Relationship of *Armillaria* genets and species distribution with habitat type and forest management practices

MEE-SOOK KIM[1], John W. Hanna[1,2], Ned B. Klopfenstein[2], Phil Cannon[3], and Geral I. McDonald[2]

[1]Dept. of Forest Resources, University of Idaho, Moscow, ID 83844
[2]USDA Forest Service, RMRS, 1221 S. Main St. Moscow, ID 83843
[3]BOISE®, 1111 W. Jefferson St. Boise, ID 83728

- The expression of Armillaria root disease results from the interactions among the genet, its host, and surrounding environmental factors.

- It is essential to identify and characterize *Armillaria* genets and species to understand interactions with host and environmental factors.

Forest Management – *Armillaria* Study

- The effect of forest management practices on genets and species diversity of *Armillaria*

- The relationships between habitat types and *Armillaria* genets/species distribution

- The identification of genetic markers from *Armillaria* genets/species correlated to their host species, habitat types, and other environmental factors
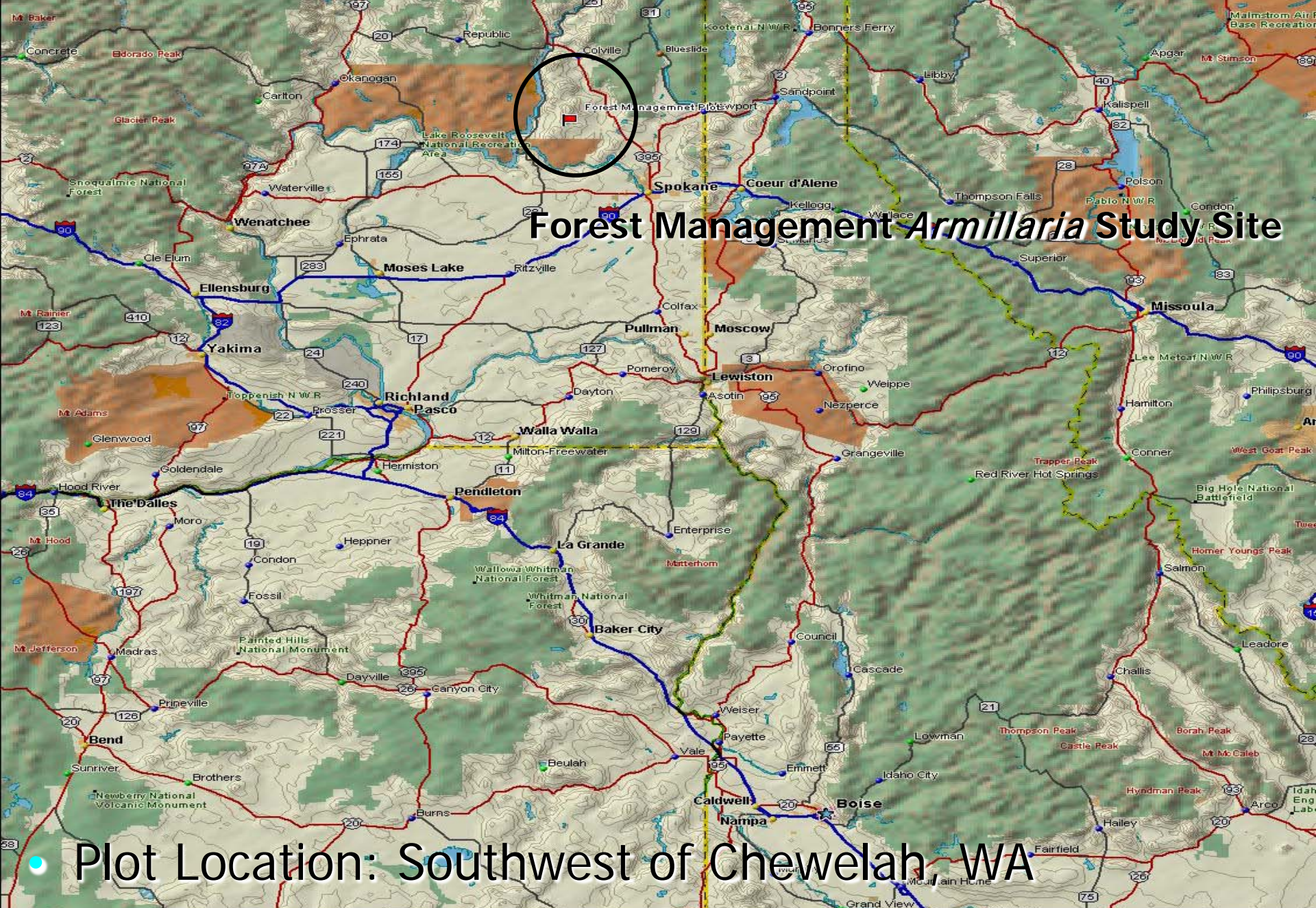
Forest Management *Armillaria* Study Site

Plot Location: Southwest of Chewelah, WA

- Plot selection
  - Wetness of the site
  - Pre-commercial thinning (PCT)
 - Fertilization

Wetness of the site

WET vs. **DRY**

Western hemlock, Western redcedar – Queencup beadlily, Twinflower

Grand fir – Ninebark
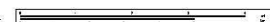
- CONTROL   WET - established 1920's.

