Whitebark Pine Genetic Restoration Program

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USFS Northern, Rocky Mountain, Southwestern and Intermountain Regions

Rationale for restoration

- Threats: blister rust, mountain pine beetle, altered fire regimes, and climate change
- Keystone and foundation species
- **Improve critical wildlife habitat**

Nadine Hergenrider

Rationale for restoration

Pine nuts = protein rich, high energy food

Bruce and Monica Robb, Birchwood, CA

Basic Knowledge Needs

- Molecular genetics (genetic diversity, inbreeding, migration and structure)
- Genecology (patterns of genetic variation, adaptive strategy)
- Identify, harness, and deploy blister rust resistant seedlings

Molecular genetics

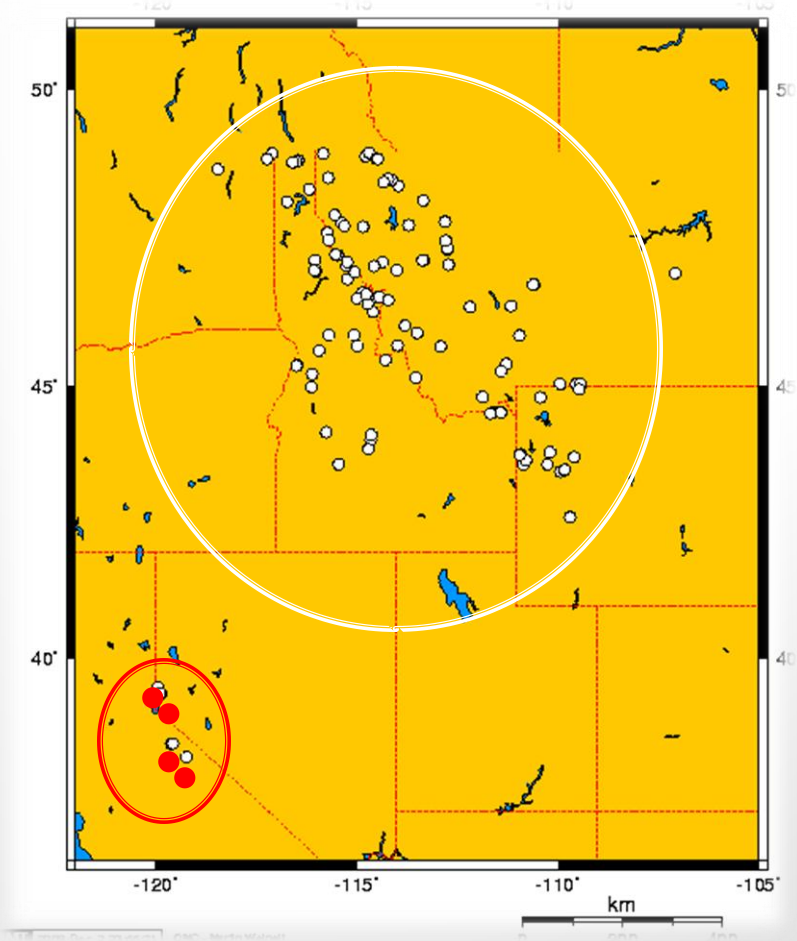
- Mitochondrial DNA, Isozyme, and Chloroplast DNA

Mahalovich and Hipkins 2011

Mitochondrial (mtDNA)

- Maternal contribution
- Measure of gene flow via seed dispersal
- Northern Rockies no subdivisions

Nadine Hergenrider



● Haplotype 2

Chloroplast (cpDNA) and genetic diversity

cpDNA (SSRs)	N	# Alleles	Expected Heterozygosity
3	160	6.7	0.516

- Paternal contribution
- Measure of gene flow via pollen
- High genetic diversity

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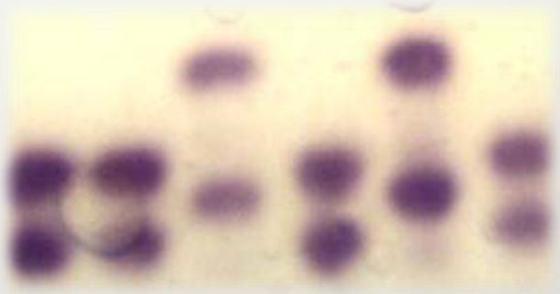
Isozymes and genetic diversity

