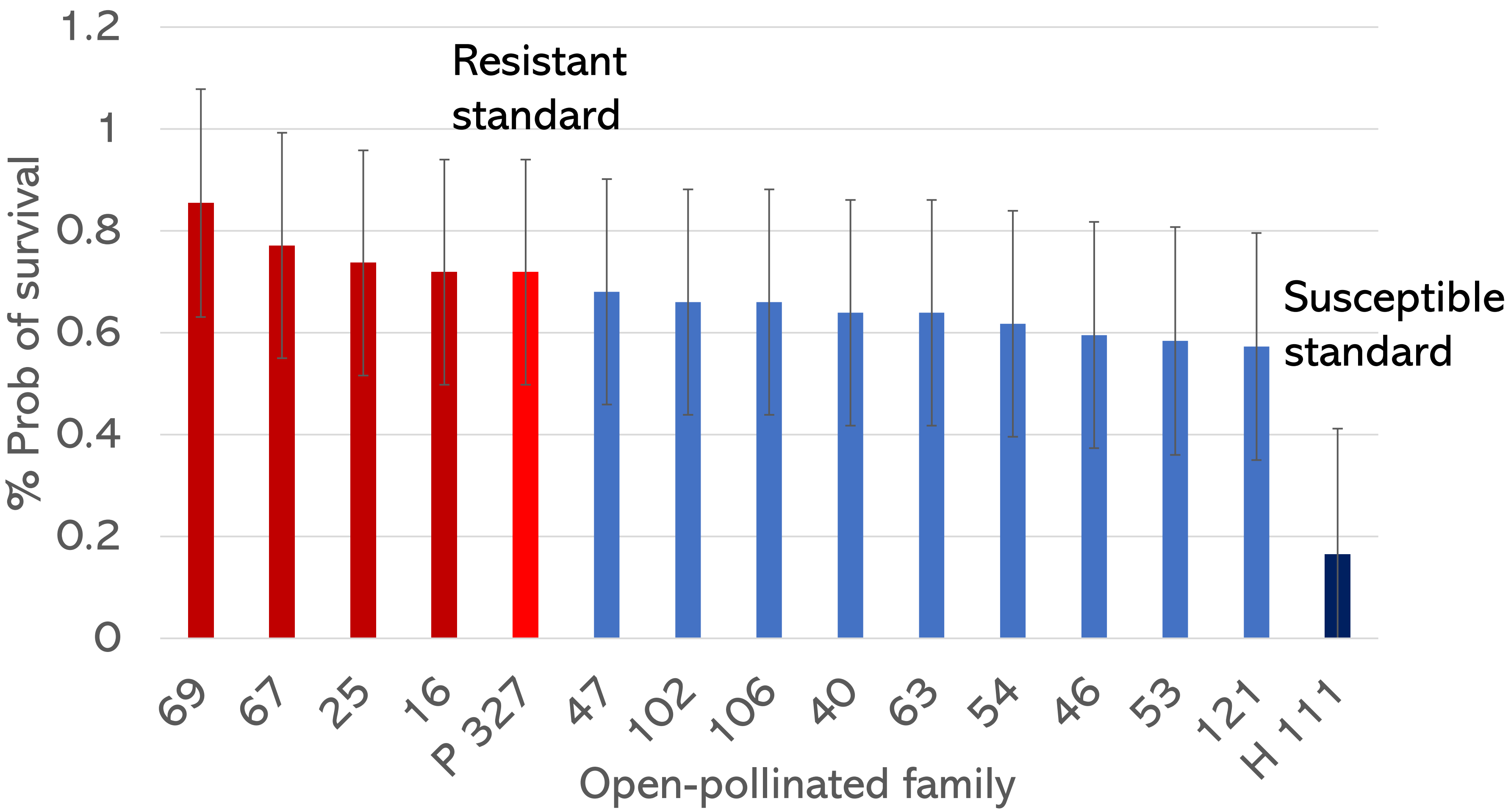
Over 14,000 initial selections have been made across breeding zones!

Goal: Seed orchards for each breeding zone

NOTE: original research concluded that there was no resistance!!

