

Forest Health Experiment  
*Armillaria ostoyae* Root Disease  
Inoculation



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Intermountain Forest Tree Nutrition Coop

Terry Shaw



# Armillaria Inoculation Study



*Armillaria* Collection



Inoculum



Inoculation



Collect Inoculated Roots



Infection Verification



Identification



## 1999-2000 *Armillaria* Collection Summary

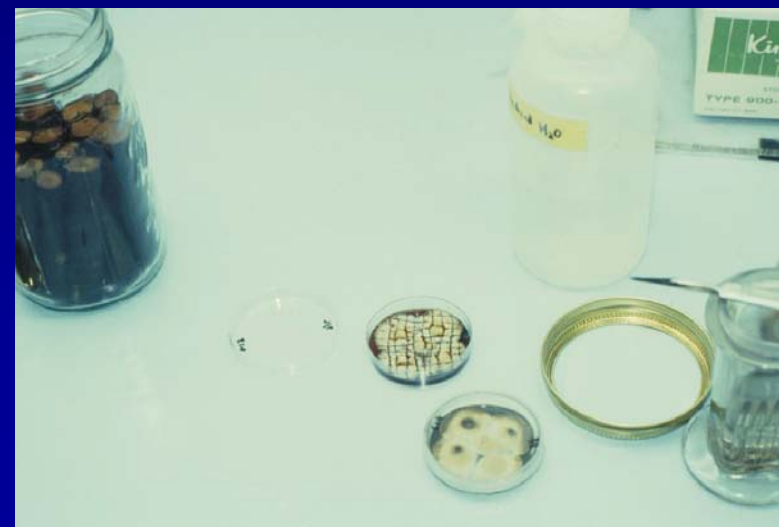
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Collection Year	1999	2000
Number of Sites	15	15
Number sites <i>Armillaria</i> collected	12	11
Number sites with <i>Armillaria ostoyae</i>	8	9



# Armillaria Collection Summary

	Collection Year	
	1999	2000
No. Samples	627	672
No. Isolates	519	650
No. Genets	111	110
No. Pairings	4140	4014





# 1999-2000 *Armillaria* Species Collection

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Species	Number of Genets
<i>ostoyae</i>	62
NABS X	16
NABS III <i>calvescens</i> NABS V <i>Sinapina</i> Complex NABS VII <i>Gallica</i>	14
NABS X/ III, V, VII	31
<i>nabsnona</i>	1

# Forest Health



## Genetic Diversity of *A. ostoyae* Inoculations

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- All but one site (Enterprise 337) received regional genet group PNW A x Rockies hybrid
  - PNW A (9)
  - PNW A x PNW B hybrid(6)
  - PNW A x Rockies hybrid(2)
- Both genets at Enterprise are group PNW A x PNW B hybrids
- Both genets at Springdale (346) are group PNW A x Rockies hybrids

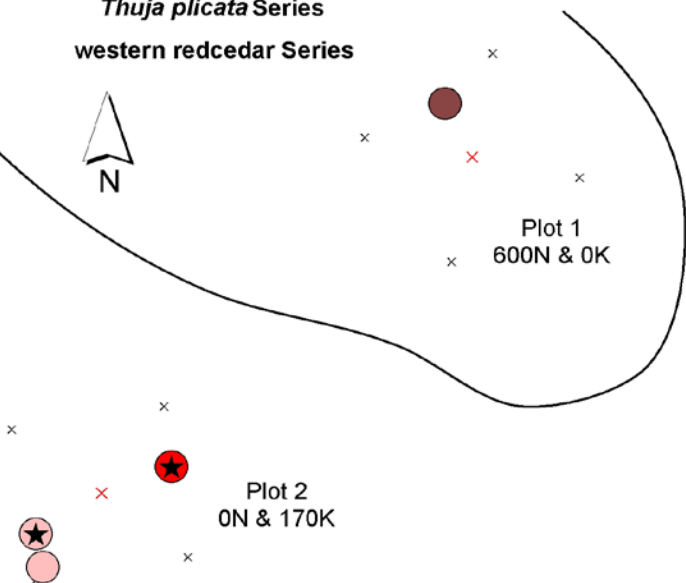
# Spirit Lake - Study Site

## Intermountain Forest Tree Nutrition Co-op

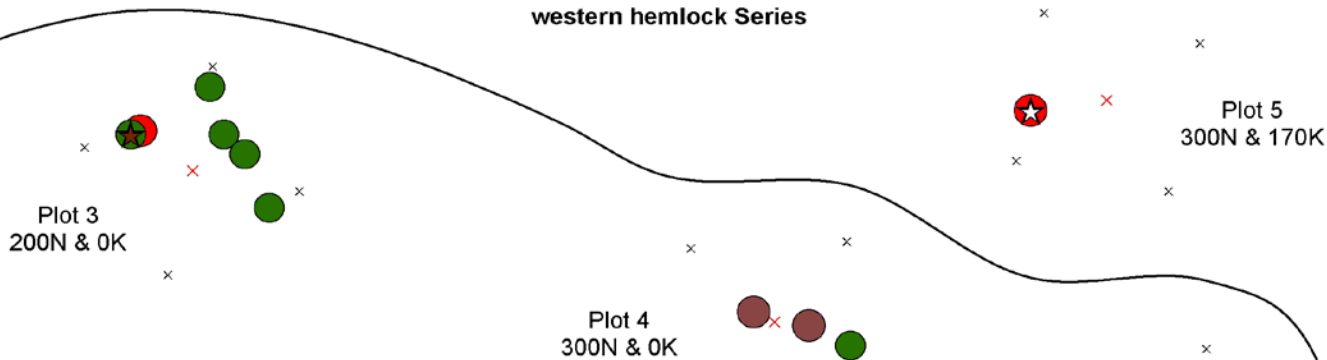
### Legend

- |   |                                 |   |  |
|---|---------------------------------|---|--|
| × | plot corners                    | ☆ | Barkfan on dead tree                                 |
| × | plot centers                    | ★ | Barkfan on live tree (active pathogen)               |
| ● | Genet 1 NABS   <i>A.ostoyae</i> | ★ | Wood Sample (Note: from <i>Holodiscus discolor</i> ) |
| ● | Genet 2 NABS   <i>A.ostoyae</i> |   |  |
| ● | Genet 3 NABS   <i>A.ostoyae</i> |   |  |
| ● | Genet 4 NABS X                  |   |  |

