Current and Future Alternatives to MB Fumigation Research

Will Littke

John Browning

Brad Miller

Weyerhaeuser Forestry R&D

Current Regulatory Environment:

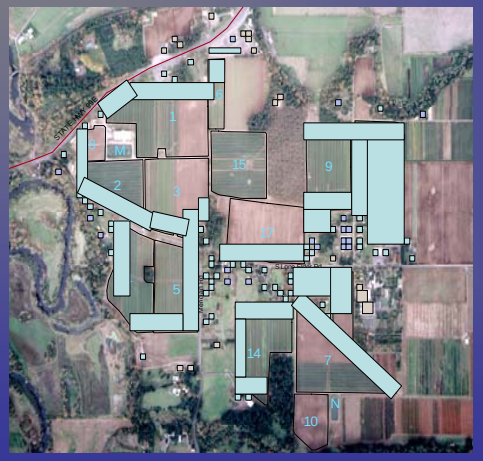
The proposed EPA fumigation buffers and other restrictions, ***if unchanged will dramatically alter bare-root conifer nursery production and setback gains made through years of fumigation research.***

- **Impacts:**
- **Buffer zones: current impact estimated at 35-47% decrease in average fumigant acres**
- **Maximum lower rates for chemicals such as Chloropicrin**
- **Fumigation plans and GAP's**
- **Monitoring**
- **Buffer zone credits for tarps and other fumigation application practices**

WA

Fall

Spring



OR

Current Fumigation Trial Results

Focus on Iodomethane and VIF Tarp

2007 USDA/ARS Study Mt. Vernon WA

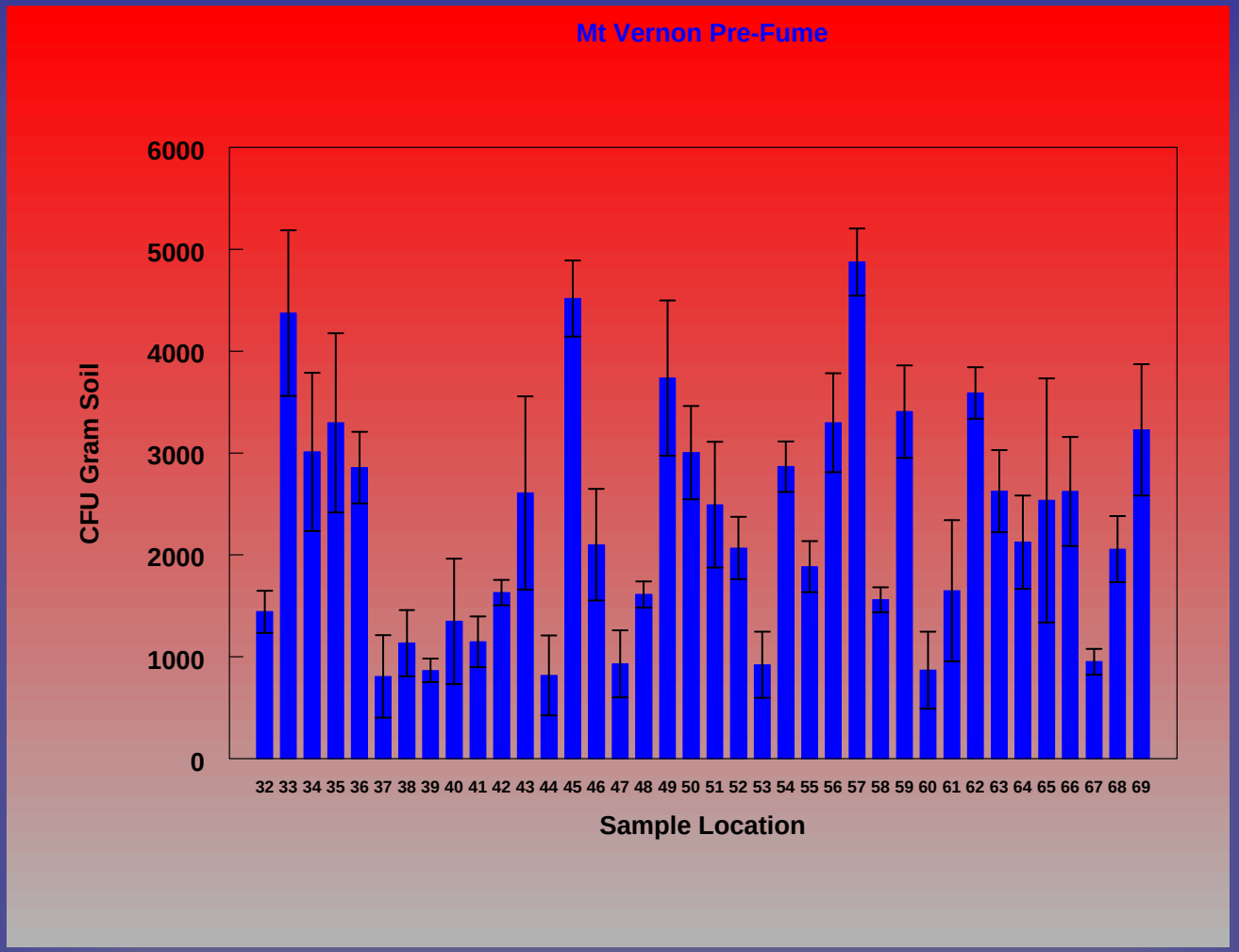
2008 USDA/ARS Study OSU Nursery COOP OR

2007 USDA/ARS Study Mt. Vernon WA

Objective:

1) **Row cover fumigation demonstration trial (*semi-replicated*)**
2) **VIF (Plant Blockade) and HDPE tarp comparison**
3) **350 lbs/ac and 175 lbs/ac Iodomethane 50:50 with Chloropicrin**
4) **Telone-C35 comparison**
5) **Soil Fusarium and buried root pathogen inoculum (Pythium, Fusarium, Cylindrocarpon and Phoma) at 15 and 30 cm depth)**