Slide provided by Richard Sniezko, USFS-Dorena Genetics Resource Center

Percent probability of survival (binary trait) of eastern white pine resistance to white pine blister rust



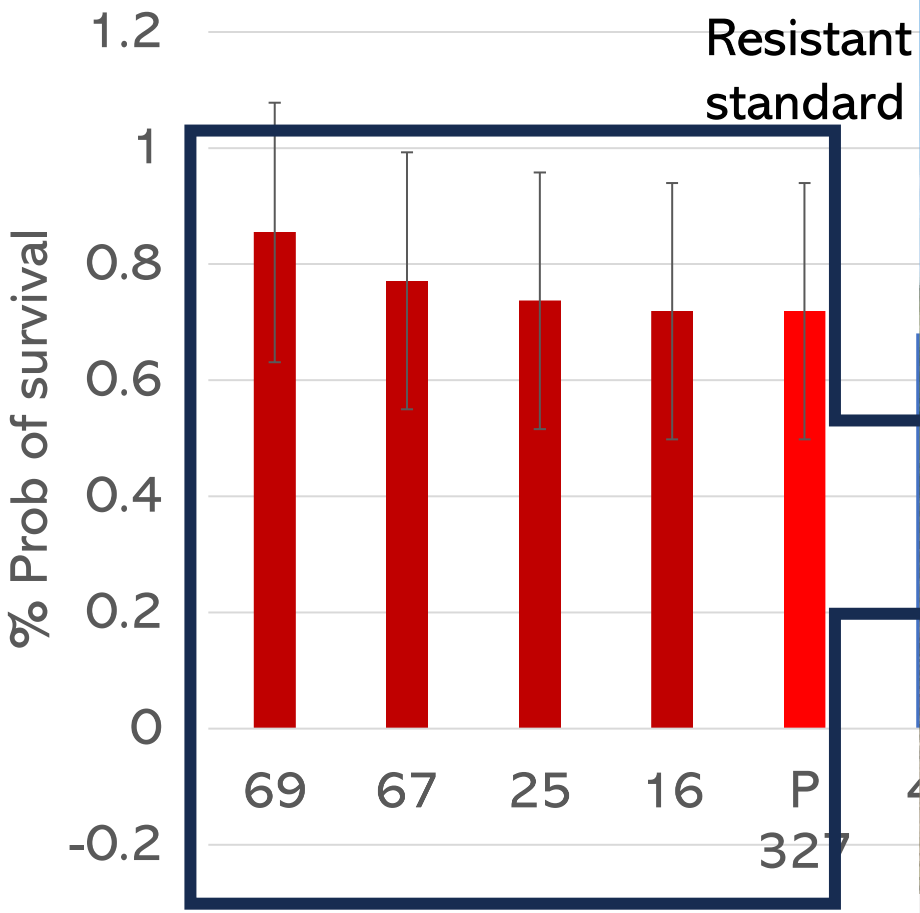
Derived from Pike et al. 2018

Survival improvement of of 30-50% over susceptible standard.

Goal: grafted seed orchards for Superior National Forest and north shore of Minnesota

NOTE: resistance was not supposed to occur in eastern white pine!

Percent probability of survival (binary trait) of eastern white pine resistance to white pine blister rust