# Loci	N	Polymorphic Loci (%)	# Alleles	Expected Heterozygosity	Whitebark Pine Citations
16	**147**	**100**	**4**	**0.271**	Mahalovich and Hipkins (2011)
13	14	49	1.6	0.204	Yandell (1992)
20	30	85	3	0.102	Jørgensen and Hamrick 1997
19 (16)	**9 (21)**	**79 (75)**	**2.1 (2.5)**	**0.154 (0.254)**	Bruederle and others 1998 (GYE)
10	~510	70	2.0	0.262	Krakowski and others 2003

Earlier studies show low diversity; more a function of sampling and geographic location. Later work (2011, 2003) indicate higher levels of diversity



General lack of inbreeding

F_{IS}	Inbreeding in individuals relative to the zone to which they belong	-0.016
F_{ST}	Inbreeding in zones relative to the total population (low genetic structure)	0.026
F_{IT}	Inbreeding in individuals relative to the total population	0.011
N_m	Number of migrants	9.354
t	Mean outcrossing rate	0.99

Oldest known whitebark pine

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- 1,285 years old
- Railroad Ridge
  Sawtooth NF
- Homozygous for 12 of 13 loci
- Only sample with
  a rare allele

Perkins and Swetnam 1996

Inland West comprehensive genetics profile (GYE Example)

- Prioritization of areas for conservation
- High frequency of polymorphic loci

LAT N	LONG W	ELEV (ft)	Site	Unit
45.042	-109.430	8,900	Hellroaring	Custer
46.969	-107.091	9,028	Fish Creek	Bridger-Teton
42.645	-109.705	10,200	Blue Ridge	Shoshone
44.808	-110.442	8,936	Washburn	Yellowstone

Management application of molecular genetics study

F. Jay Haynes, YNP Archives

Management implications

- Operational cone collections: continue to follow minimum of 20 trees separated by 200 feet for an outcrossed species
- Seed inventory: minimum of three, bulked lots by seed zone and 400-ft elevation band for an effective population size (N_e) of 60
- Seven seed zones consolidated to five
- Revised seed zones shall be used for seed procurement planning, seed transfer, and experimental designs for orchards, genetic tests and breeding

(FSH 2409.26f Seed Handbook)

Genecology
Harnessing Rust Resistance

- Patterns of genetic variation in adaptive traits and assignment of species adaptive strategy

Mahalovich *in press*, Mahalovich and others 2006

Genecology (genetics + ecology)

- Focus on limiting traits critical to whitebark pine (blister rust resistance, cold hardiness)
- Greenhouse, nursery, and field locations

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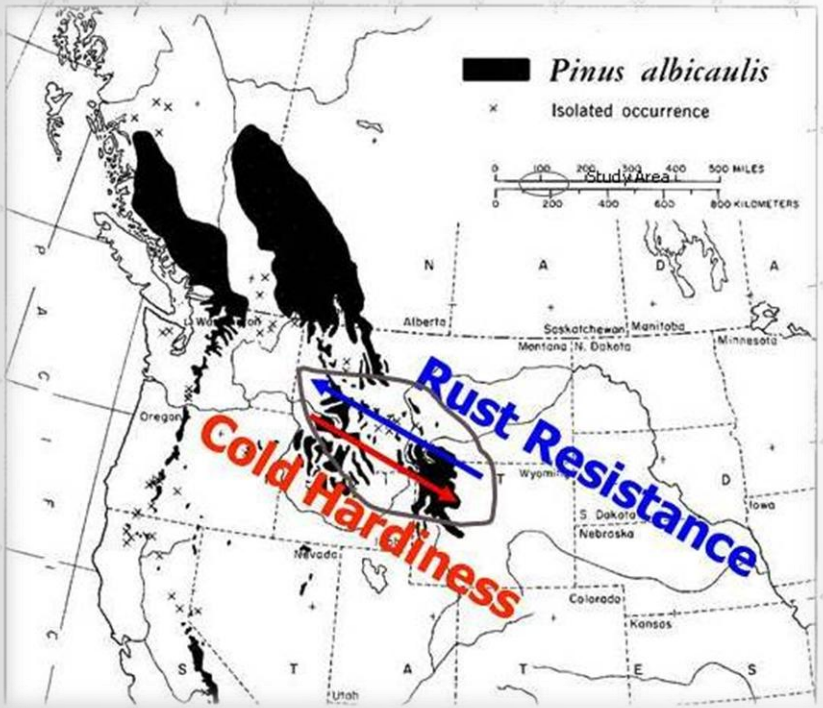
Modeled after the Northern Region's western white pine program