Pre-Fume Conditions

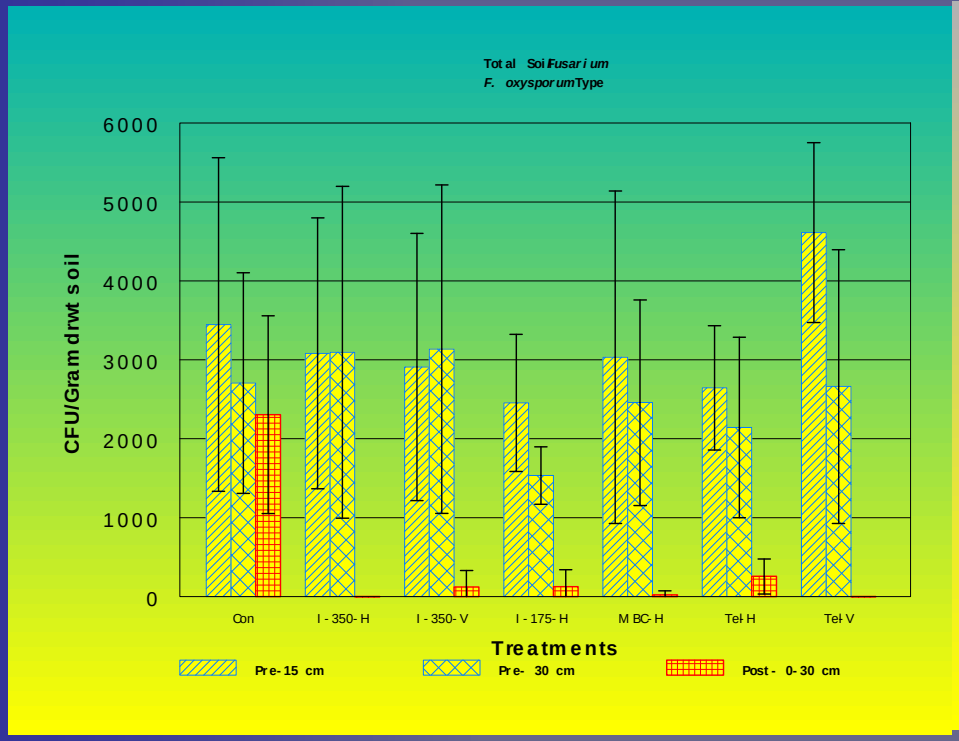


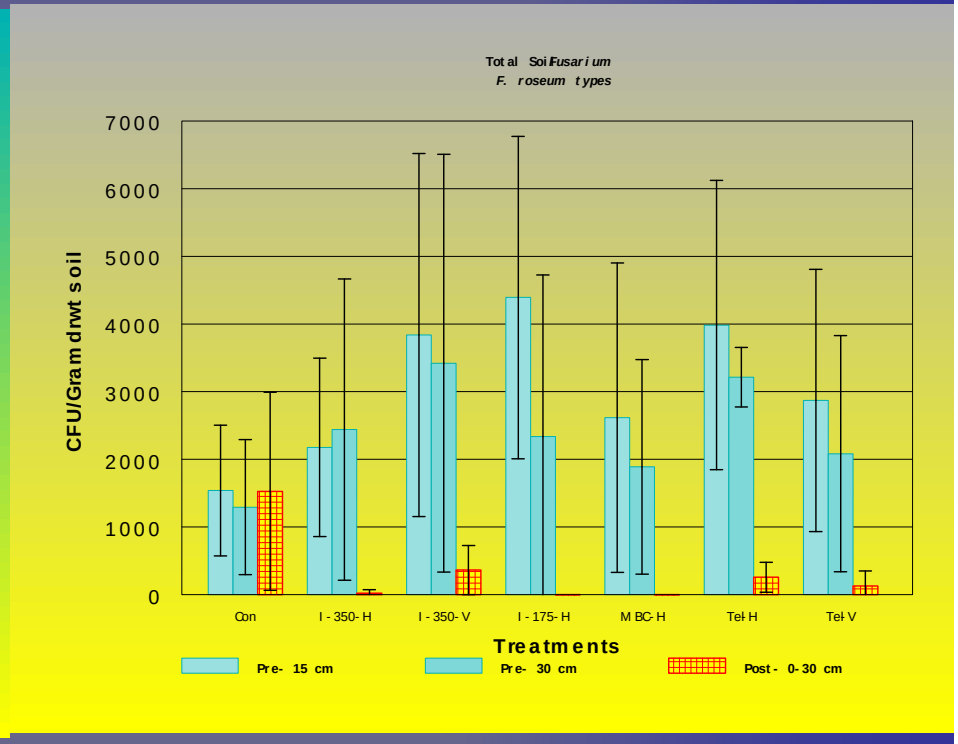
Pre-fumigation Fusarium oxysporum levels in the study site exceed the typical levels (~1000 CFU/g) found in conifer seedbed soils at fumigation.

Treatment
Control
Iodomethane +Pic (50:50) 350 lbs/ac HDPE
Iodomethane +Pic (50:50) 350 lbs/ac VIF (Blockade)
Iodomethane +Pic (50:50) 175 lbs/ac HDPE
MBC (67:33) 350 lbs/ac HDPE
Telone C-35 350 lbs/ac HDPE
Telone C-35 350 lbs/ac VIF (Blockade)

2007 USDA/ARS Study Mt. Vernon WA

Soil Inoculum Potential as measured on Komada's Media





1-month post fumigation all treatments significantly reduced soil Fusarium propagules in the F. oxysporum and F. roseum groupings in the 0-30 cm soil depth.

2007 USDA/ARS Study Mt. Vernon WA

Buried nursery seedling root inoculum potential (Komada's Media)

-roots excised from diseased nursery stock and buried in hardware cloth bags

-sampled pre- and post fumigation (June to September)

Percent control based on pre- and post fume isolation success

Treatments	Tarp	F. oxysporum	Pythium spp.	Cylindrocarpon spp.	Phoma spp.
Control	None	0	0	0	0
Iodomethane 50:50 350	HDPE	87	95	76	89
Iodomethane 50:50 175	HDPE	60	100	21	72
Iodomethane 50:50 350	VIF	100	100	100	100
MBC 67:33 350	HDPE	78	100	49	78
Telone C35 350	HDPE	87	100	54	90
Telone C35 350	VIF	93	100	81	98

Inoculum buried at 15 and 30 cm depth

1-month post fumigation treatments varied in the control of buried conifer root inoculum containing various root pathogenic fungi.

2007 USDA/ARS Study Mt. Vernon WA

2008 USDA/ARS Study WA & OR

Objective:

1) **Replicated treatments in three nursery facilities.**
2) **VIF and HDPE tarp comparison**
3) **Iodomethane comparisons with MBC , DMDS, and Metam Sodium**
4) **Soil Fusarium and buried pathogen inoculum (Pythium, Fusarium) at 15 and 30 cm depth)**
5) **PCR analysis of soil and root pathogenic fungi**
6) **Crop production economics**

Study Plan Elements

- ✓ **Regulatory issues trumped fumigant efficacy comparisons**
- ✓ **Modified treatments to limit Pic applications to 120 lbs per acre**
- ✓ ***Treatments equate to a 100' buffer under the Summer 2008 RED's***
- ✓ **PIC-Clor 60 dropped because rate limitation imposed by RED's**
- ✓ **DMDS added even though there is little study evidence supporting its use and efficacy in bare root conifer nurseries**
- ✓ **Focus on VIF tarping even though troubles with gluing have not yet been resolved**
- ✓ **All MB alternatives in the trial are applied with Pic.**
  - **-Methyl iodide + Pic**
  - **-DMDS + Pic**
  - **-Metam sodium + Pic**

