HOW DEEP IS TOO DEEP?

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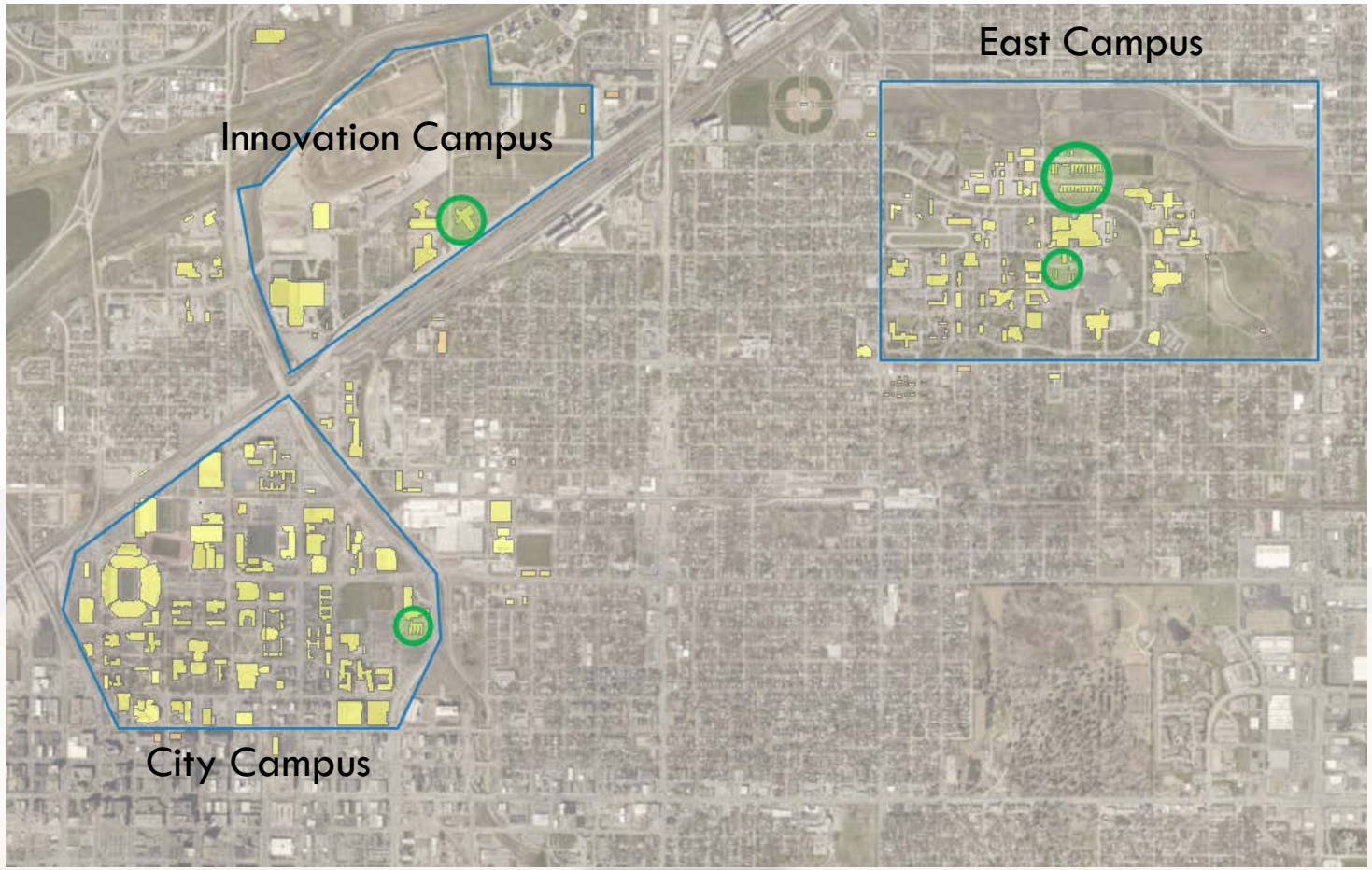
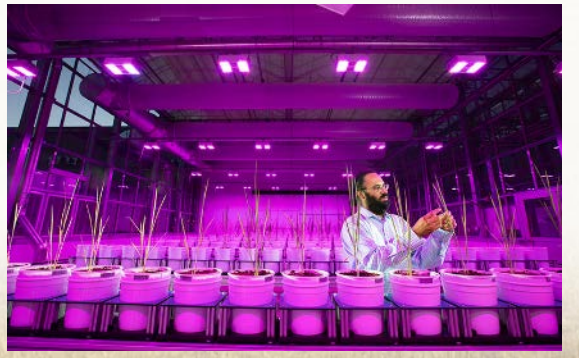
A VALIDATION STUDY OF GREENHOUSE WASTE DECONTAMINATION AT UNL