    DRY - established 1930's

- THINNED   WET

    harvested by over-story removal in 1978, PCT in 1987, selectively harvested in 1987.

    DRY

    harvested by over-story removal in 1985, PCT in 1986

- THINNED & FERTILIZED   WET

    PCT in 1987 and fertilized in 1999 (nitrogen, potassium)

    DRY

    PCT in 1978 and fertilized in 1995 (nitrogen, potassium, sulfur, boron)
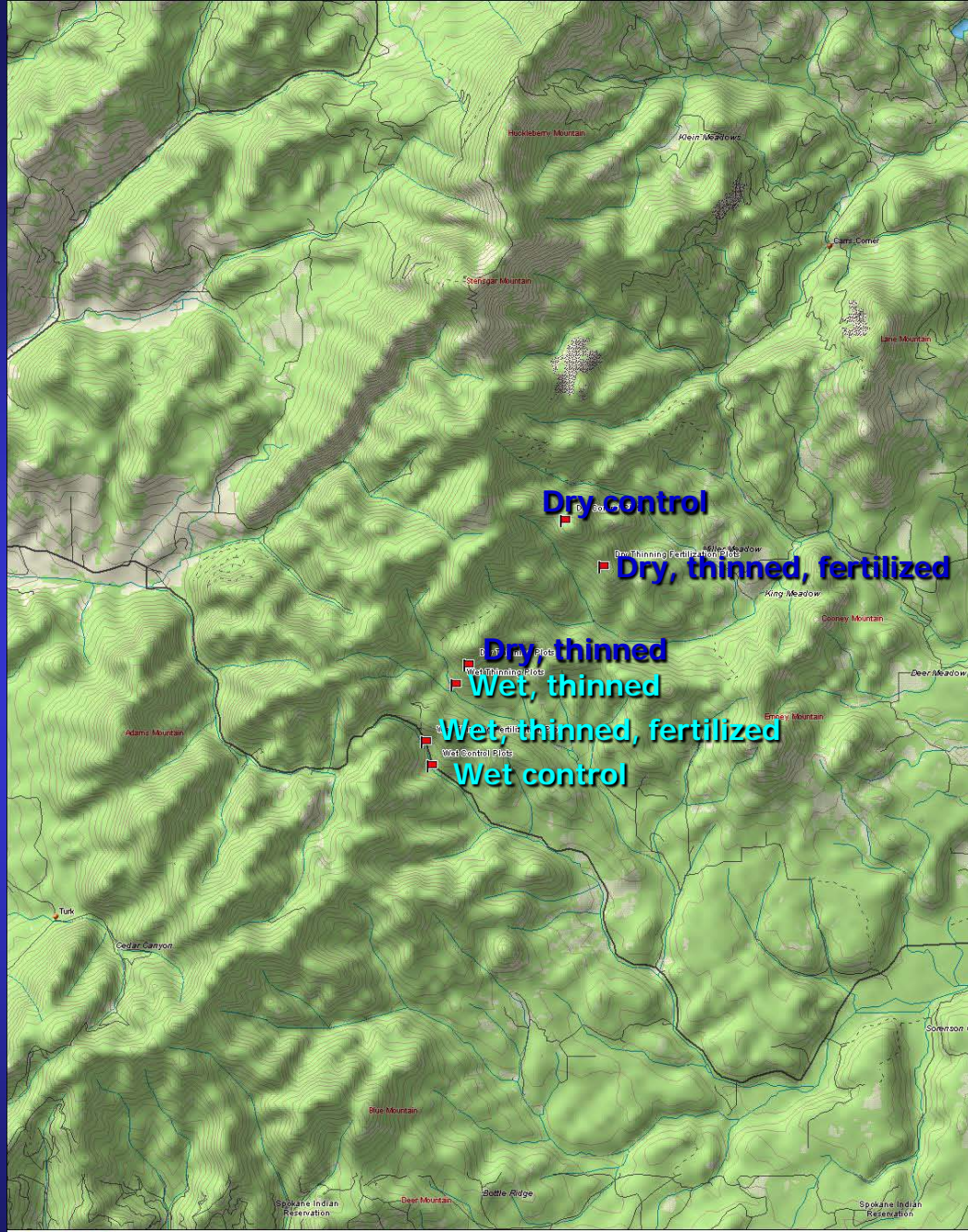
Dry control

Dry, thinned, fertilized

Dry, thinned

Wet, thinned

Wet, thinned, fertilized

Wet control

Armillaria Plots

- Control wet (redcedar-hemlock/clintonia-twin flower) undisturbed sites (3 plots)
- Control dry (grand fir/ninebark) undisturbed sites (3 plots)
- Wet PCT with no fertilization (3 plots)
- Dry PCT with no fertilization (3 plots)
- Wet PCT with fertilization (3 plots)
- Dry PCT with fertilization (3 plots)