Methods

All treatments applied via shank injection 9” depth

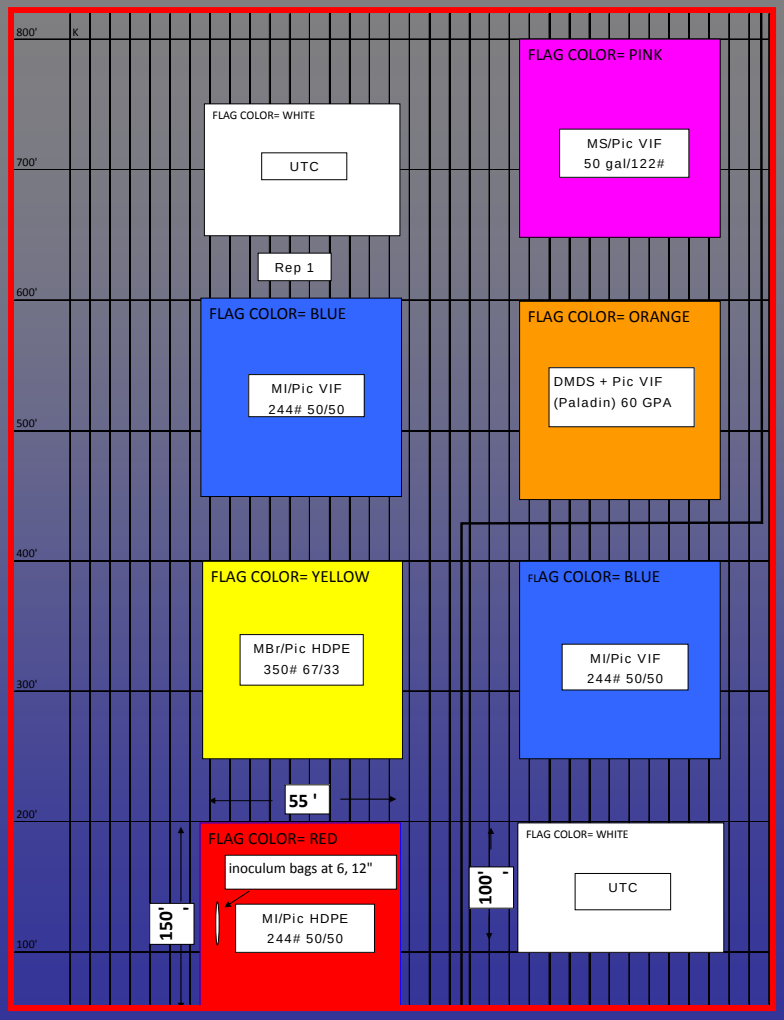
Treatments	Rate of Application	Film Type[1]
T1 - Methyl Iodide + Chloropicrin	244 lbs/A (50/50)	VIF
T2 - Metam Sodium + Chloropicrin	50 gal/A + 122 lbs/A	VIF
T3 - Methyl Iodide + Chloropicrin	244 lbs/A (50/50)	HDPE
T4 - DMDS + Pic (Paladin)	60 GPA (453 lbs+120lbs)	VIF
T5 - Methyl Bromide + Chloropicrin	350 lbs/A (67:33)	HDPE
T6 - Untreated Control		HDPE

2008 USDA/ARS Study WA & OR

Methods

Randomized treatment design 120 foot by 55 foot plots

4 treatment replicates per facility

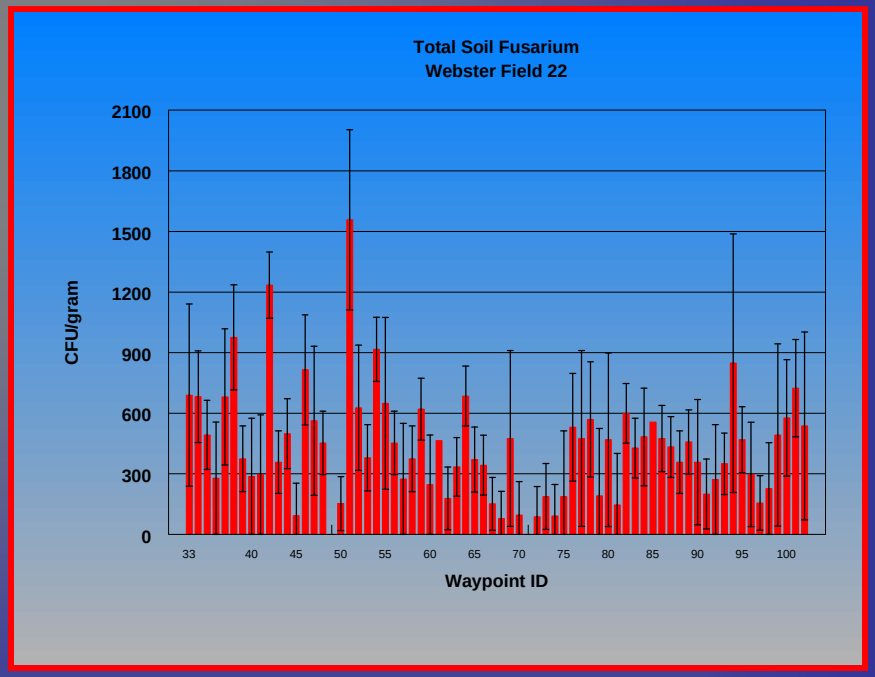


Results- Soil & Root Pathogen Assays

- Pre-fume pathological analysis indicate three distinct seedling pathogens present in the study fields; Fusarium, Pythium, and Cylindrocarpon
- PCR work on Pythium and Phytophthora (Jerry Wieland USDA/ARS Corvallis, OR)
- Fusarium PCR results still being tallied (Anna Leon- MS Student UW, Seattle)

Webster	#	Canby	#	Aurora	#
Pythium diclinum	3	*Pythium diclinum*	3	*Phytophthora pseudotsugae*	4
Pythium irregulare	1	*Pythium macrosporum*	1	*Pythium diclinum*	3
Pythium spiculum	1	*Pythium salvaticum*	3	*Pythium macrosporum*	1
		Pythium spiculum	1	*Pythium salvaticum*	2
Unidentified isolates	4	Unidentified isolates	45	Unidentified isolates	34

Isolation and identification of specific Pythium species



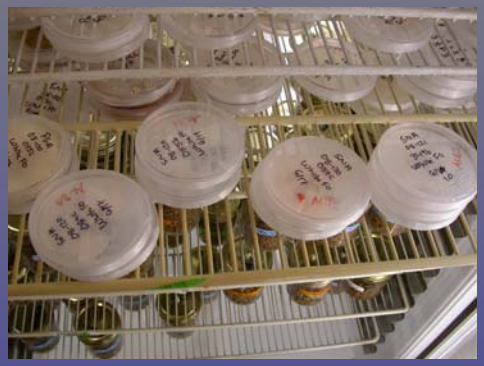
Quantifying pre- and post fume soil Fusarium across sample points

2008 USDA/ARS Study WA & OR

Results- Buried Inoculum

- Specific Fusarium and Pythium isolates placed in soil at 15cm and 30cm depth
- PCR identification of specific isolates is currently in progress
- Recovery post-fume and treatment analysis is underway