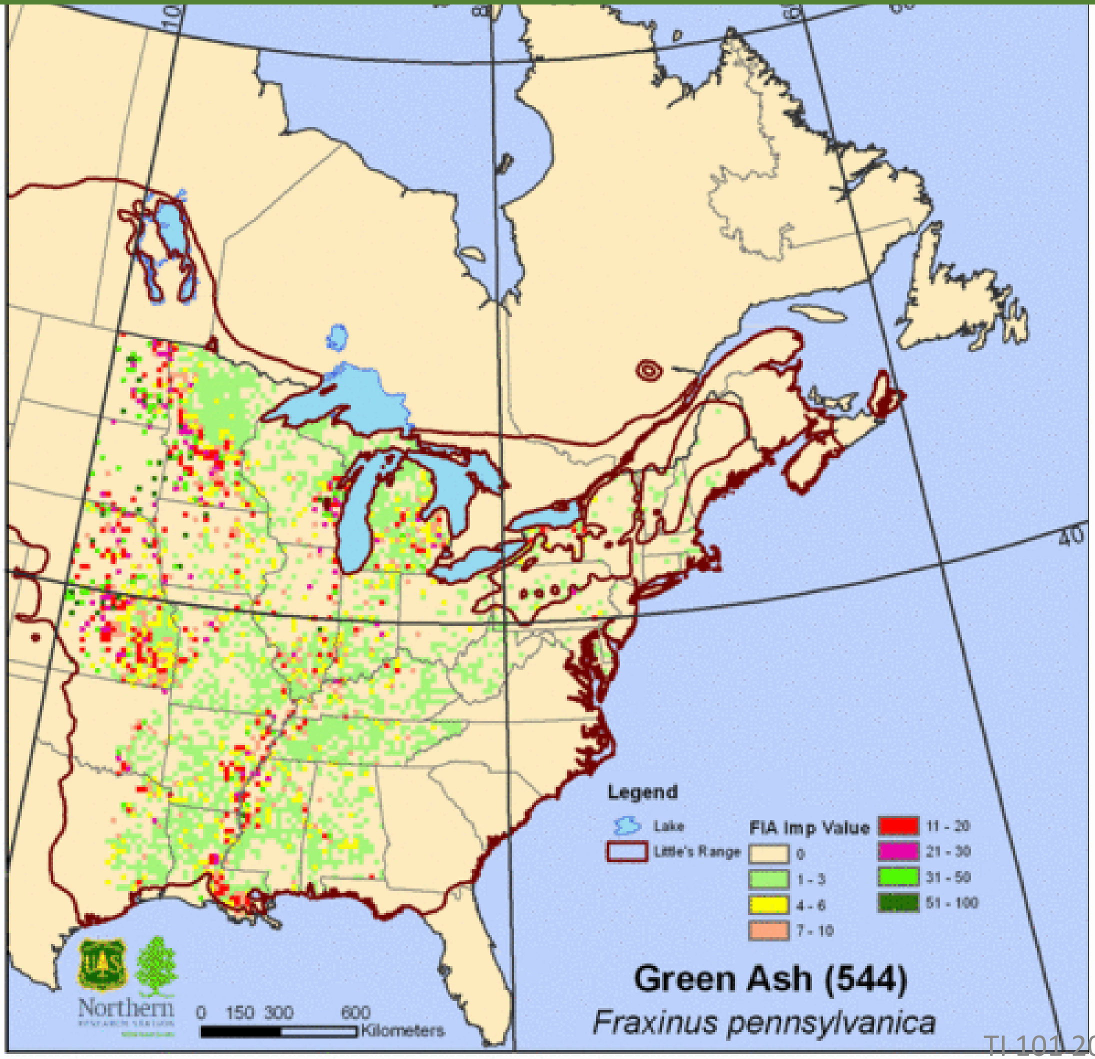
We grafted these mother trees for this seed orchard

Clonal orchard at General Andrews nursery – Minnesota DNR – at Willow River.