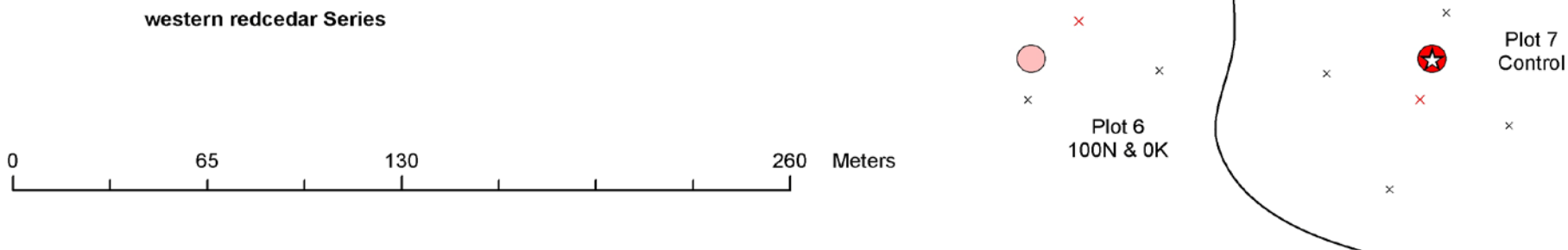
*Thuja plicata* Series  
western redcedar Series



*Tsuga heterophylla* Series  
western hemlock Series



*Thuja plicata* Series  
western redcedar Series



0 65 130 260 Meters



# *Armillaria* Inoculum

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Inoculum Blocks



*Armillaria* Field Ready







# IFTNC Inoculation Sites

2000

2001

Site	Region	Site	Region
Bovill (335)	NID	Spirit Lake (336)	NID
Enterprise (337)	NEO	Grasshopper (341)	NID
Huckleberry (354)	NID	Upper Pataha (342)	SEWA
Stanton (355)	NID	Springdale (346)	NEWA
Soldier (357)	NID	Whiskey Butte (348)	NID
Furport (360)	NEWA	Pivash (349)	NID
Hanson (361)	NEWA	Sportsman Access (356)	NID
Haverland (362)	NEWA	Skookum Lake (358)	NEWA
		Dick's Creek (359)	NID

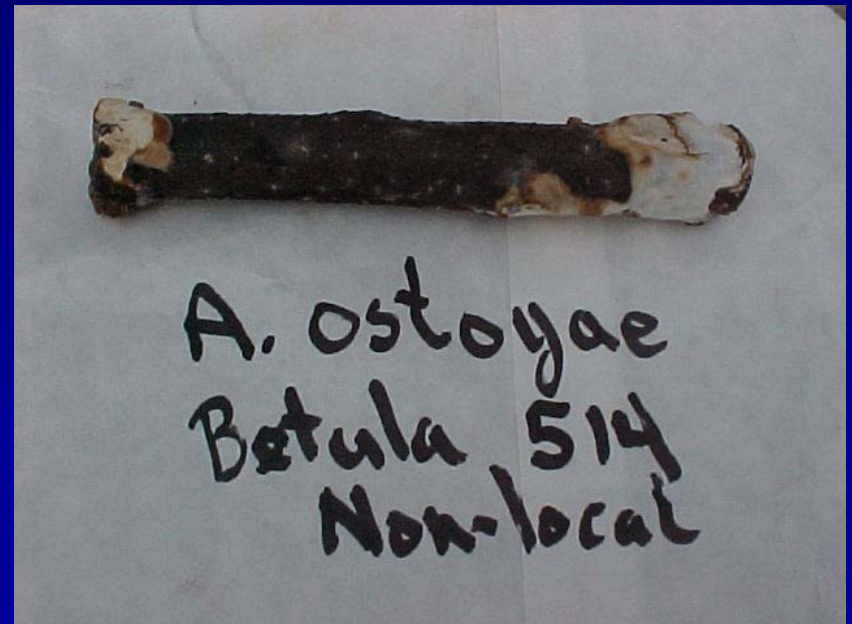
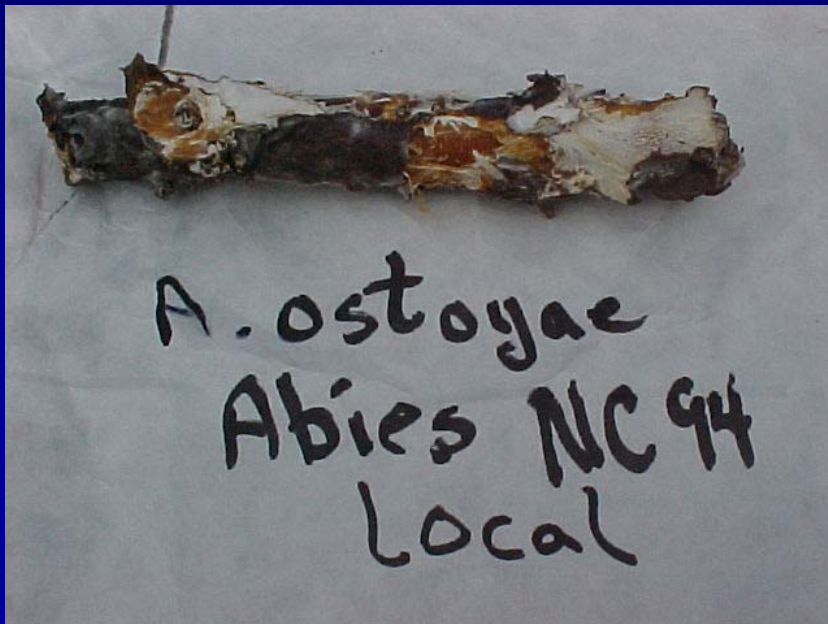
# Forest Health Root Inoculation