Total: 6 treatments x 3 plots/treatment = 18 plots

Armillaria Plots

- Plot size: 0.04 ha (20 x 20 meters)
- Each plot was mapped using GPS, and individual trees were mapped with a laser range finder and angle encoder.
- Within each plot, three individuals (three different diameter classes) for <u>every tree</u> and hardwood shrub species present were surveyed for the occurrence of *Armillaria* (rhizomorphs, mycelial fans, or wood decay) and pathogenic colonization by *Armillaria* (e.g., resinosis)

  - Host information

  - Ecological information associated with *Armillaria* isolates
- Plant communities
- Topography, elevation, slope, aspect, and landform

Boise Cascade Wet Control #1

N

ROAD-GPS-POINT-VC

CORNER-2-VC1
×

BOTTOM-CENTER-POINT-VC1
×

CORNER-1-VC1
×

THPL3-VC1 SALIX2-VC1
GPS-POINT-VC1

PSME1-VC1

ABGR1-VC1

LAOC1-VC1

ABGR2-VTHPL2-VC1

ABGR3-VC1

THPL1-VC1

PSMESME3-VC1
PICO1-VC1

SALIX1-VC1

TSHE1-VC1

PICO3-VC1

CORNER-4-VC1
×

TSHE3-VGR1
TSHE2-VC1

PICO2-VC1

CORNER-3-VC1
×

Scale = 1 : 19.93(inch:feet)

BC #'s 191,192,192F,193,195,196,198,200,201,202

BC #
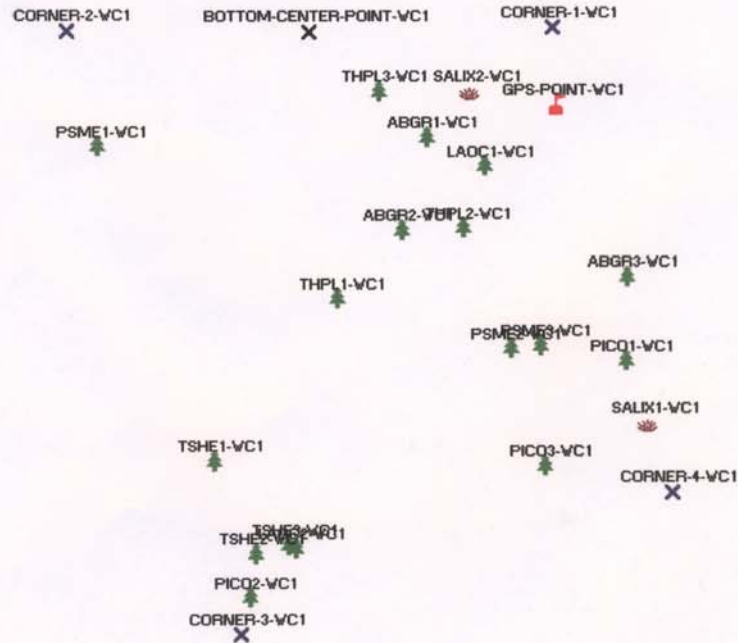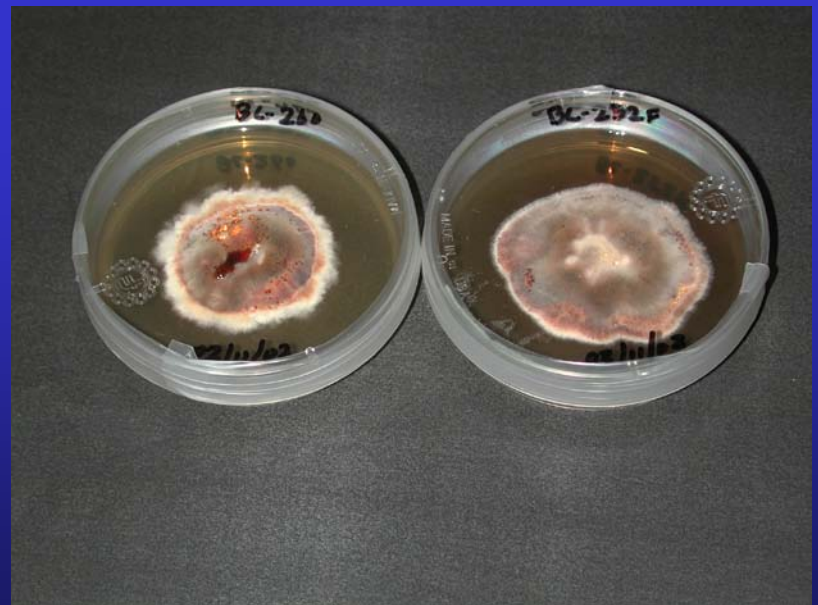
An Example of Somatic Incompatibility Test