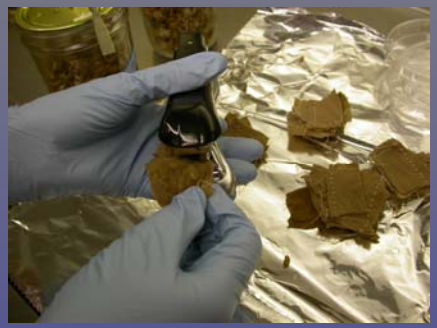
Sequence of inoculum development



Fusarium isolates recovered from soil and roots

PCR analysis

Inoculum grown on rye seed



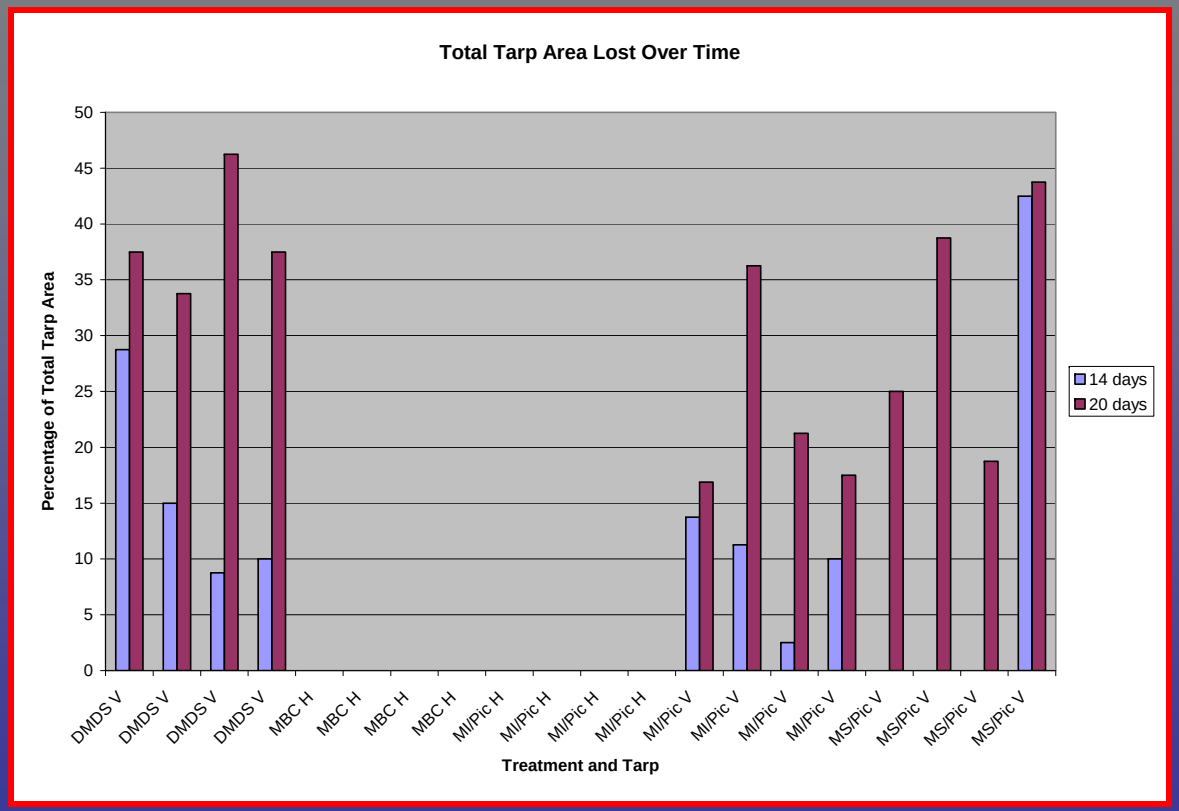
Inoculate rye placed in nylon bags for burial

Re-isolation post-fume

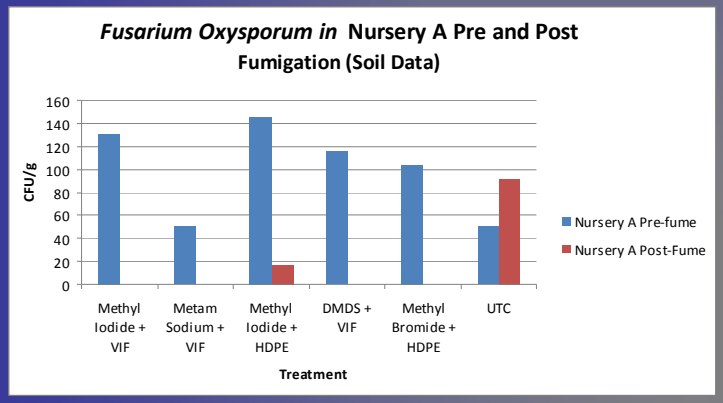
2008 USDA/ARS Study WA & OR

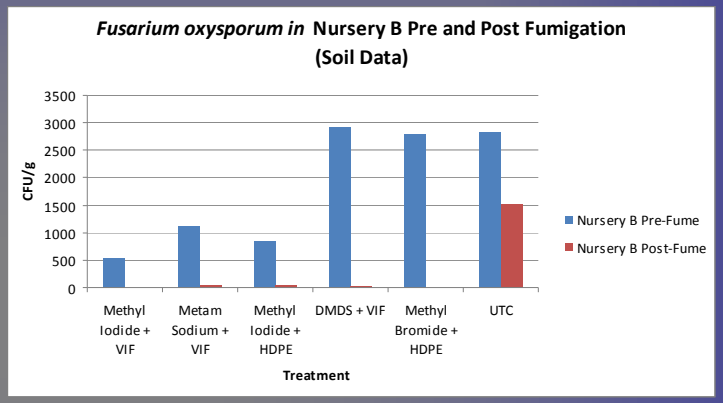
Results- Tarp Performance

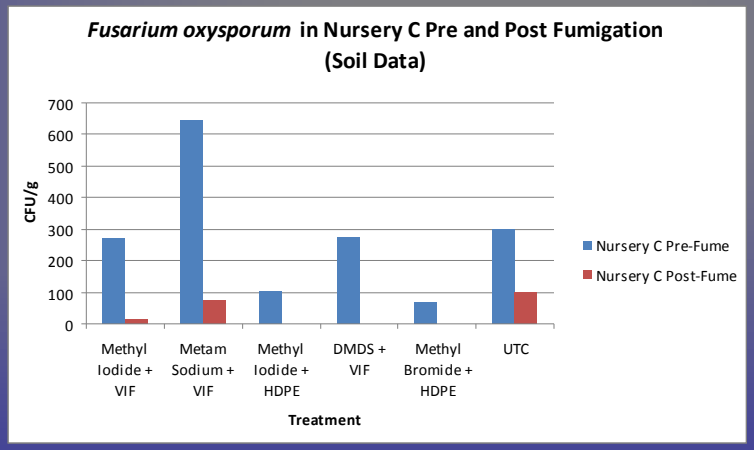
- VIF tarp that was used was the only type commercially available at the time of the experimentation.
- HDPE tarp remained intact
- VIF was intact through the fumigation period but rapidly deteriorated ~14 days



- *Pre-fume soil Fusarium levels were low by fumigation standards.*
- *1-month post-fume these levels were below the detection level.*
- *Buried inoculum results have not yet been fully analyzed*