## Pathogenetic *A. ostoyae* Isolates

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- Local *A. ostoyae* Isolate
- Regional *A. ostoyae* Isolate



# Forest Health Core Treatment Design

## *A. ostoyae* Inoculation Design



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 <p>0#N/a + 0#K/a</p>	<p>300#N/a + 0#K/a</p>
   <p>0#N/a + 170#K/a</p>	   <p>300#N/a + 170#K/a</p>  



# Armillaria Inoculation

Each tree received one local isolate and one regional isolate



Five trees per plot were inoculated



Secured with plastic wrap

Finished product



# Inoculated Root Collection Excavation



Three years after inoculation





# Infection Verification

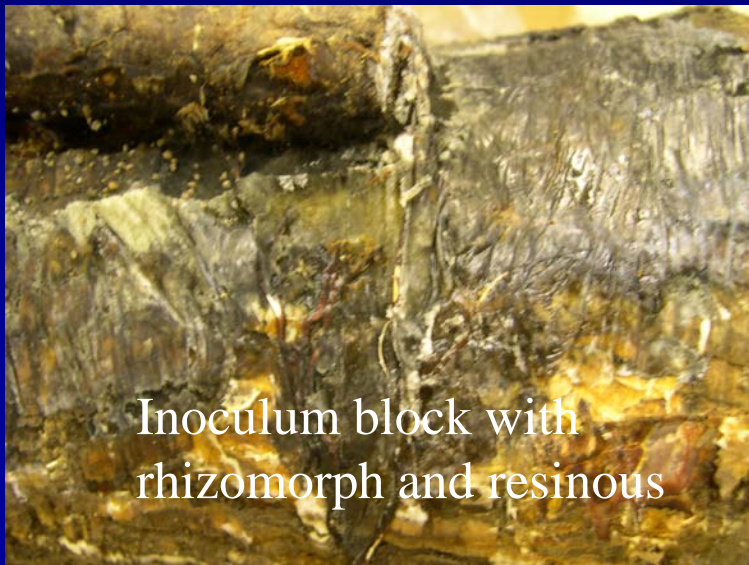
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Rhizomorph w/ mycelial fan



Rhizomorph attached to inoculum block



Inoculum block with rhizomorph and resinous



Rhizomorph/wound contact

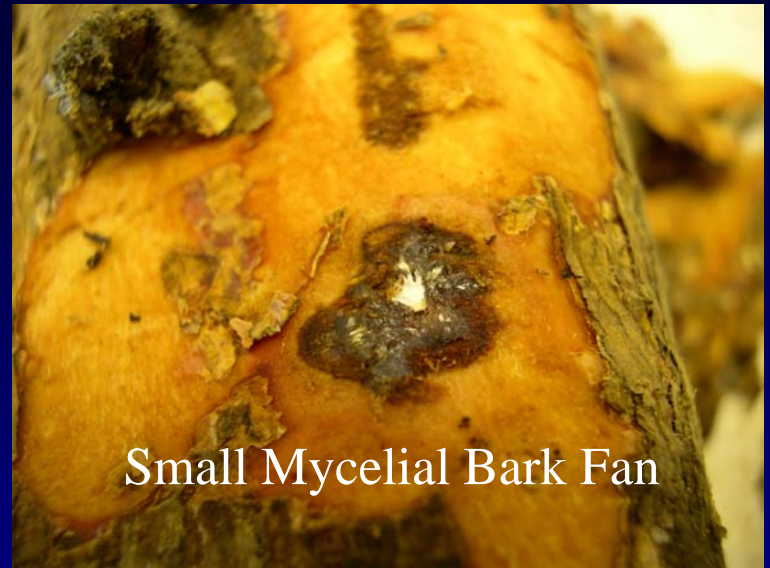


# Infection Verification

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“Invader” Rhizomorph



Small Mycelial Bark Fan



Brown Necrotic Area



Mycelial Bark Fan



# Infection Verification Methods

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# Infection Verification Methods Ratings



- Block
  - Viable, infection rhizomorph or mycelial fan
- Root Mycelial Fan Rating
  - Absent or present
  - Surface or under root bark (outer or inner bark, cambium)
  - Origin – rhizomorph or mycelial fan
  - Origin – block or invader
- Root Rhizomorph Rating
  - Absent or present
  - Attached to surface or not attached to surface
  - Origin – block or invader
  - Absent or present
  - Attached or not attached
  - Origin – block or invader

# Infection Verification Results by Installation



Installation	Rock Type	Vegetation Series	Isolates	Numbers of Roots
Bovill (335)	Meta	Cedar	53,514	6
Enterprise (337)	Basalt	Douglas-fir	542,546	11
Huckleberry (354)	Meta	Grand fir	514	3
Stanton (355)	Meta	Cedar	-	0
Soldier (357)	Basalt-Mix	Hemlock	166,514	5
Furport (360)	Glacial-Mix	Grand fir	580	4
Hanson (361)	Glacial-Mix	Cedar	463	8
Haverland (362)	Granite-Mix	Grand fir	-	0