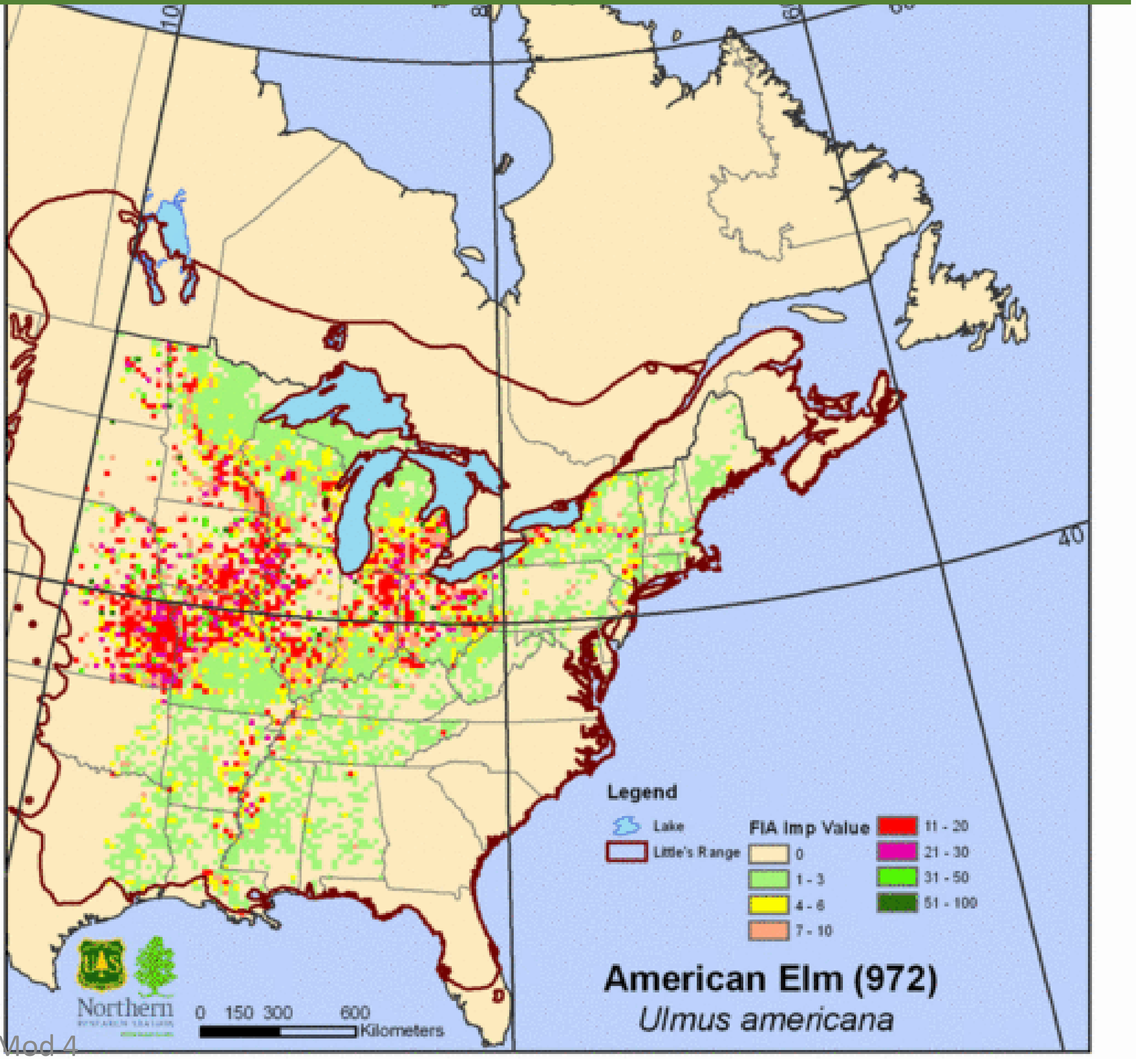
Genotype by environment interactions

Performance of a genotype at one site or seed zone may not be consistent across locations!