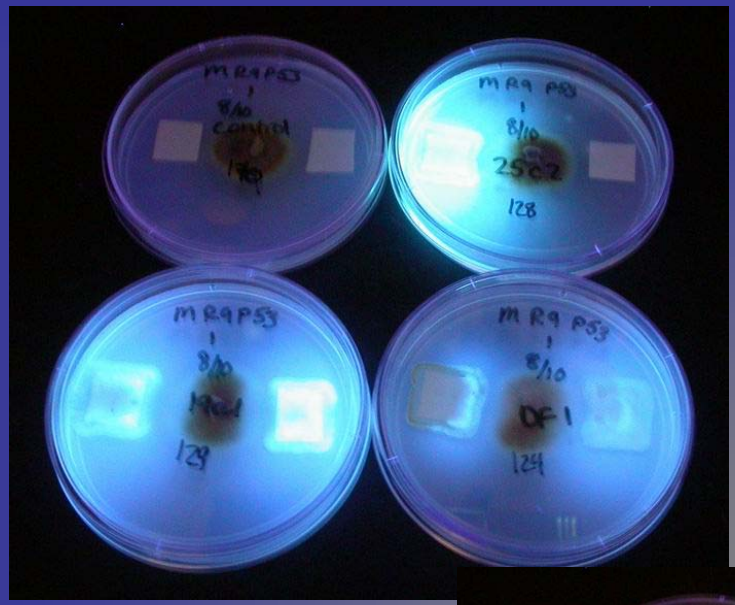


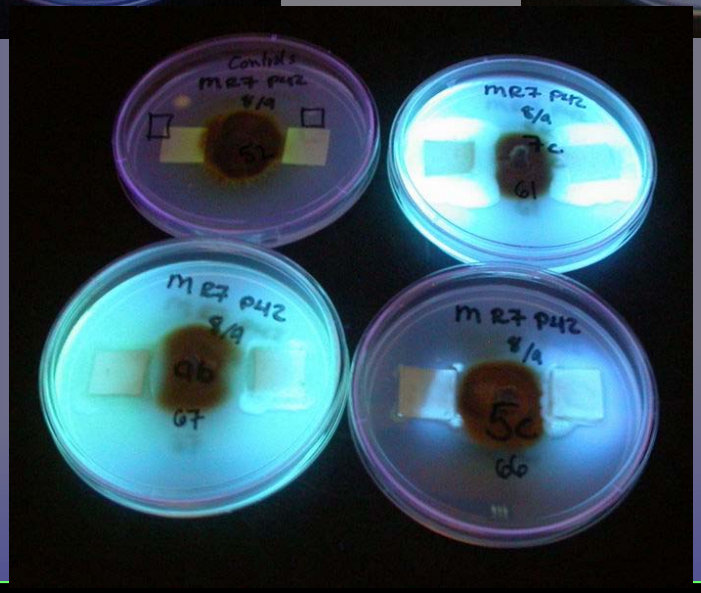


2008 USDA/ARS Study WA & OR

Other Observations

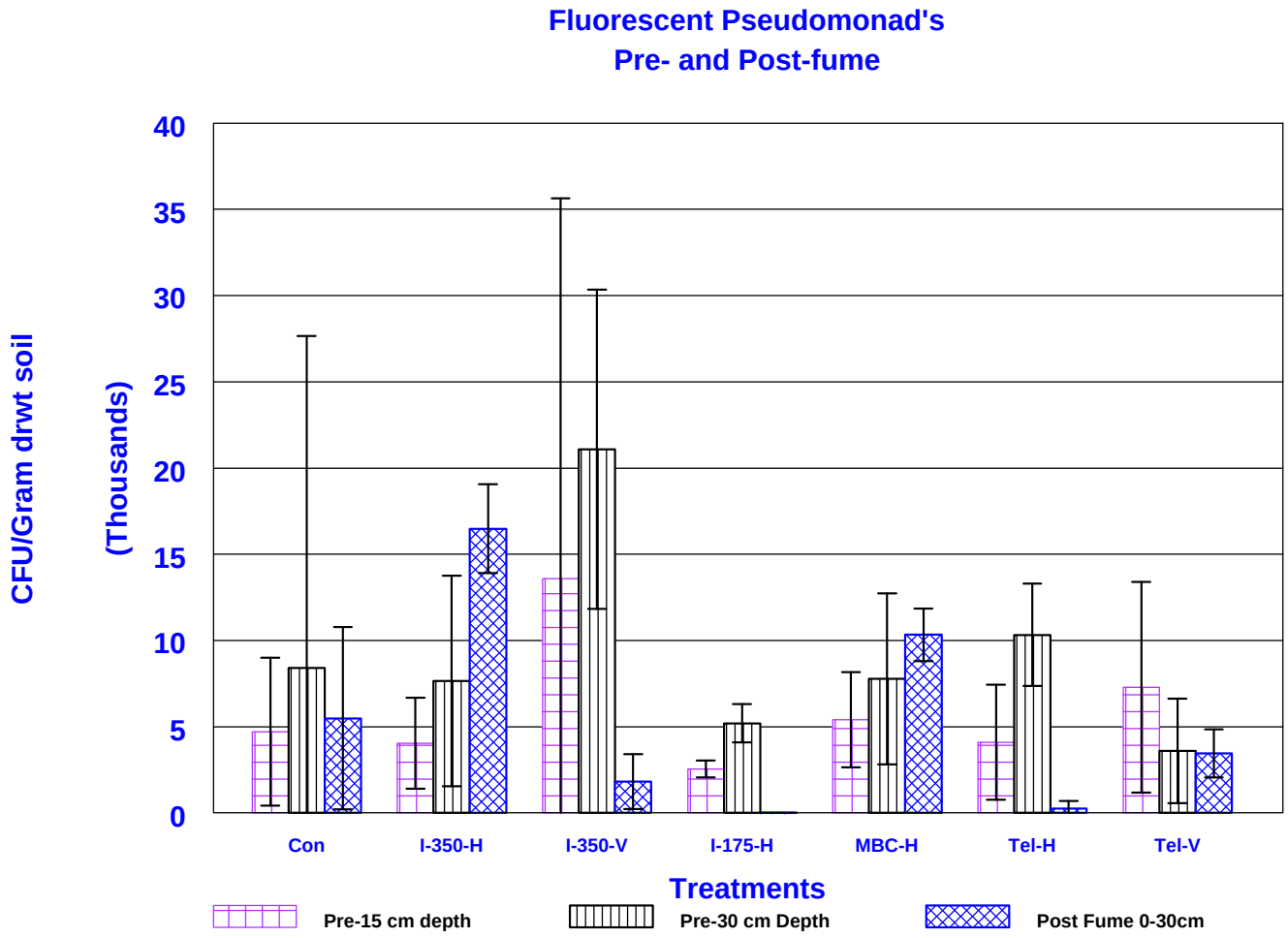
Fluorescent Pseudomonas growing on King's B media





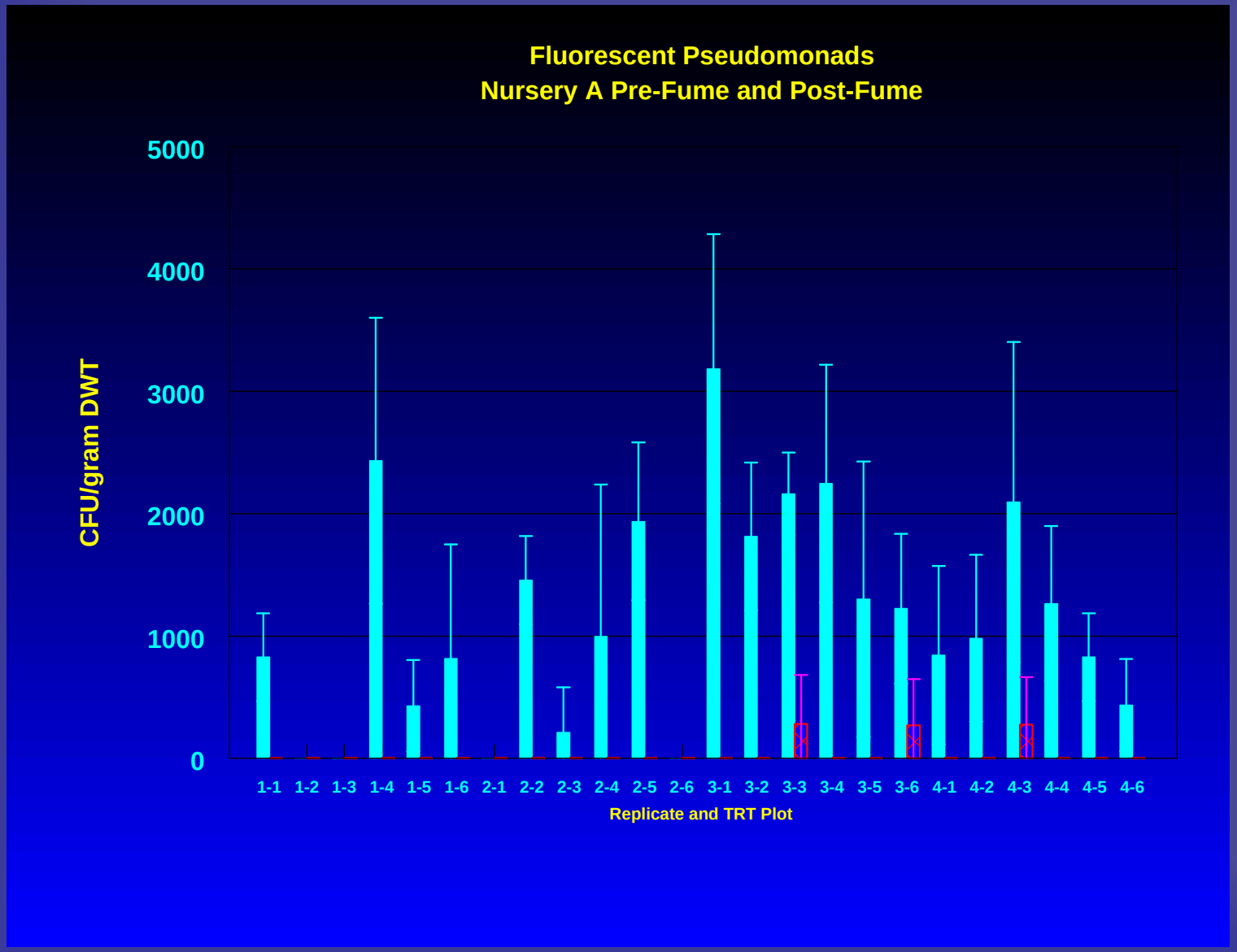
FP cultures have assessed for levels of biocontrol potential against various Fusarium, Pythium and Cylindrocarpon isolates. Some are antagonistic others not!