# Infection Verification

## Isolate Identification

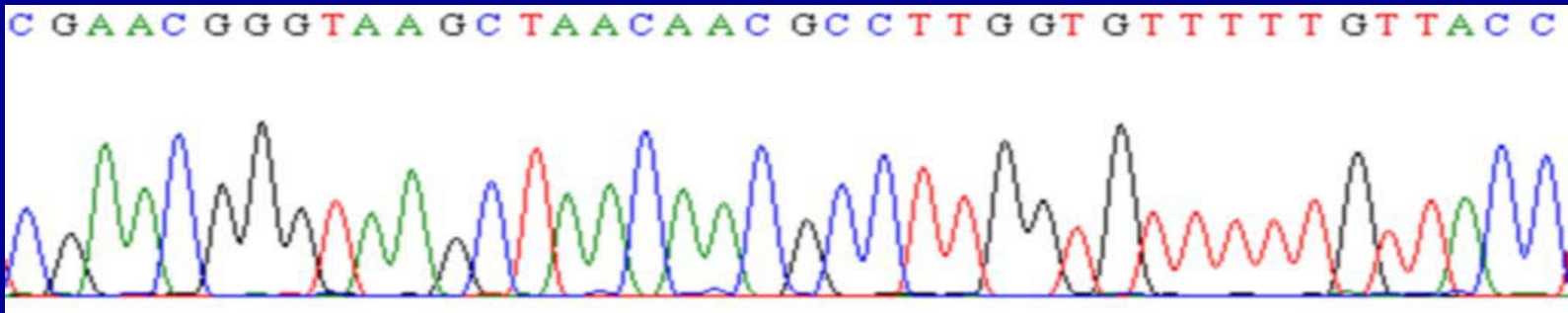
### Somatic Incompatibility – DNA Sequencing



Somatic  
Incompatibility



DNA Sequencing



# Infection Verification Isolate Identification



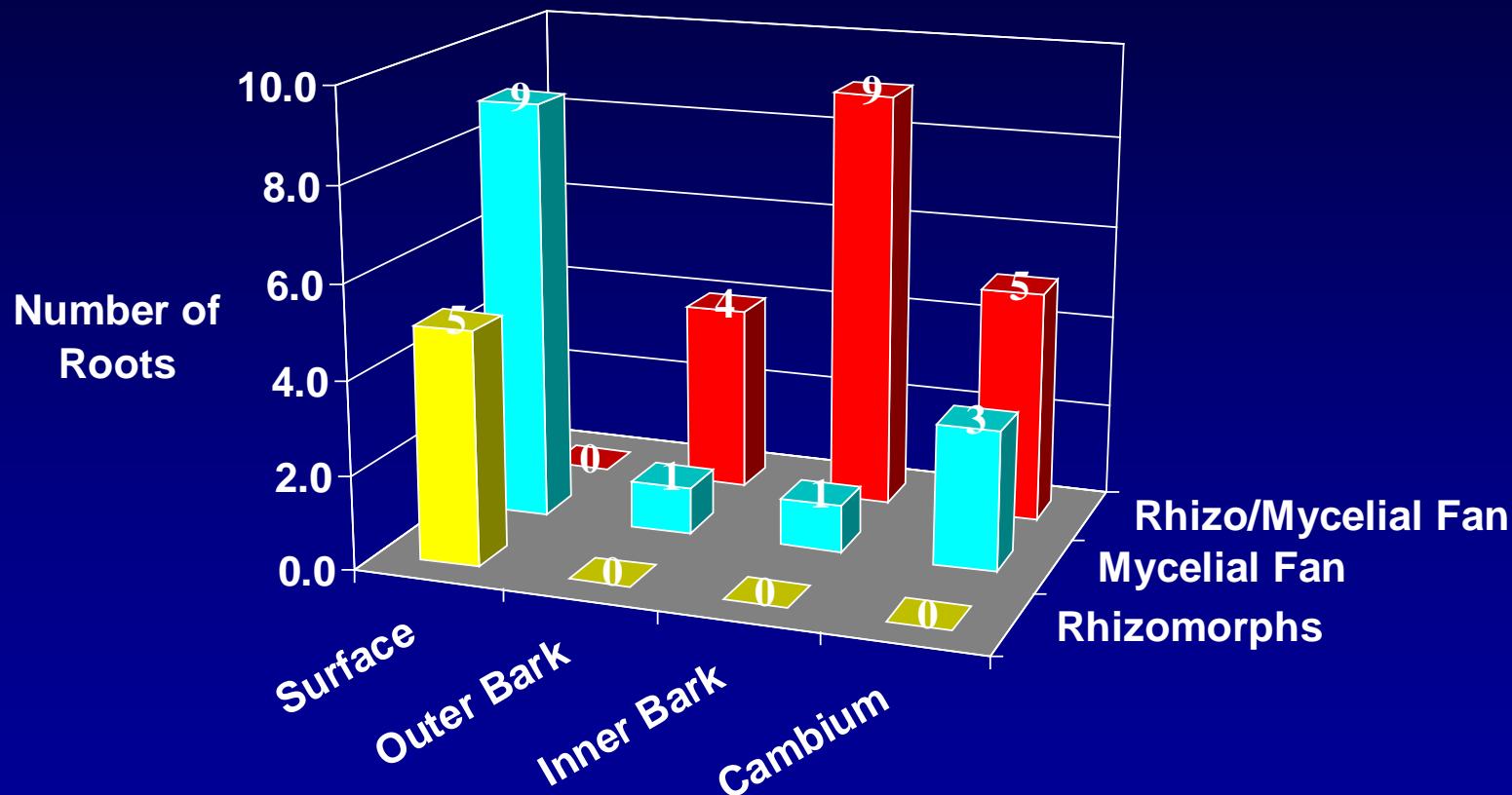
## Somatic Incompatibility – DNA Sequencing

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- Somatic Incompatibility
  - 35 of the 37 infection ratings fused with archive samples during somatic incompatibility testing
- DNA Sequencing
  - DNA sequencing identified “invader” species
    - NABS X (6)
    - NABS X / NABS III, V, VII complex hybrid (7)
    - NABS III,V,VII complex (7)
    - PNW A (8)
    - PNW A x PNW B hybrid (1)