Occurrence of *Armillaria* on conifers and shrubs growing on two different habitat types in eastern Washington

Habitat Type	*Armillaria* occurrence # of trees	No *Armillaria* occurrence # of trees	χ^2
WET (TSHE-THPL/CLUN-LIBO)	95 (70%)	41 (30%)	6.36*
DRY (ABGR/PHMA)	48 (53%)	42 (47%)	

*df = 1, α = 0.05, $p < 0.05$; TSHE: Western hemlock, THPL: Western redcedar, CLUN: queencup beadlily, LIBO: Twinflower, ABGR: Grand fir, PHMA: Ninebark

Occurrence of *Armillaria* on conifers and shrubs growing on <u>WET</u> habitat type

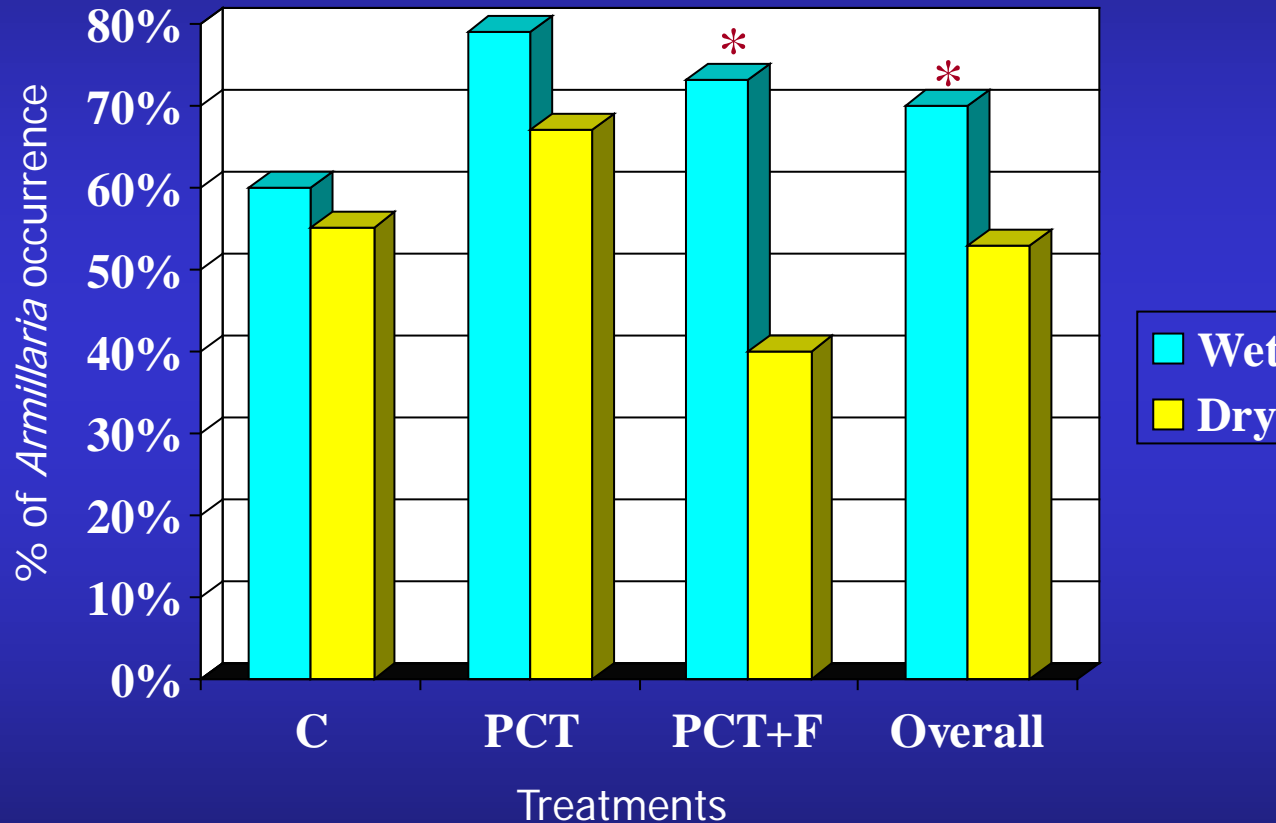| Wet habitat type (TSHE-THPL/CLUN-LIBO)	*Armillaria* occurrence # of trees	No *Armillaria* occurrence # of trees	χ^2
Wet, Control	32 (60%)	21 (40%)	4.13^{ns}
Wet, Thinned	34 (79%)	9 (21%)	
Wet, Thinned, Fertilized	29 (73%)	11 (27%)	

^{ns} df = 2, α = 0.05, p = 0.13; TSHE: Western hemlock, THPL: Western redcedar, CLUN: queencup beadlily, LIBO: Twinflower

Occurrence of *Armillaria* on conifers and shrubs growing on <u>DRY</u> habitat type