Pre- and post-fume populations of FP were variable by location and treatment



2007 USDA/ARS Study Mt. Vernon WA

In the current study FP populations were nearly eliminated post-treatment

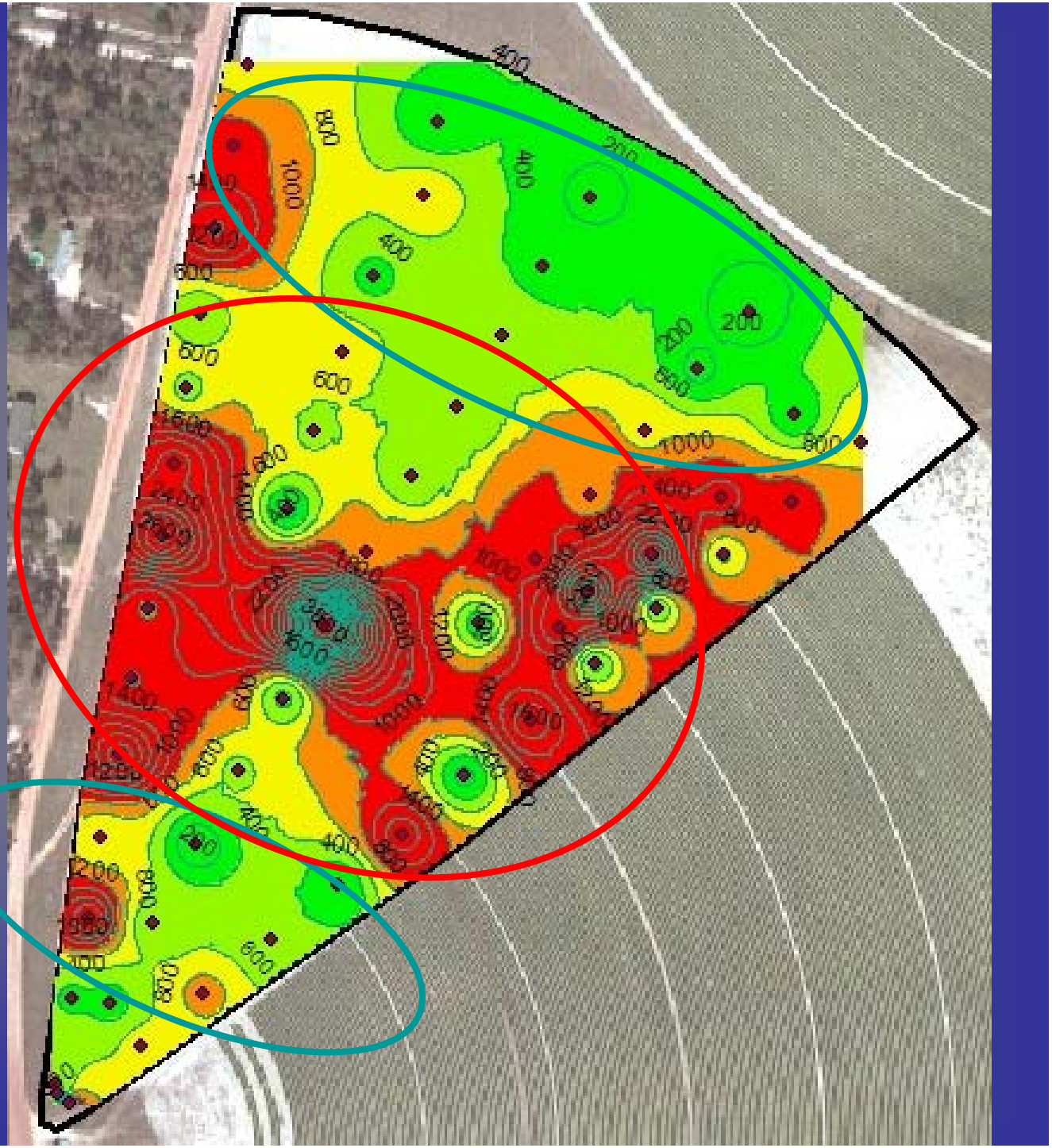


Pre Post

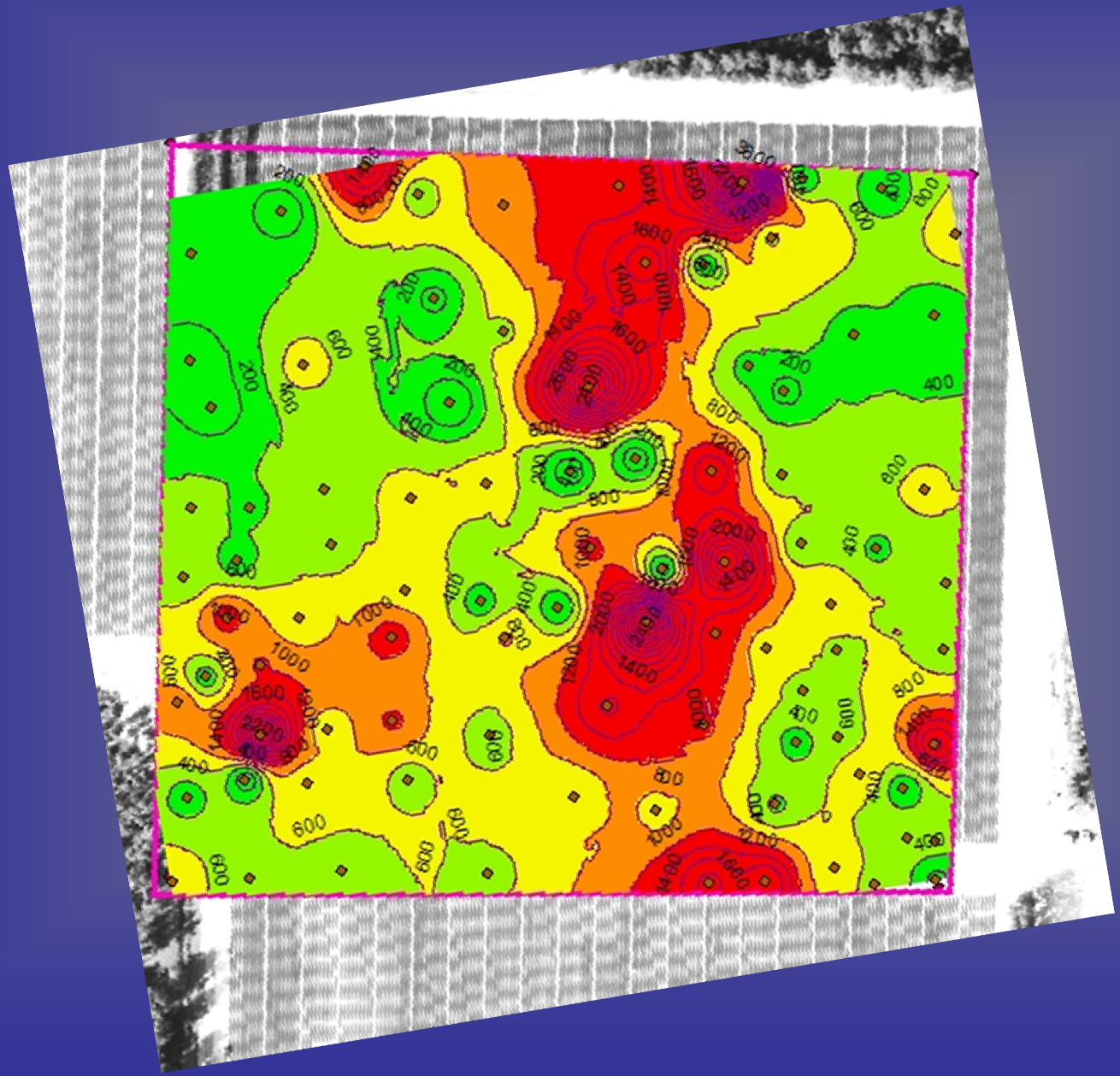
2008 USDA/ARS Study WA & OR

What determines soil pathogen distribution within a nursery block?

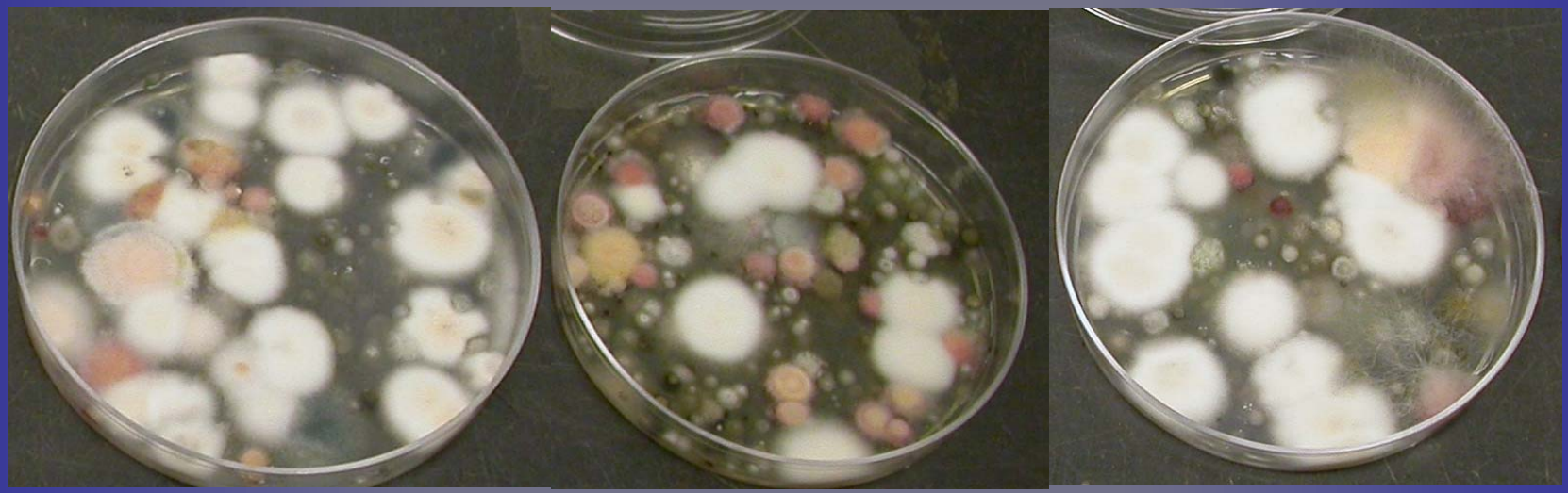
Is there a need for differential fumigation?



GPS Sampling and tracking of soil pathogens- may lead to other application possibilities



What species are present and do we need to *kill them all?*



Soil assays showing typical Fusarium isolation plates

Current assay methods equate all colonies as being pathogenic?

PCR tracking will show us who is there, who survived fumigation and what they do in the post fumigation crop phase.

How susceptible are specific pathogens to fumigants?

Species	What was Tested	CT Value (Various Units)	Reference
Glomus fasiculatus and G. contrictus	Resistant chlamydospores in soil	96,000 to 98,400 microliters MB/Liter ***(mg hr/L = g hr/M3)***	Menge et al 1978