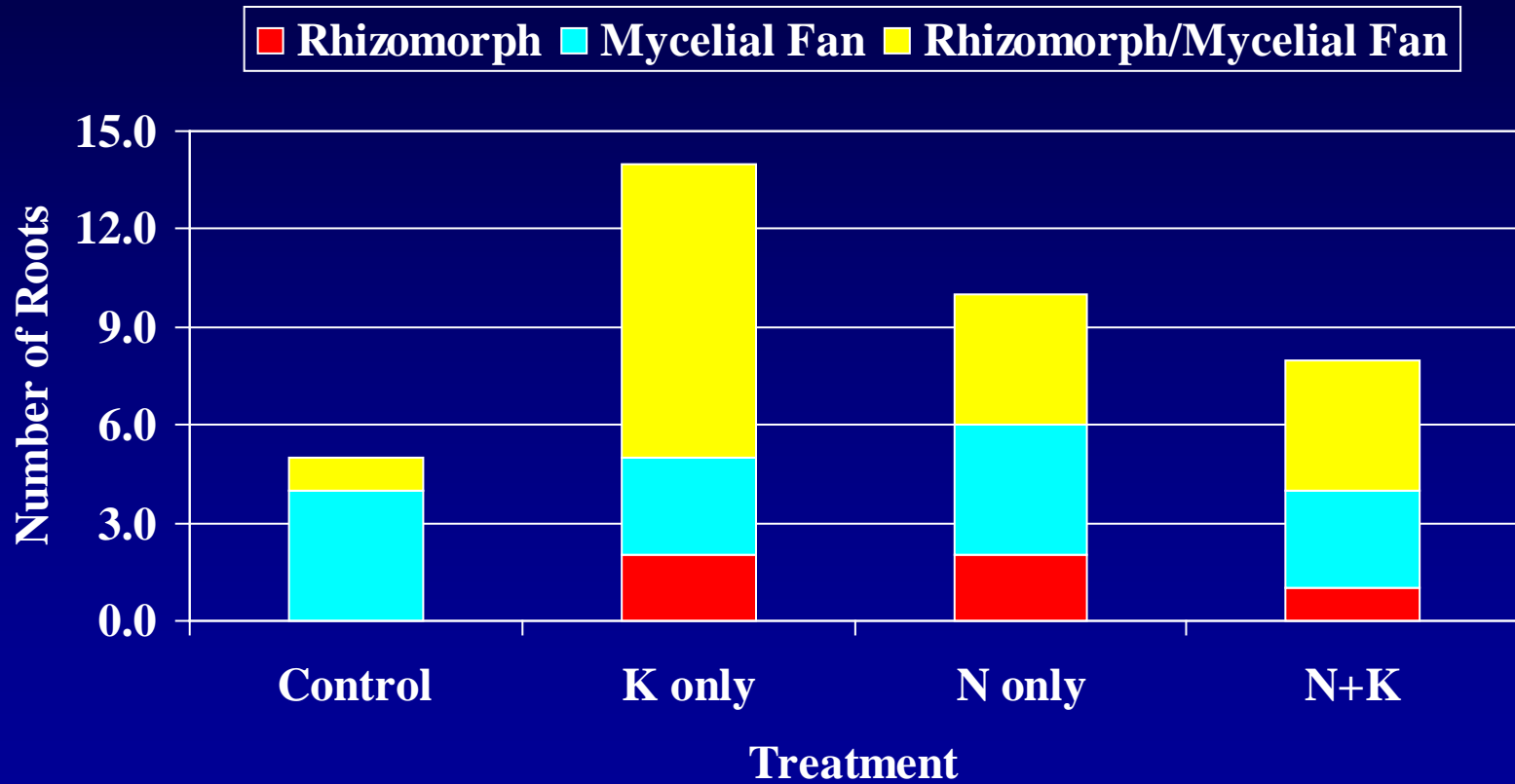


# Infection Rating by Form and Root Location

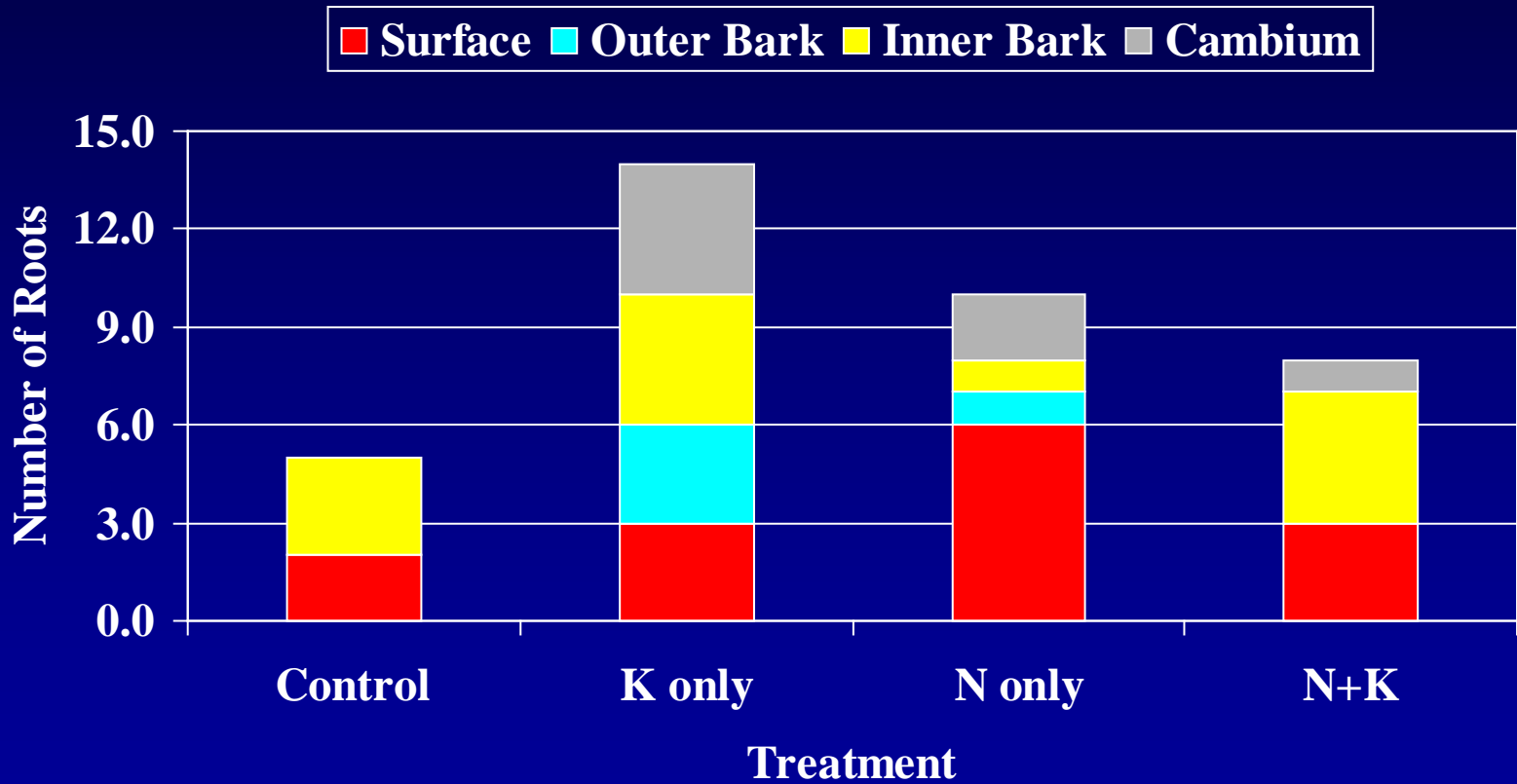