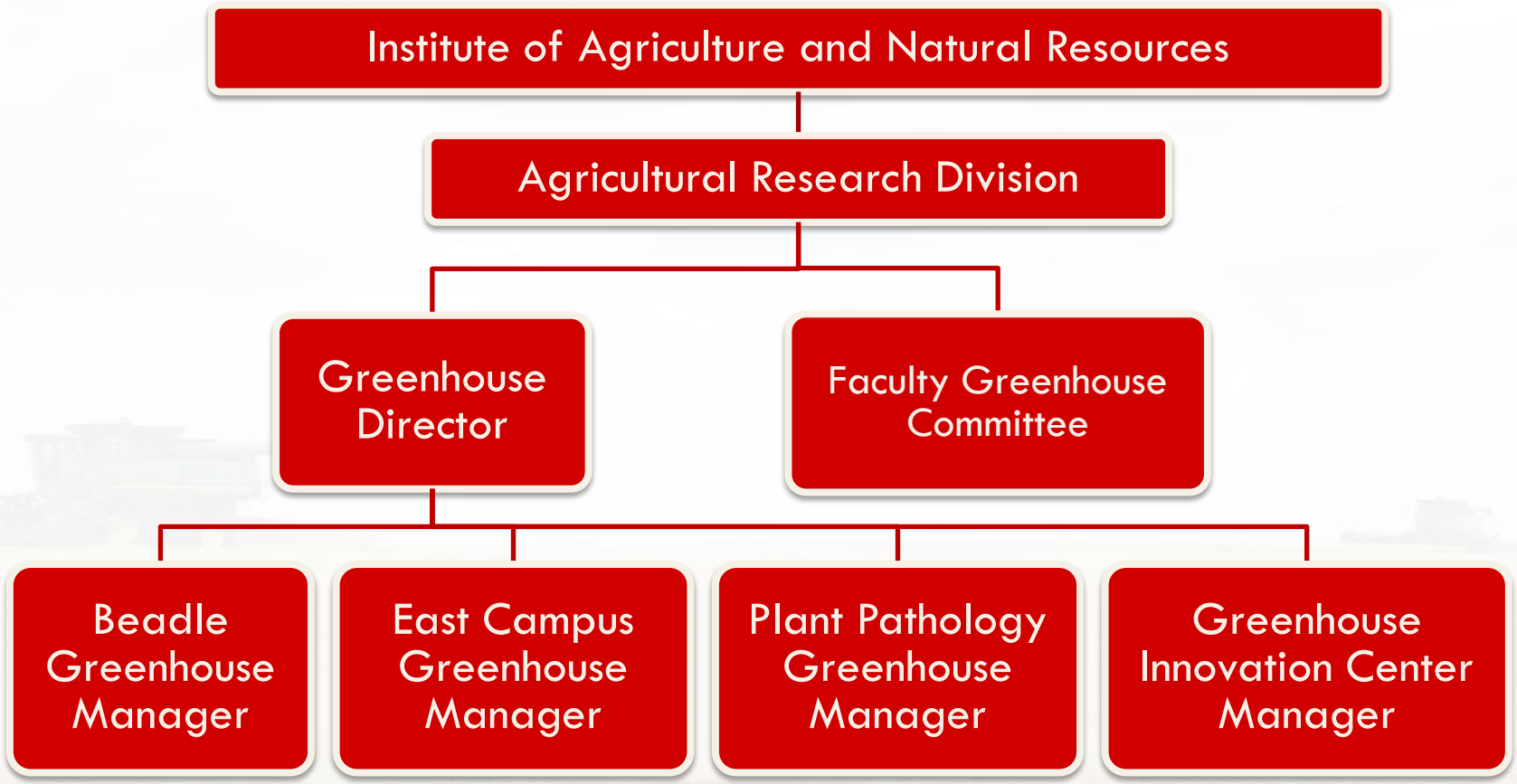
Greenhouses @ UNL



Greenhouse Facilities



Greenhouse Management at UNL



Presentation Objectives:

- ✓ Explain the three methods of plant and soil decontamination used at the University of Nebraska –Lincoln (UNL).
- ✓ Share the challenges associated with decontamination of large volumes of organic material from greenhouses.
- ✓ Share how UNL optimized autoclave cycles for efficient soil decontamination using steam sterilization in an autoclave.