Seed source (family)	Individual-tree
Survival	Survival
Reduced spot frequency/meter	No spots
Reduced early stem symptoms	Needle shed
Bark reaction	Short shoot
Canker tolerance	Bark reaction
Height	Height
Cold Hardiness	

Utilized cone collections from 1992-1997

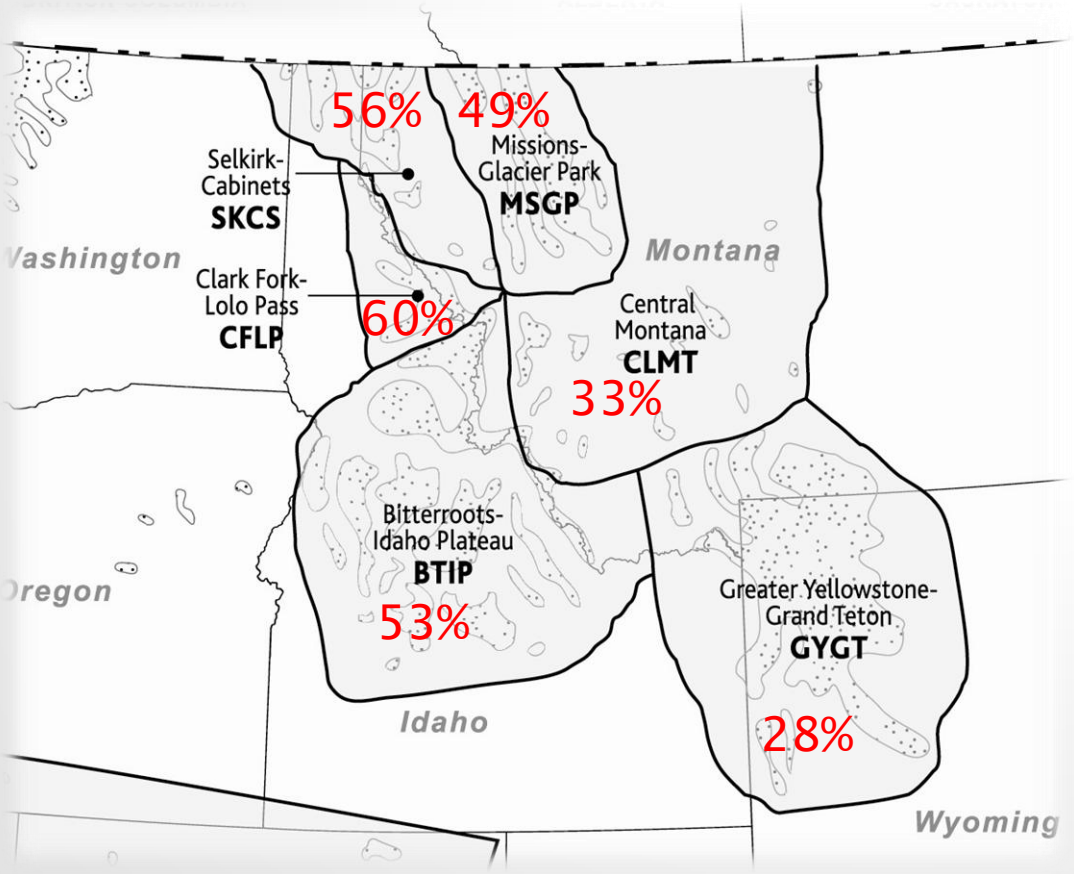
Mahalovich 2010

Adaptive Trait Relationships



- Best rust resistance northwestern MT to eastern WA
- Most cold hardy Greater Yellowstone Ecosystem
- Most mountain pine beetle tolerance central Idaho