Armillaria ostoyae	Mycelium in spruce wood blocks	1279 mg hr/L	Rhatigan et al. 1998
Armillaria mellea	Mycelium in petri dishes	779 mg hr/L	Munnecke et al. 1978
Heterobasidion annosum	Mycelium in spruce wood blocks	3010 mg hr/L	Rhatigan et al. 1998
Lachnellula willkommii	Mycelium in spruce wood blocks	1230 mg hr/L	Rhatigan et al. 1998
Ceratocystis wagneri	Mycelium in spruce wood blocks	4750 mg hr/L	Rhatigan etl al. 1998
Ceratocystis fagacearum	Mycelium in petri dishes	1920 mg hr/L	Liese and Reutze 1981
Antrodia carbonica	Mycelium in petri dishes	2093 mg hr/L	Ricard 1966
Fusarium oxysporum	Mycelium in petri dishes	2688 mg hr/L	Ebben et al. 1983
Phomopsis sclerotioides	Mycelium in petri dishes	> 2688 mg hr/L	Ebben et al. 1983
Phytophthora cinnamomi	Mycelium in petri dishes	461 mg hr/L	Munnecke et al. 1978
Pythium ultimatum	Mycelium in petri dishes	469 mg hr/L	Munnecke et al. 1978
Rhizoctonia solani	Mycelium in petri dishes	795 mg hr/L	Munnecke et al. 1978
R. solani	Mycelium in petri dishes	< 1911 mg hr/L	Ebben et al. 1978
Verticillium albo-atrum	Mycelium in petri dishes	1390 mg hr/L	Munnecke et al. 1978
V. albo-atrum	Mycelium in petri dishes	2688 mg hr/L	Ebben et al. 1978