Purpose of the Study

- Verify sterilization method parameters in detail.
- Evaluate effectiveness of parameters in sterilization. If the parameters are not appropriate, then optimize the procedure.
- Provide results our findings as a guidance to Institutional Biosafety Committee and Greenhouse Safety Committee for adaptation of the approved procedure for all greenhouses.

Plant and Soil Disposal Methods

- At UNL three methods are utilized:

AUTOCLAVING

STEAM PASTEURIZATION

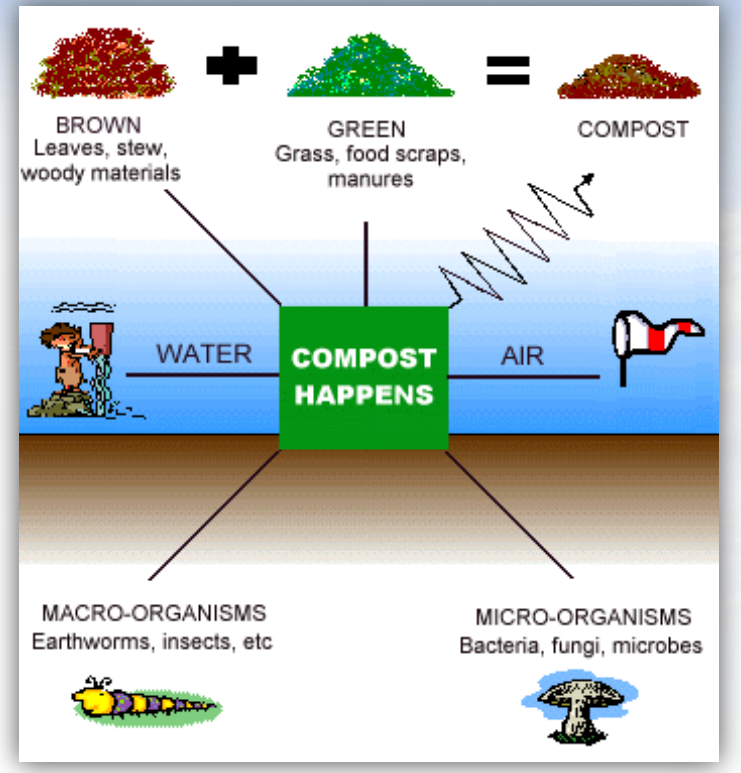
COMPOSTING

Steam Pasteurization:

- Introduction of steam to kill bacteria, fungi and viruses, or inactivate plants
- Soil or pots are pilled up inside the unit
- Surface is covered with tarp
- The unit is connected to source of steam
- Steam is turned on for duration set up by greenhouse

Aerobic Composting:

- Natural process of decomposition of organic matter by microorganisms
- Forming a pile of plants and soil
- Heat from the sun will help some organism grow and multiply using sugars and amino acids readily available.
- Increased temperature helps growth of other organism
- Raising temperatures 65°C will kill pathogens and weeds seeds



Materials:

- *G. stereothermophilus* biological indicators (log 5)
- Soil commonly used in greenhouses
- 20 gal. galvanized trash cans
- Collected plant waste without leaves/stems

Study Design:

- BIs placed soil of varying depths using centrifuge tubes attached to bamboo rods
- BIs placed at 0”, 4”, 8”, 12” from the bottom of the trash can or pasteurizer wagon
- Autoclave parameters:
  - 121°C at 16psi for 60 min. (*initial*)
- Steam pasteurization:
  - 80°C for 3 hr.