WBP Blister rust resistance 47%



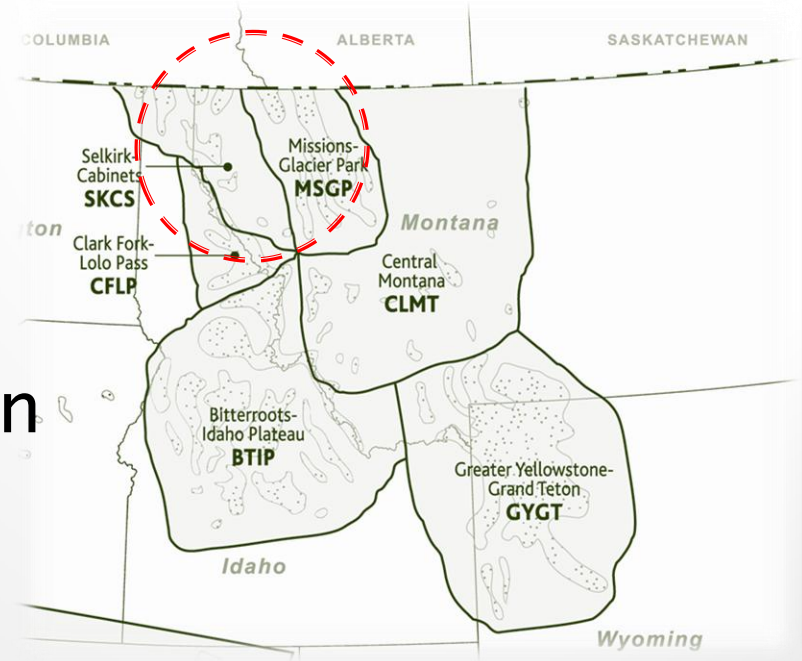
Moderate heritability = 0.68

Key Findings

- Most of the differentiation was explained by seed sources from the GYGT seed zone.
- Gentle clines in elevation for height and rust resistance.
- Generalist to intermediate adaptive strategy ($\pm 1.85^0$ latitude, $\pm 2.15^0$ longitude, $\pm$400 ft elevation)
- Adaptation to heterogeneous environments does not appear to be as strongly related to phenotypic plasticity as western white pine.

Management implications

- Seed zone consolidation (6→5)
- Rust resistance traits (7→6)
- Apply restricted selection index to handle unfavorable correlations
- Rust screening stock type (3→2-yr container)



FSH 2409.26f Seed Handbook
GYA WBP Restoration Strategy

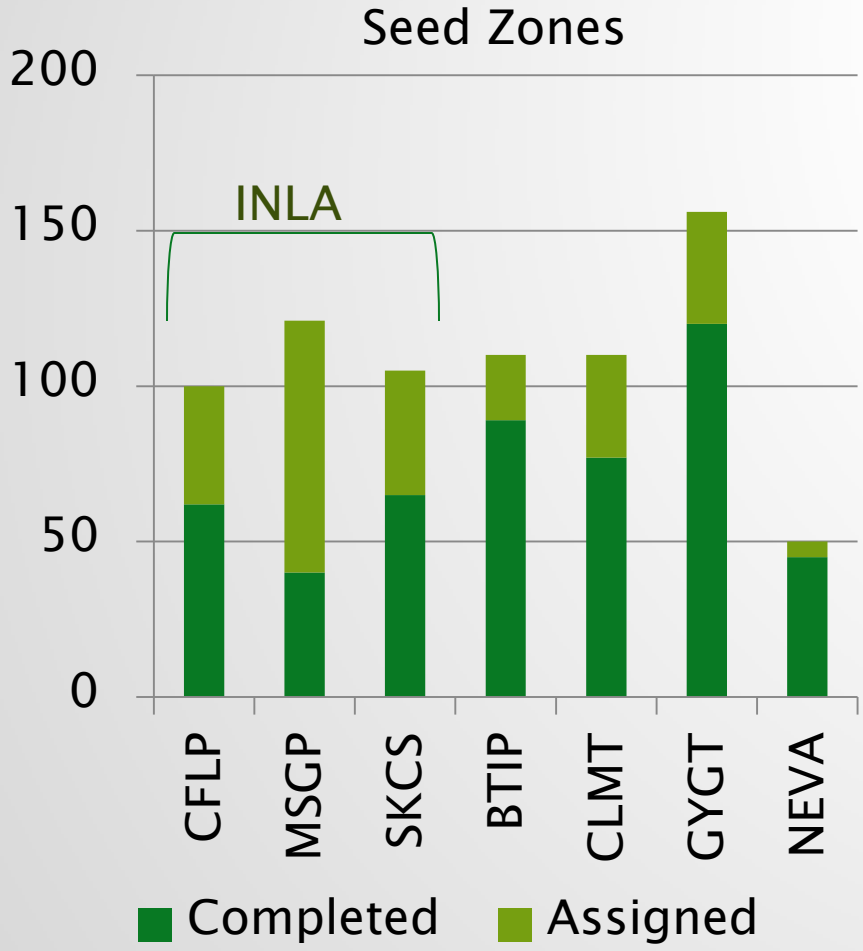
Inland West Whitebark Pine Genetic Restoration Program