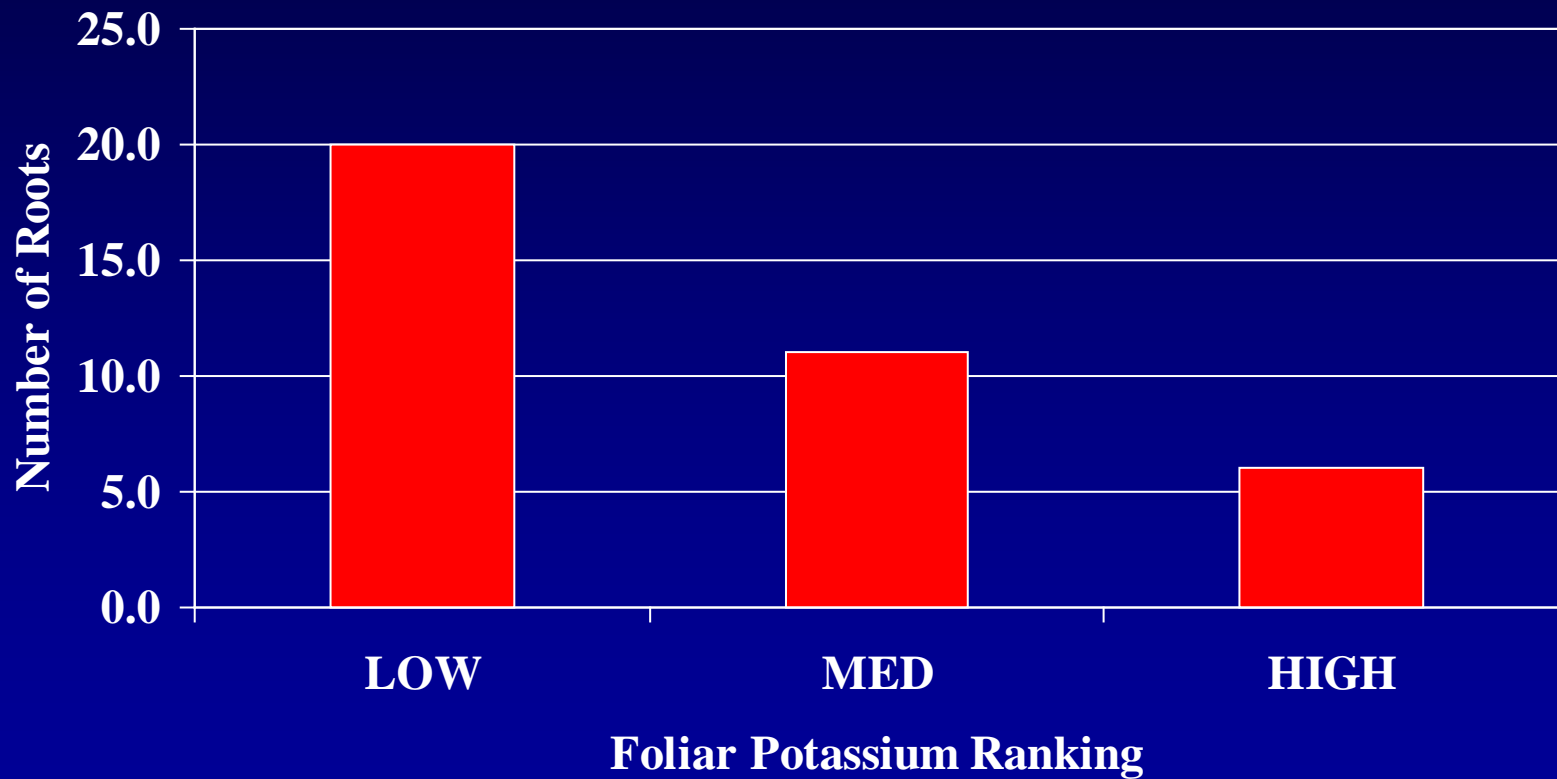
# Infection Rating by Treatment and Form