Dry habitat type (ABGR/PHMA)	*Armillaria* occurrence # of trees	No *Armillaria* occurrence # of trees	χ^2
Dry, Control	18 (55%)	15 (45%)	4.09^{ns}
Dry, Thinned	18 (67%)	9 (33%)	
Dry, Thinned, Fertilized	12 (40%)	18 (60%)	

* df = 2, α = 0.05, p = 0.13; ABGR: Grand fir, PHMA: Ninebark

Occurrence of *Armillaria* on conifers and shrubs growing on two different habitat types in eastern Washington

C: Control, T: Pre commercially thinned (PCT), PCT+F: Pre commercially thinned and Fertilized, *df = 1, α = 0.05, *p* < 0.05

Incidence of culturally verified *Armillaria* on conifers within WET (TSHE-THPL/CLUN-LIBO) /DRY (ABGR/PHMA) habitat types

Conifer species	# of plants inspected	# with *Armillaria*	Proportion with *Armillaria*	χ^2
Ponderosa pine	18	6	33%	47.17*
Lodgepole pine	9	1	11%	
Douglas-fir	44	29	66%	
Grand fir	44	39	87%	
Western larch	37	26	70%	
Western redcedar	20	4	20%	
Western hemlock	10	8	80%	
Shrubs[1]	42	29	69%	

[1]Oceanspray, Serviceberry, Ninebark, Rocky Mountain Maple, Salix, Redstem ceanothus, Pacific yew, Buffaloberry, Blue elderberry

*df = 6, α = 0.05, $p < 0.05$; TSHE: Western hemlock, THPL: Western redcedar, CLUN: queencup beadlily, LIBO: Twinflower, ABGR: Grand fir, PHMA: Ninebark

Incidence of culturally verified *Armillaria* on conifers within <u>WET</u> (TSHE-THPL/CLUN-LIBO) habitat types

Conifer species	# of plants inspected	# with *Armillaria*	Proportion with *Armillaria*	χ^2
Ponderosa pine	2	2	100%	45.35*
Lodgepole pine	7	1	14%	
Douglas-fir	16	12	75%	
Grand fir	32	30	94%	
Western larch	27	22	81%	
Western redcedar	20	4	20%	
Western hemlock	10	8	80%	

*df = 6, α = 0.05, $p < 0.05$; TSHE: Western hemlock, THPL: Western redcedar, CLUN: queencup beadlily, LIBO: Twinflower

Incidence of culturally verified *Armillaria* on conifers within DRY (ABGR/PHMA) habitat types

Conifer species	# of plants inspected	# with *Armillaria*	Proportion with *Armillaria*	χ^2
Ponderosa pine	16	4	25%	10.69*
Lodgepole pine	2	0	0%	
Douglas-fir	28	17	61%	
Grand fir	12	9	75%	
Western larch	10	4	40%	

* df = 4, α = 0.05, $p < 0.05$; ABGR: Grand fir, PHMA: Ninebark

List of *Armillaria* genets from northeastern WA identified by somatic incompatibility test

Habitat types Treatments	*Armillaria* genets	Total # of genets
Wet, Control	BC99, BC103, BC113	3
Wet, Thinned	BC204, BC205, 207	3
Wet, Thinned, Fertilized	BC157, BC160	2
Dry, Control	BC1, BC3, BC13, BC18F, BC26	5
Dry, Thinned	BC34, BC38, BC50, BC52	4
Dry, Thinned, Fertilized	BC65, BC72, BC84, BC92, BC94	5