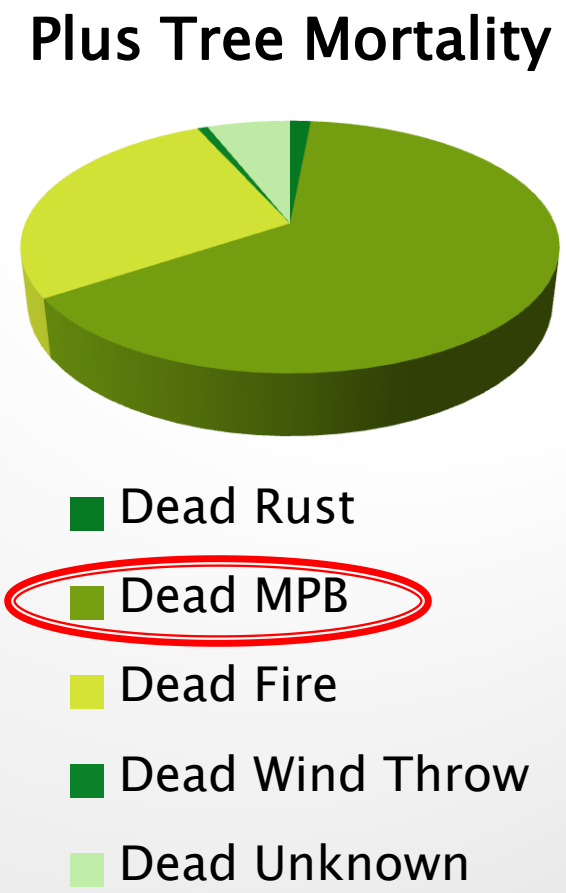
- Est. 2000

Plus tree identification
Cone collections

Requires wire cages to protect cone crop, tree climbing, and manual seed extraction