# Infection Rating by Treatment and Location on Root



# Infection Rating by Foliar Potassium Status

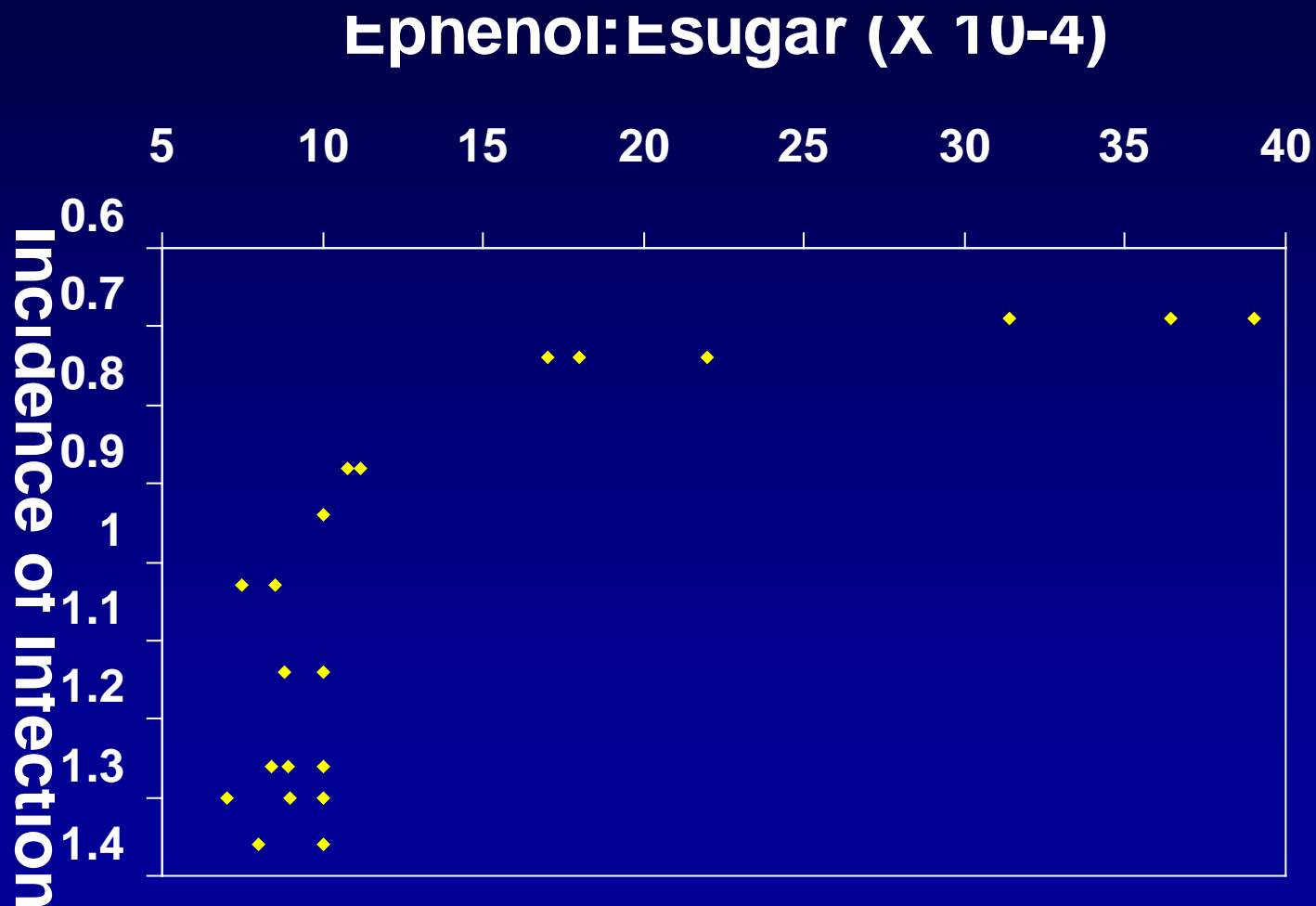






# Armillaria Infection Rate

## Relationship to Thermochemical Budget



Adapted from Entry *et al* 1991



# IFTNC Root Chemistry

Field root bark collection



Freeze-drying samples



Freeze-dried sample



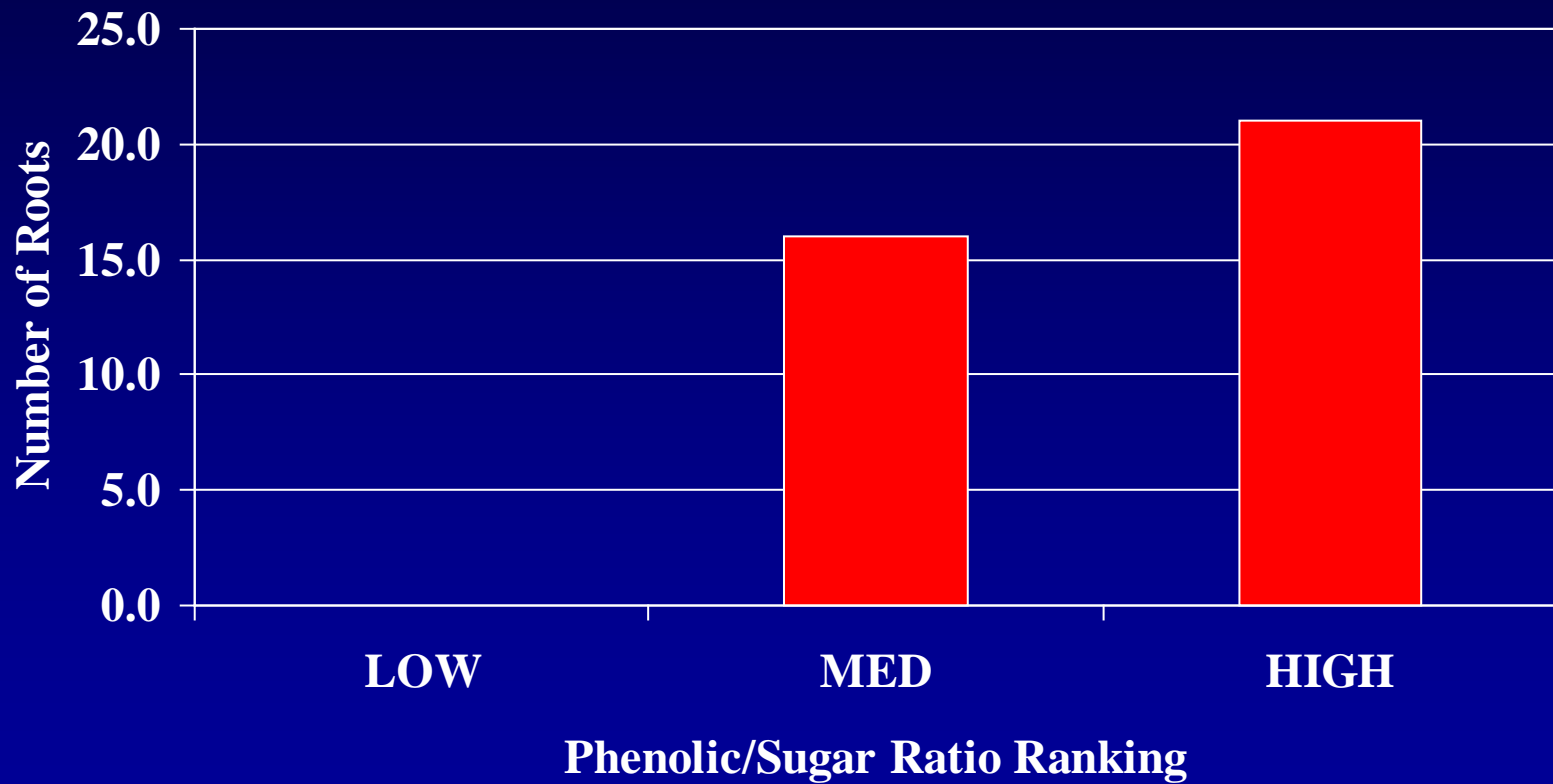
Outer Bark

Inner Bark

Root Chemistry