Neighbor-joining tree of *Armillaria* genets from eastern WA and *Armillaria* reference species based on DNA sequences of intergenic spacer region
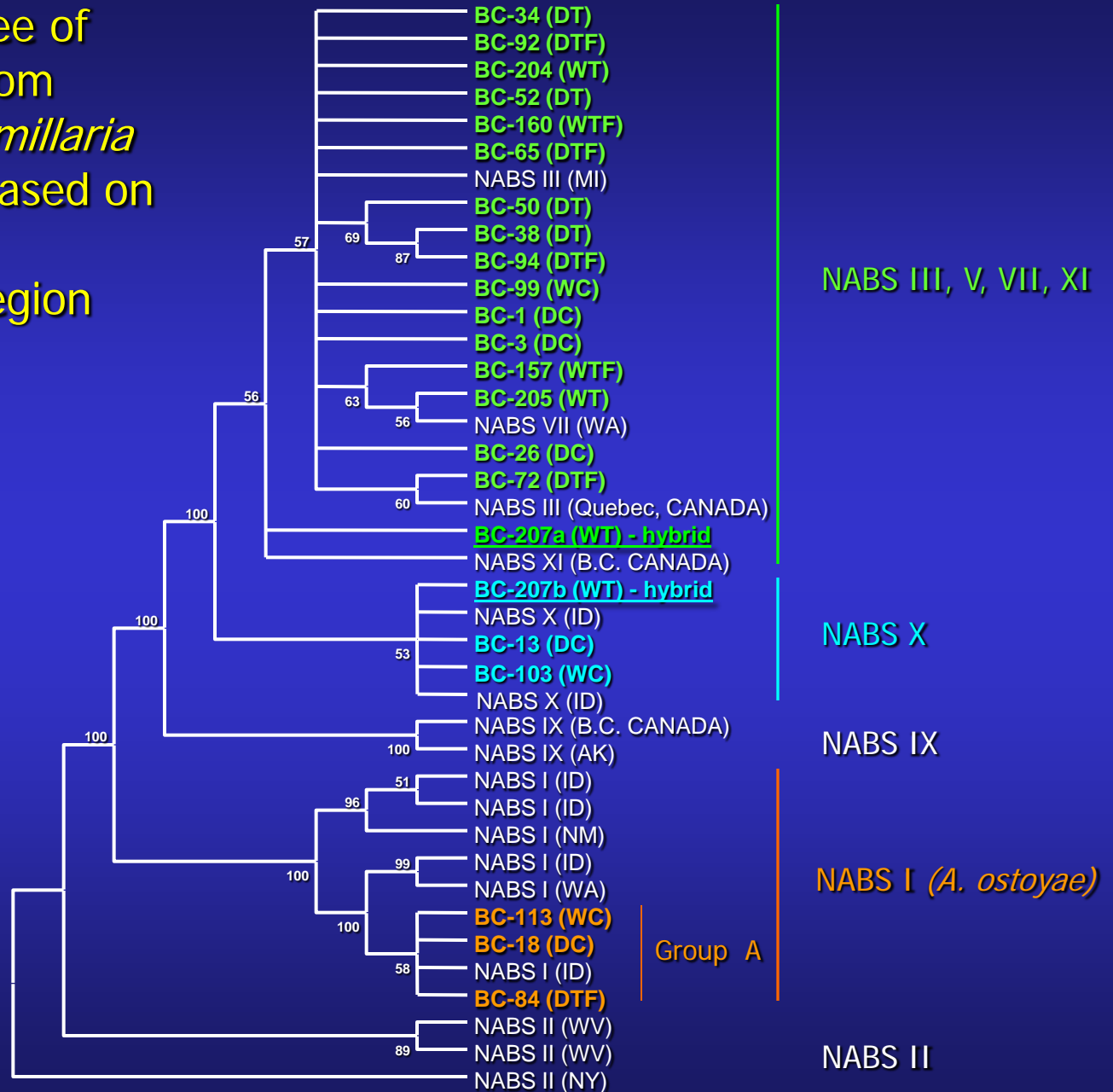
Current status of *Armillaria* genets and species derived from northeastern WA

| Treatment	# of genets	# of species	Species ID
Wet, Control	3	3	*A. ostoyae* NABS III, V, VII NABS X
Dry, Control	5	3	*A. ostoyae* NABS III, V, VII NABS X
Wet, Thinned	3	2	NABS III, V, VII NABS X (hybrid)
Dry, Thinned	4	1	NABS III, V, VII
Wet, Thinned, Fertilized	2	1	NABS III, V, VII
Dry, Thinned, Fertilized	5	2	*A. ostoyae* NABS III, V, VII

Preliminary Observation - I

- *Armillaria* occurrence was higher in the wet habitat type (TSHE-THPL/CLUN-LIBO) than the dry habitat type (ABGR-PHMA).

- No differences in *Armillaria* occurrence were found within habitat type (control vs. thinned vs. thinned and fertilized).

- Incidence of *Armillaria* was different among conifer species.

- A total of 22 genets were recovered from 289 isolates using somatic incompatibility tests.

- Three *Armillaria* species were identified from 22 genets using DNA sequences of intergenic spacer region.
    A. ostoyae, NABS III-V-VII-XI complex, NABS X

Preliminary Observation - II

- The dry habitat type contained more genets than the wet habitat type (14 vs. 8).

- Undisturbed control plots (both wet and dry) have more diverse *Armillaria* spp. than disturbed plots.

- NABS X, which is a potential protector against pathogenic *A. ostoyae,* did not occur in disturbed plots (WT – hybrid NABS X).