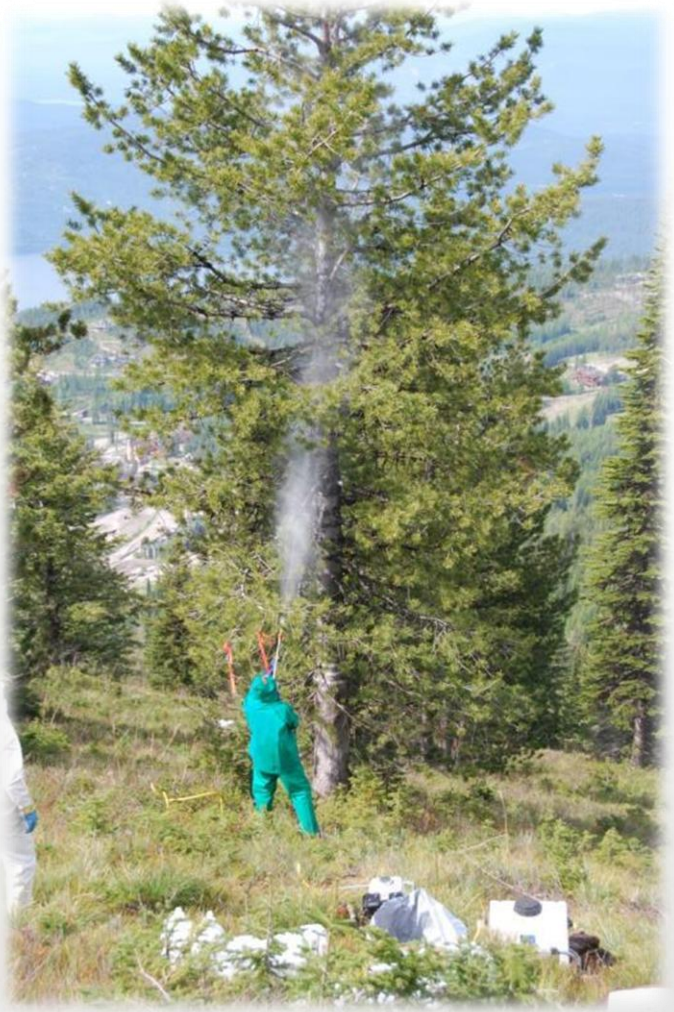
Cone Collection Accomplishments





Mountain pine beetle protection of elite/plus trees

Verbenone Pouches



Backcountry carbaryl mule sprayer
MTDC

Collecting Aeciospores
Inoculating the Ribes Garden

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Dave Foushee

Field work in preparation for rust screening

Rust screenings (artificial inoculations) Detached leaf method

Dave Foushee

Dave Foushee

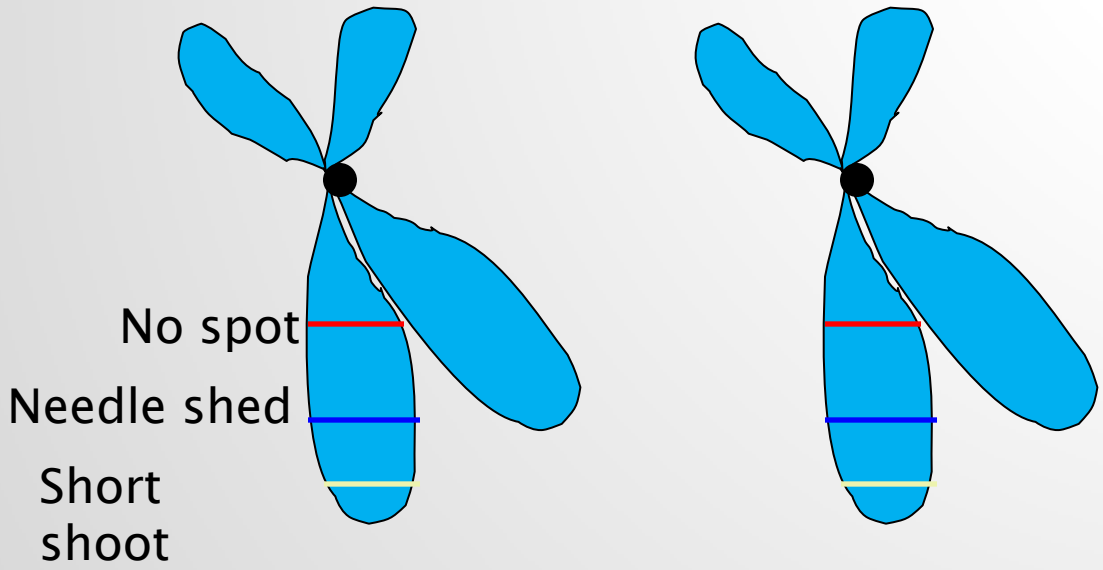
Rust Screenings

- 657 plus trees under evaluation (~95,000 seedlings)

Dave Foushee

Packaging rust resistance

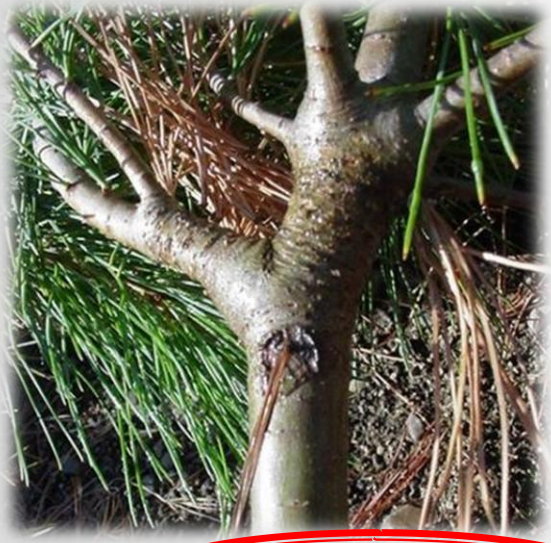
- *Pinus* spp. 12 chromosomes
- Assume traits are on the same chromosome
- Expression is sequential
- Both Mendelian and polygenic traits



Any given seedling has one individual-tree trait and four polygenic traits

Three-year screening process

Developing resistance rather than immunity



Bark reactions

Genetically improved material will still show spotting, branch flagging, and cankers, as some of these need to be expressed for the resistance response to kick in