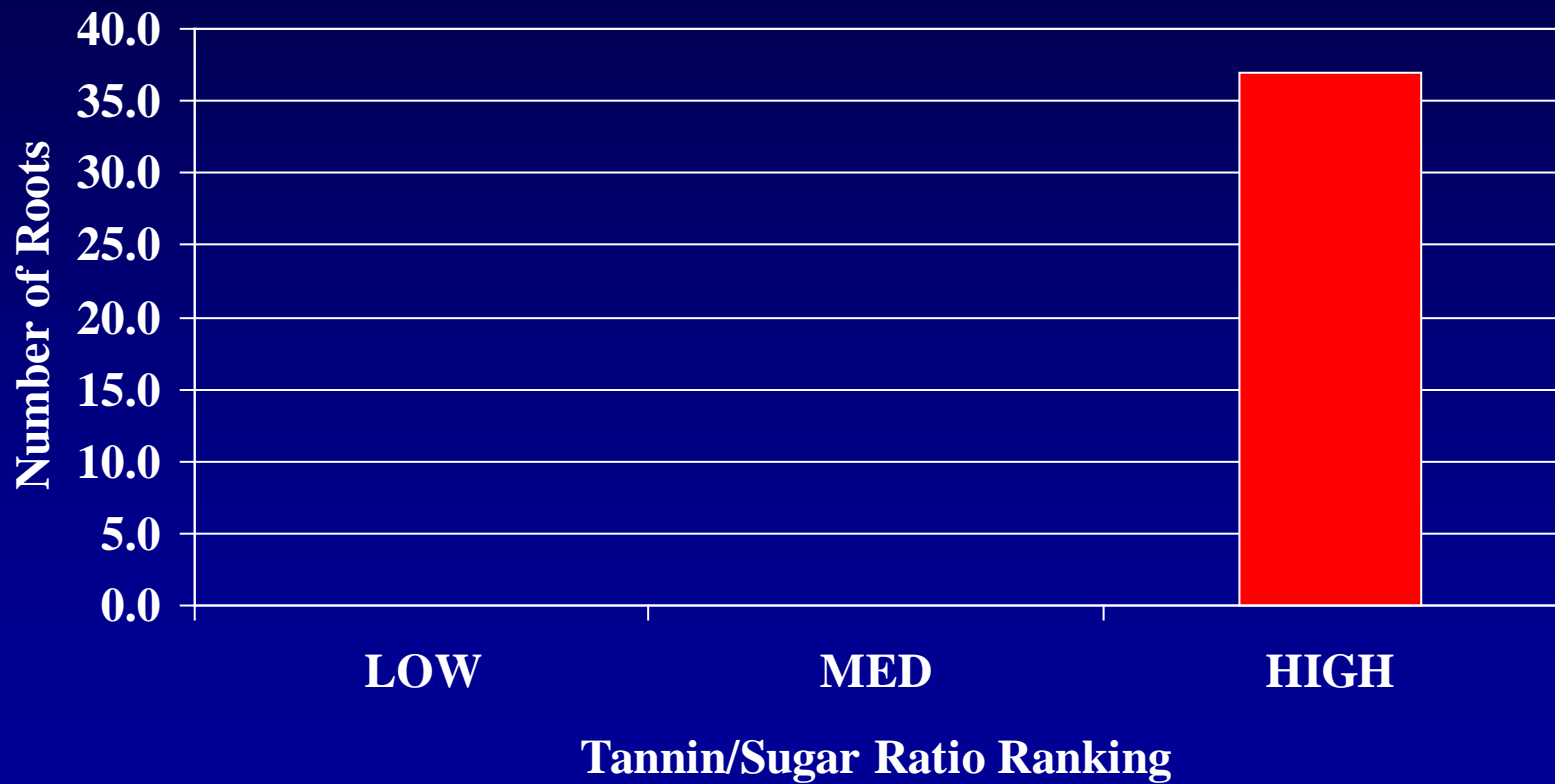


# Infection Rating by Phenolic/Sugar Ratio





# Infection Rating by Tannin/Sugar Ratio



# Forest Health

## *A. ostoyae* Inoculation

### What's Left



- Spring/2004 - collect inoculated roots from 9 Forest Health sites for verification of *A. ostoyae* infection



# Special Acknowledgement

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USDA Forest Service

Rocky Mountain Research Station

Pathology Unit

Moscow, ID