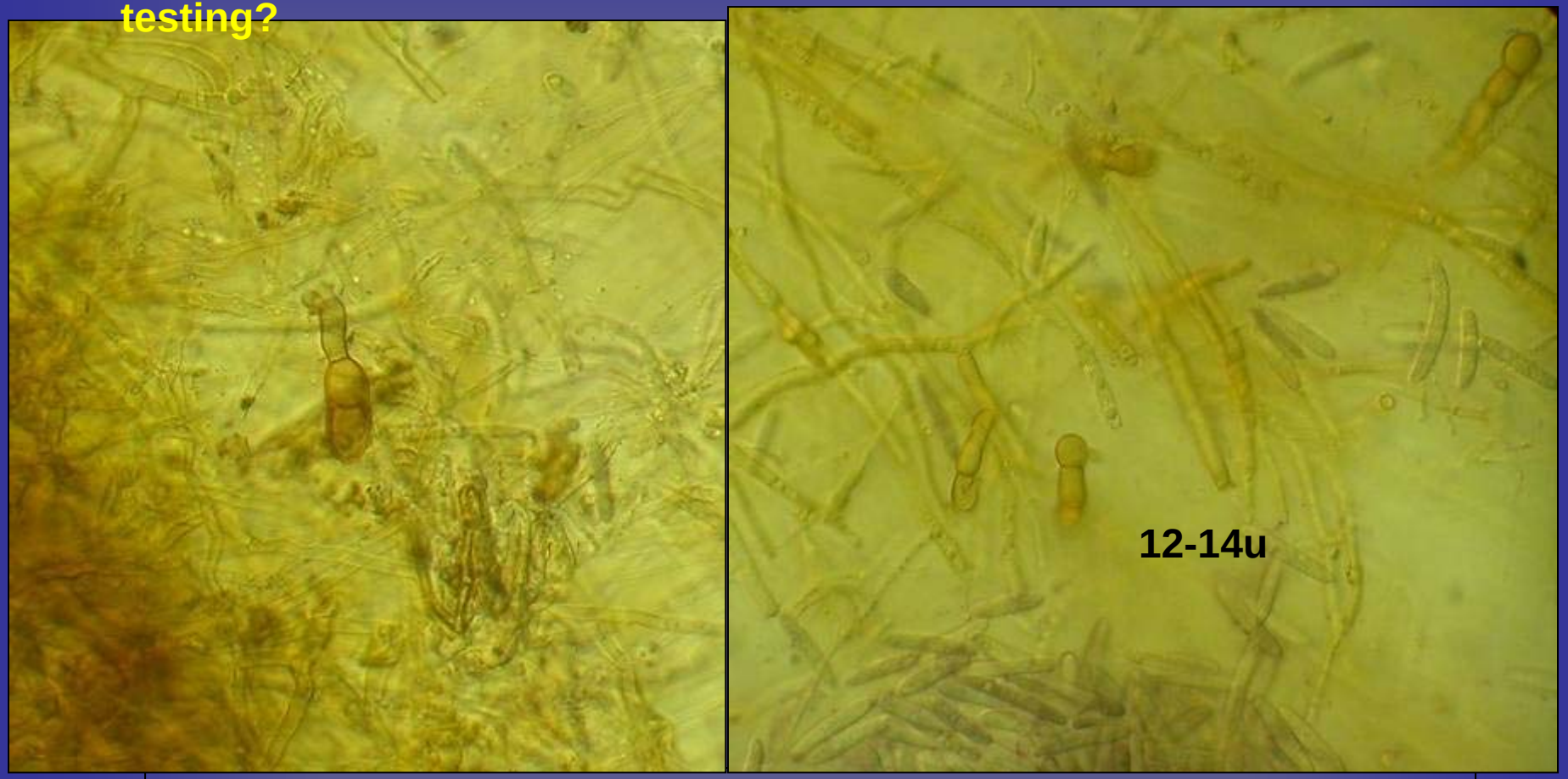
CT value= time weighted exposure average of concentration

How susceptible are specific pathogens to fumigants?

Species	What was Tested	CT Value (Various Units)	Reference
Glomus fasiculatus and G. contrictus	Resistant chlamydospores in soil	96,000 to 98,400 microliters MB/Liter ***(mg hr/L = g hr/M3)***	Menge et al 1978
Armillaria ostoyae	Mycelium in spruce wood blocks	1279 mg hr/L	Rhatigan et al. 1998
Armillaria mellea	Mycelium in petri dishes	779 mg hr/L	Munnecke et al. 1978
Heterobasidion annosum	Mycelium in spruce wood blocks	3010 mg hr/L	Rhatigan et al. 1998
Lachnellula willkommii	Mycelium in spruce wood blocks	1230 mg hr/L	Rhatigan et al. 1998
Ceratocystis wagneri	Mycelium in spruce wood blocks	4750 mg hr/L	Rhatigan etl al. 1998
Ceratocystis fagacearum	Mycelium in petri dishes	1920 mg hr/L	Liese and Reutze 1981
Antrodia carbonica	Mycelium in petri dishes	2093 mg hr/L	Ricard 1966
Fusarium oxysporum	Mycelium in petri dishes	2688 mg hr/L	Ebben et al. 1983
Phomopsis sclerotioides	Mycelium in petri dishes	> 2688 mg hr/L	Ebben et al. 1983
Phytophthora cinnamomi	Mycelium in petri dishes	461 mg hr/L	Munnecke et al. 1978
Pythium ultimatum	Mycelium in petri dishes	469 mg hr/L	Munnecke et al. 1978
Rhizoctonia solani	Mycelium in petri dishes	795 mg hr/L	Munnecke et al. 1978
R. solani	Mycelium in petri dishes	< 1911 mg hr/L	Ebben et al. 1978
Verticillium albo-atrum	Mycelium in petri dishes	1390 mg hr/L	Munnecke et al. 1978
V. albo-atrum	Mycelium in petri dishes	2688 mg hr/L	Ebben et al. 1978

CT value= time weighted exposure average of concentration

Pathogens with resistant chlamydospores may be more difficult to control- Would they be better yardsticks than current soil propagule testing?



Cylindrocarpon didymum chlamydospores.

Is pathogen succession a reality? 1,2--? fumigation rotations out

Optimization of Fumigation Efficacy- what the literature says

- Soil conditions which affect porosity modify biocide concentrations and effect efficacy on target pests.
- Variables: soil moisture, soil porosity, tarping, injection depth and rate of injection.

- <u>Soil Moisture Factors</u>
- - movement in wet soil is possible if pore space is available
- - some moisture in very sandy soil is needed to retard rapid loss of fumigant
- - saturated soils are not fumigable since pore space is blocked. This can occur at > 25% of FC in soils heavier to silt and clay. Optimal values for sandy loams are 10-15% .
- -diffusion through water is 10 to 30 thousand times slower than air.
- -tills which restrict drainage also restrict fumigant penetration.

Optimization of Fumigation Efficacy- what the literature says

- **Injection Depth:**
- **- affects the point of maximum CT values and downward diffusion pattern or lateral spread.**
- **- downward movement through gravitational forces is much greater than lateral movement (width of injection tines), although deeper injection is a head-start on achieving downward diffusion.**
- **- diffusion and concentration then determined by soil factors and rates used, and tarp efficacy.**
- **- ripped soils have even more requirement for controlled soil moisture, since channels allow exit points for gas relative to penetration of clods.**

Immediate Next Steps

- Complete isolation and PCR work associated with the buried inoculum samples
- Complete analysis of fumigation efficacy on specific pathogen groupings and isolates
- Sample from treatment plots through spring planting season and growing year
- Sow and/or transplant the 2009 crop in treatment plots
- Determine the treatment effects on 2009 growing season seedling survival, disease levels, and season end morphometrics (caliper, height, biomass, and packable yields)
- Documentation of treatment efficacy and subsequent follow-up trials
- Submit findings to EPA after publication in appropriate journals

Conclusions:

- ✓ Most current fumigant formulations appear to control soilborne Fusarium inoculum to 30 cm depth, but other pathogens or deeper buried debris?
- ✓ Buried root inoculum is more difficult to control, providing a source of re-infection in the next rotation.
- ✓ The depth of control varies by pathogen tolerance, fumigant concentration and soil physical properties.
- ✓ GAP's are a necessity to achieve the greatest efficacy for the expense
- ✓ Issues with tarp integrity (VIF) may skew the results
- ✓ The impact on potential biocontrol organisms is relatively unknown – both pre- and post fumigation

☹ **No fumigant, not even MBC kills everything !**