Cankers

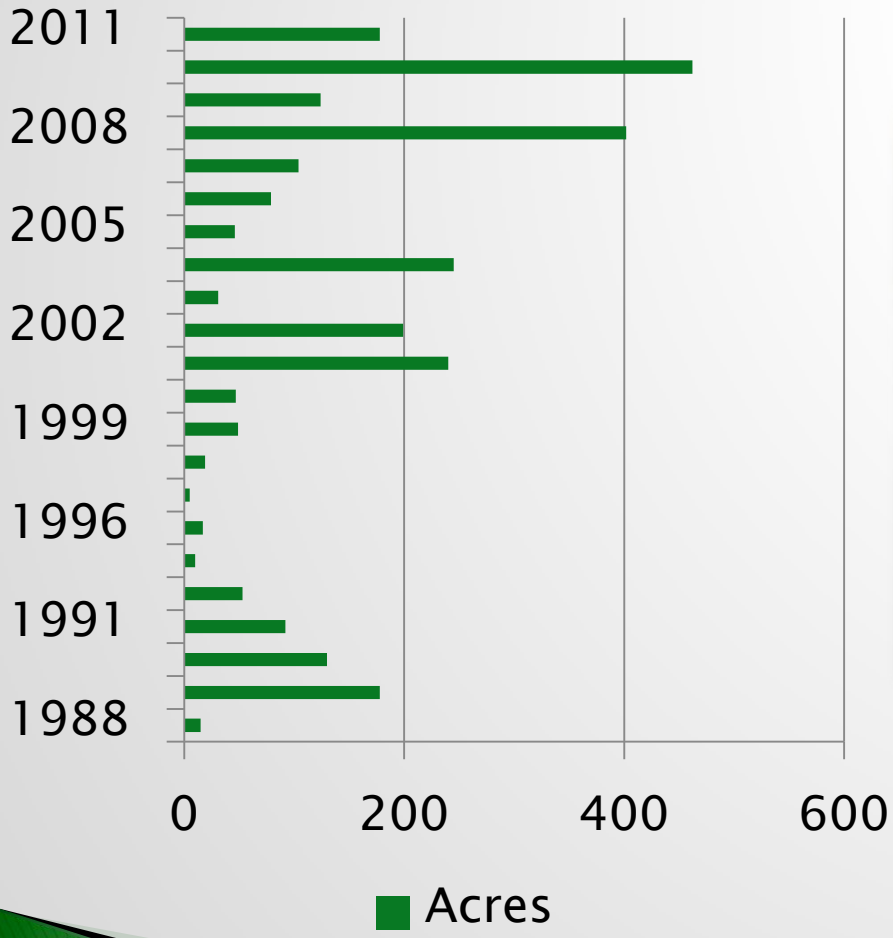
Needle spotting

Branch flagging

Payoff of genetics restoration program

- Planting genetically diverse, rust resistant seedlings

Planting accomplishments (2,874 acres)



Gallatin NF Beaver Creek

Roger Gowan

Nursery cultural practices

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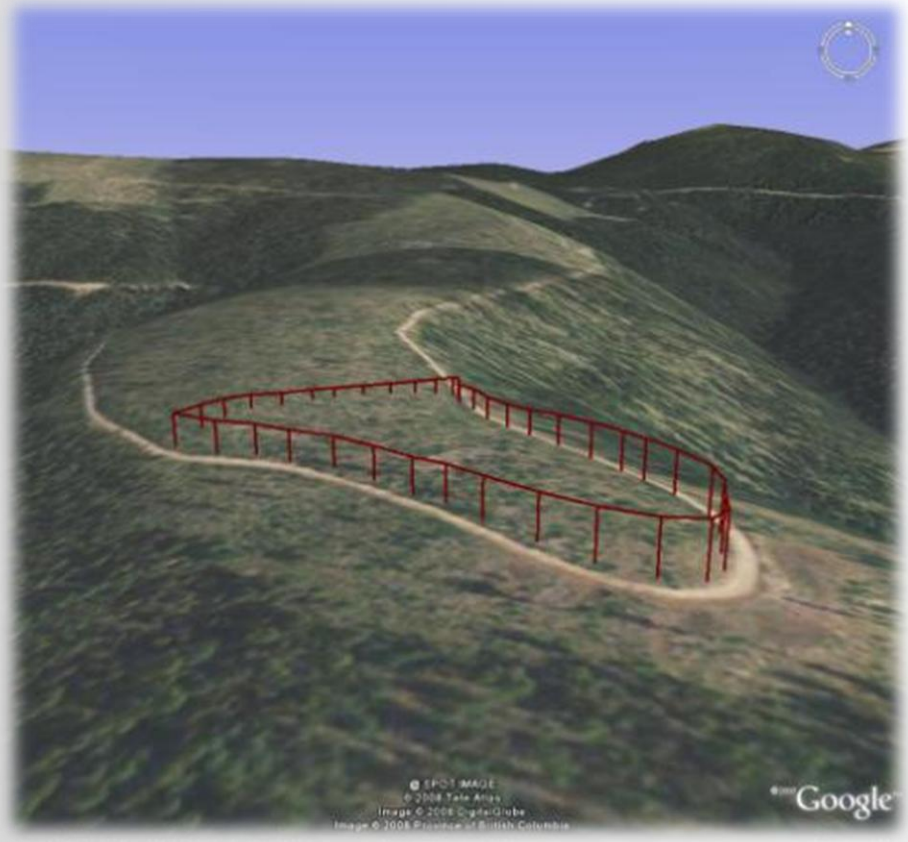
- Stock type: 1 to 2-year container
- Culling specs:
  Shoot length 3-10”
  Caliper 2.5 mm
- Average seedling cost from 1999-2011 dropped from $4.00+ to $1.75.