Many native tree species in North America have large geographic ranges

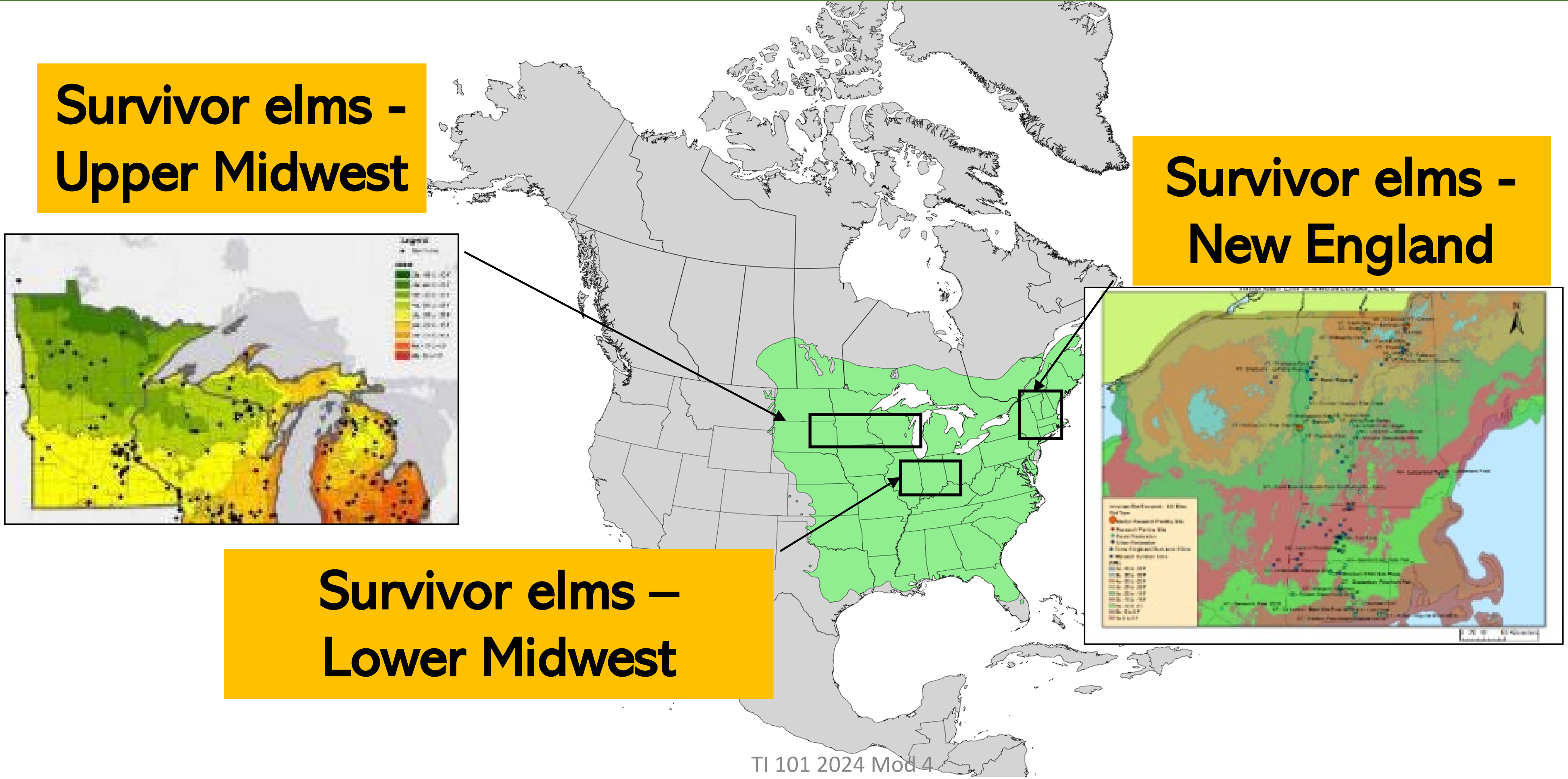


Green Ash (544)
Fraxinus pennsylvanica



American Elm (972)
Ulmus americana

Over 800 survivor elms (to Dutch elm disease) >24 inches in diameter have been reported by members of the public since 2011