Armillaria NABS I (*A. ostoyae*), X, and mixed I and X on the Ida Creek plantation
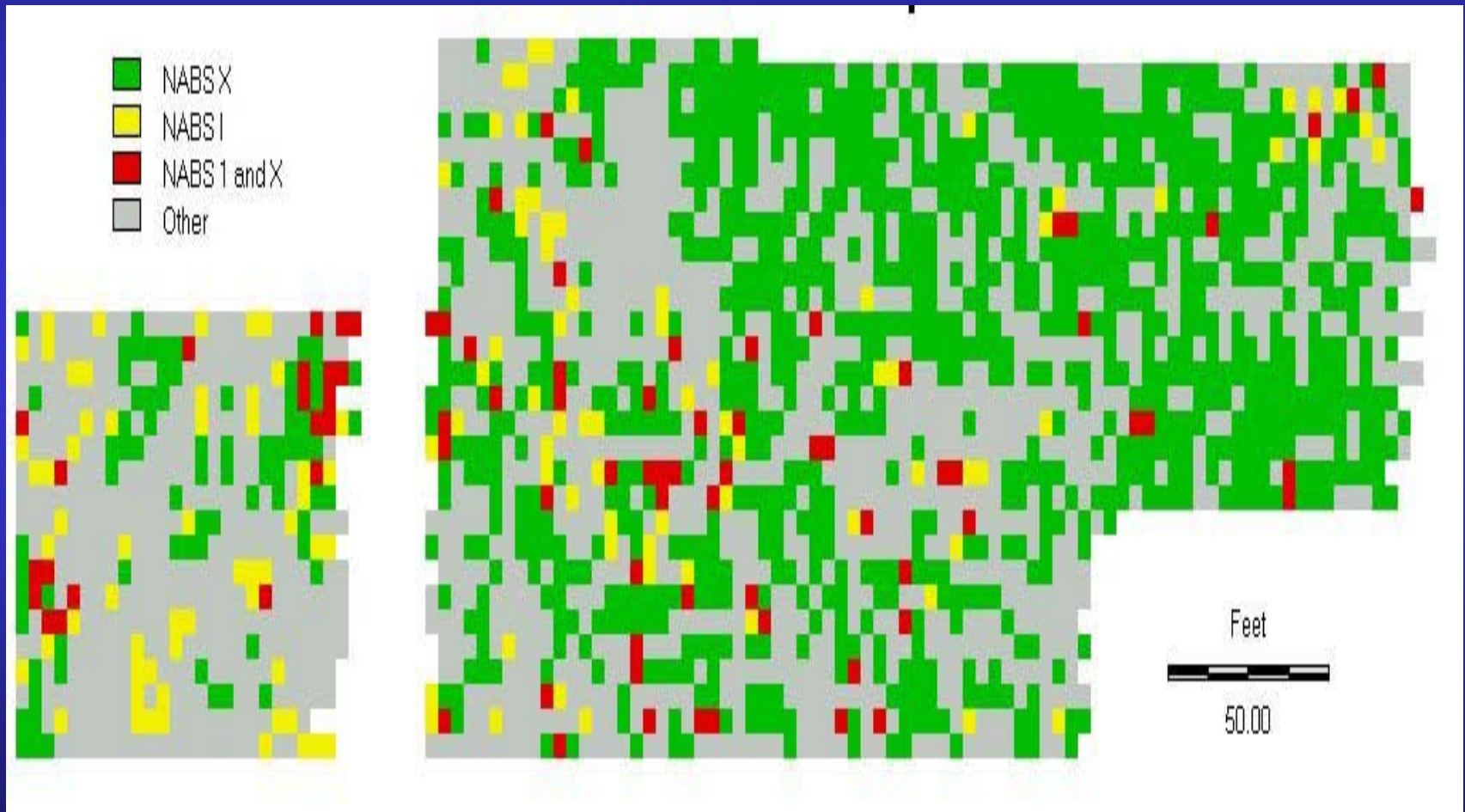
Hybridization within and among *Armillaria* spp.
## An example from BC-207 (NABS X hybrid)

• A selected NABS XI isolate exhibited compatibility with a selected NABS X isolate, and selected NABS V isolates were individually compatible with selected isolates of NABS X and NABS XI <u>in culture</u> (Kim et al. 2001, *Mycological Research*).

• We found inter- and intra-specific hybridization occurring within and among *Armillaria* spp. derived from <u>nature</u>.
　　　- intra-specific: *A. ostoyae*, NABS X
　　　- inter-specific: between *A. ostoyae* and *A. gemina* (NABS II)
　　　　　　　　　　　between NABS X and NABS VII

• The possible pathogenicity of NABS X hybrids is under investigation at the USDA Forest Service, RMRS, Forestry Sciences Lab, Moscow, ID.

Forest Management – *Armillaria* Study

- The effect of forest management practices on genets and species diversity of *Armillaria*

- The relationships between habitat types and *Armillaria* genets/species distribution

• The identification of genetic markers from *Armillaria* genets/species correlated to their host species, habitat types, and other environmental factors