North Fork seed orchard

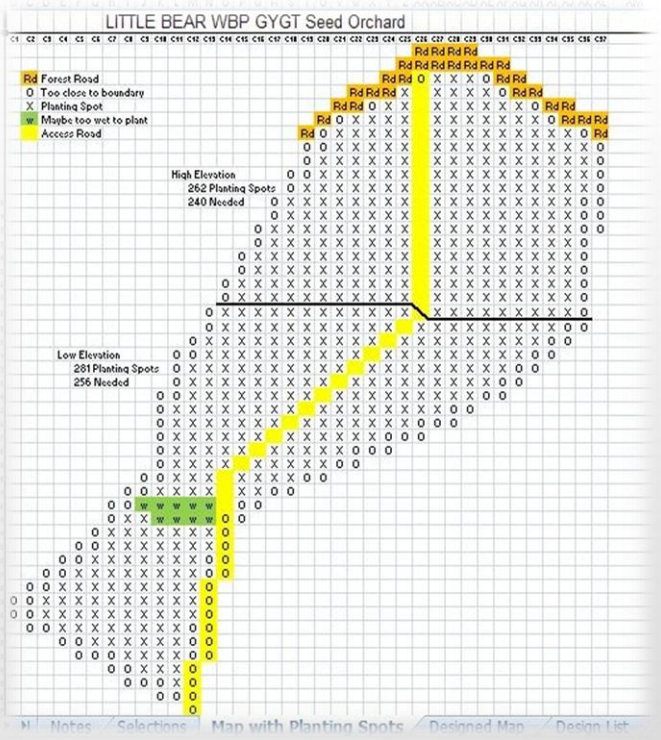


Valerie Walker

- Lolo National Forest
- Inaugural planting FY09
- Clonal (grafted) orchard
- Services Inland NW Seed Zone

Seed orchards

- Clearwater, Gallatin, Lewis & Clark, and Lolo NFs
- Located by habitat type
- Rescheduled spring scion collection to late fall
- GYGT zone additional cold hardiness design consideration



Seed Availability/Orchard Yields

1st year conelet July 2010

3- to 6-year old rootstock

30- to 200-year old scion

Anticipate first cone crops in the next five years; thereafter, promote flowering with GA 4/7

Accomplishments

- Program of Work
  Upcoming Assignments

FY11 Accomplishments*

Rust Screening	Scion (# trees)	Spores (# sites)	Pollen (# trees)	Cones (# trees)
CLMT/GYGT 1st and 2nd inspection	10 (0)	22 (6)	45 (17)	235 (ask me)
BTIP/SKCS Inoculated				
CFLP/MSGP Sown				

As of 10-14-2011
* ( ) GYGT

Long-term performance test

- Bulked control lot collection FY12-FY13 (20 trees)
- Little Bear location (GYGT)
- Site prep FY13
- Layout (RCB)
- Plant FY14
- Map FY14

Dave Foushee

Micro-environmental sensors climate monitoring

FY12-FY13 Installation; one per collection area

Status of our knowledge

- Genetically diverse with no marked inbreeding
- Has genetic variation in adaptive traits
- Moderate to high heritabilities that can respond to selection
- Good germination in older seedlots
- Cost effectively grow rust-resistant seedlings

Acknowledgements

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