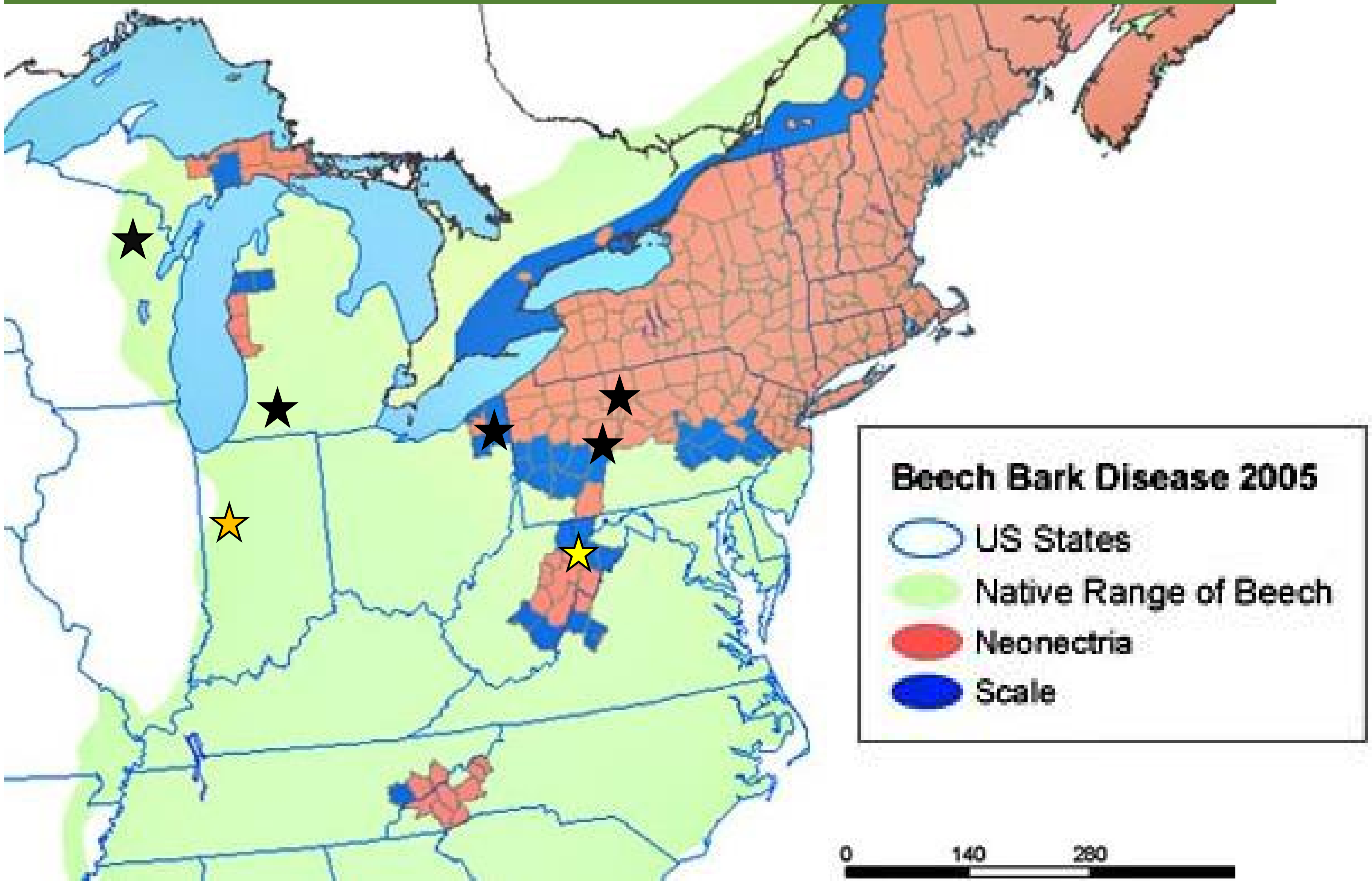


Survivor elms - Upper Midwest

Survivor elms - New England

Survivor elms – Lower Midwest

Regional American Beech Seed Orchards



Slide: J. Koch, USFS Northern Research Station

Summary: steps in resistance breeding:

Select: Identify resistant germplasm (trees that appear to have partial resistance)

Accession: Preserve & propagate resistant germplasm

Phenotype: Verify resistance through 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

Slide from Dave Carey, USFS Northern Research Station

Other successes?

Acacia koa in Hawaii is threatened by koa wilt disease, caused by *Fusarium oxysporum.* Resistance breeding efforts are demonstrating early successes.

Photo from Dudley et al. 2020

Chestnut blight resistance, in American chestnut, has been pursued by American Chestnut Cooperators' Foundation (ACCF) for over 50 years.

Ed Greenwell (ACCF): *All of the trees shown are blight tested, at least 23 years old by now and look quite good. Left photo: courtesy of the Virginia Department of Forestry. Right photo: courtesy of ACCF.*

Other successes?

The American Chestnut Foundation (TACF) has utilized a citizen-science approach to improve resistance in the species. Breeding program originated with backcrosses to Chinese chestnut.

Science Strategies: 3 BUR

Breeding, **B**iotechnology,
and **B**iocontrol
United for **R**estoration

THE
AMERICAN CHESTNUT
FOUNDATION™

They have a large network of 16 chapters: CT, MA/RI, ME, VT/NH, IN, NY, OH, PA/NJ, KY, MD, VA, WV, AL, GA, TN, Carolinas.

Thousands of volunteers!

Many different breeding populations

A metric of success? When a species' status is downgraded on the IUCN red list!

According to the IUCN Red List of Threatened Species, the species status of Port-Orford-cedar which was listed as 'vulnerable' in 2000, has been downgraded to 'near threatened' as of 2013, with anticipation that it will be listed as a species of 'least concern' within 10 years, if current conservation actions, including planting resistant seedlings, are successful and maintained (Farjon 2013).

IUCN = International Union for Conservation of Nature and Natural Resources

Acknowledgements:
Richard Sniezko's team at Dorena, NRS team at Ohio: Jennifer Koch, Leila Pinchot, Kathleen Knight, Charlie Flower, Mary Mason, Dave Carey, Anna Conrad. Therese Poland, Toby Petrice, Kirsten Lehtoma, Nate Seigert, Scott Rogers, Paul Berrang, Carrie Sweeney, Melanie Moore, Jim Warren, Keith Woeste, Linda Haugen, Andy Meier, Ed Greenwell and the team at ACCF, Manton Foundation, Great Lakes Restoration Initiative, The Nature Conservancy, The American Chestnut Foundation. So many others!!!

Thank you for participating in this webinar series!!!

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We'd like to add 6 more modules to this series: please send us feedback on specific topics that you would like to hear!