AFLP
(**A**mplified **F**ragment **L**ength **P**olymorphism)
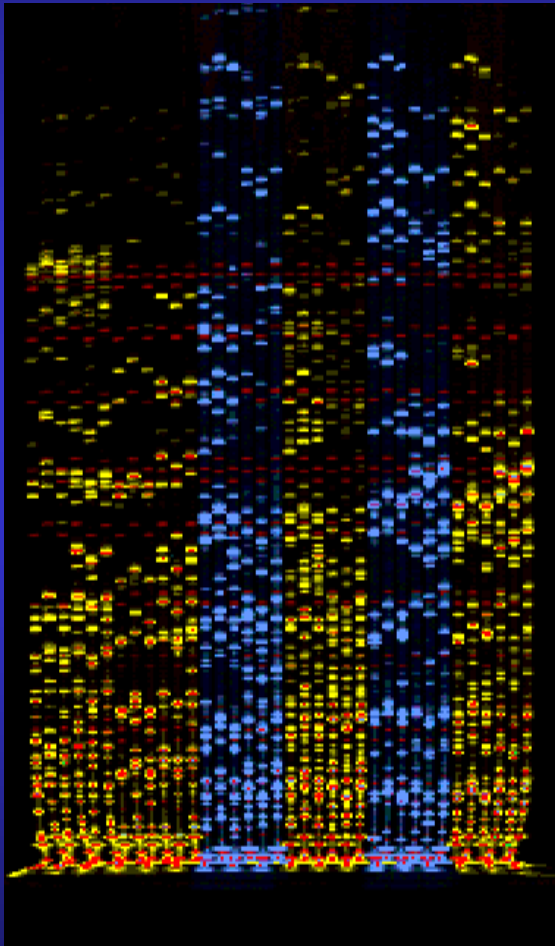
AFLP gel image of *Armillaria sinapina* and X genets

- Prepare DNA extraction and generate genetic markers from 22 BCC *Armillaria* genets

- Complete analyses of genetic marker data on the basis of geographic and selected environmental parameters

- Integrate genetic marker data with GIS based systems

Develop predictive models for *Armillaria* occurrence based on pathogenicity, soil nutrition, plant factors, and other available environmental information

Armillaria genets from

- Preexisting plots located 11 western states
- IFTNC Forest Health Study sites
- Boise Cascade lands

Plots and *Armillaria* Isolate Collection
USDA Forest Service, Rocky Mountain Research Station, Forestry Sciences Lab, Moscow, ID

- ca. 300 plots in the 11 western states

- Samples from 50 locations in Wisconsin, Iowa, Tennessee, and Nebraska

- 10,000 isolates belonging to 1,100 genets from
  A. gallica, *A. ostoyae*, *A. sinapina*, *A. mellea*,
  A. nabsnona, *A. cepistipes*, and NABS X

Armillaria Collection
at the USDA Forest Service, RMRS, Forestry Sciences Lab, Moscow, Idaho
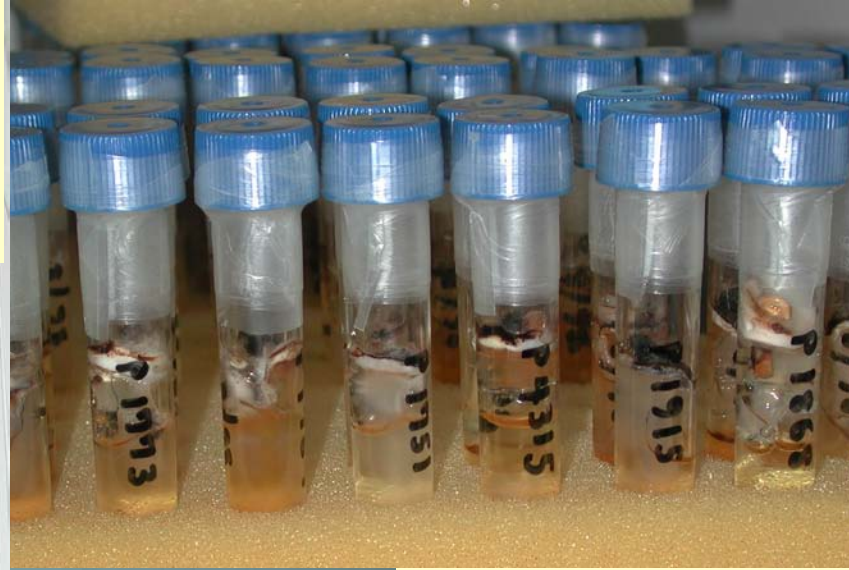
- Location
- Host information
- Habitat typing
- Ecological info. of *Armillaria* isolates
- Physical data
   Light
   Temperature
   Soil data
   Stand data

Advantages of this novel experimental approach

These studies allow application at the landscape level by integrating two rapidly developing, highly informative technologies:
- Molecular biology
- Remotely sensed data analysis (Geographic Information Systems)

This integrative approach should provide insights into critical forest-management issues within a few years. Traditional studies would require decades to address similar issues.

Applications to Management

- Determine appropriate management practices for specific sites
    - species selection
    - thinning and harvest methods
    - site preparation
    - manipulation of soil nutrition
    - management of soil water relations
    - prescribed burns, etc.

- Increase beneficial effects of saprophytic *Armillaria* spp. and minimize effects of pathogenic *Armillaria* spp.

Acknowledgments

University of Idaho
- James A. Moore
- Steven J. Brunsfeld
- Terry M. Shaw
- Raini C. Rippy

BOISE®
- Mike Brown
- Phil Anderson

USDA Forest Service
- James B. Donley
- Joanne Tirocke
- Bryce A. Richardson
- Jill A. Hoff
- Jonalea R. Tonn
- Kwan-Soo Woo