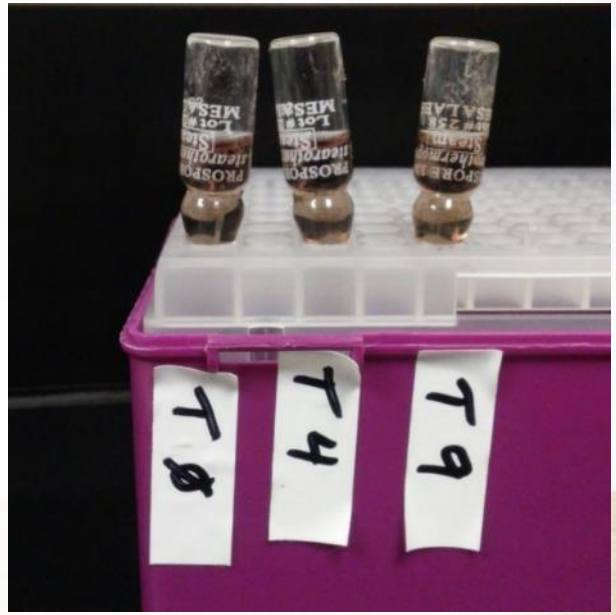
STUDY RESULTS

Autoclave: Plant and moist soil

Autoclave : Plant and moist soil

- Parameters: 121°C, 16 PSI, 60 min, soil depth 13”

20 gallon trash can	Depth of BI	24hrs	48hrs
Soil and Plant	0 inches	Pass	Pass
Soil and Plant	4 inches	Pass	Pass
Soil and Plant	9 inches	Pass	Pass

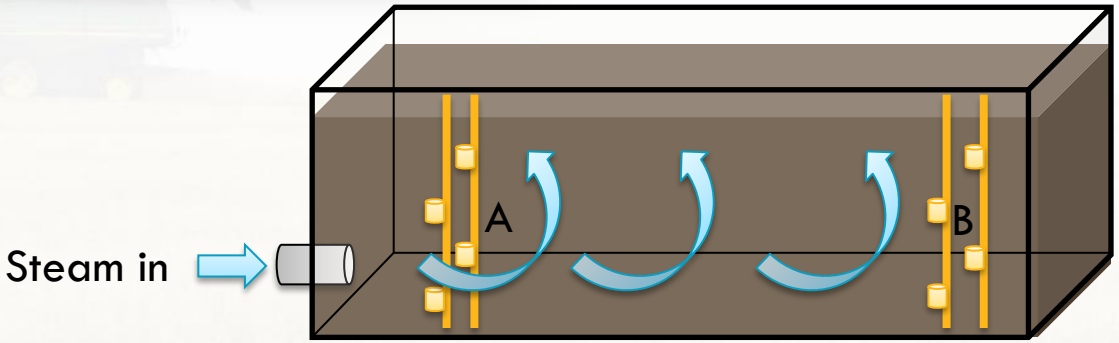


Steam Pasteurization:
Soil containing cut stems and roots

- Preparing pots for steam sterilization

Steam Pasteurization Setup

Video



BIs at Depths of:
0”, 4”, 8”, 12” at two locations

Steam Pasteurization Results

BI location A	BI incubation results	BI location B	BI incubation results
0 inches deep	Fail	0 inches deep	Fail
4 inches deep	Fail	4 inches deep	Fail
8 inches deep	Fail	8 inches deep	Fail
12 inches deep	Fail	12 inches deep	Fail

Steam Pasteurization:
Soil containing cut stems and roots

- Common pasteurized plants include: corn, soybean, sorghum and tobacco
- BI testing failed – time and temp not sufficient to kill spores
- 14 pots were selected to test for plant viability after pasteurization.
  - Grown under standard greenhouse conditions.

Viability testing of plants after pasteurization

Viability testing of plants after pasteurization

Week one: No Growth

Week two: No Growth

Pasteurization Control Group:
Plants from compost pile (no steam treatment)

Day 0

Day 14: Growth

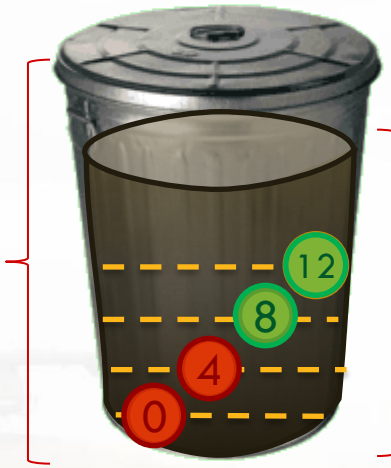
Viability testing of plants after pasteurization

Plant/soil	Week one	Week two
Corn (4 pots)	No Growth	No Growth
Soybean (4 pots)	No Growth	No Growth
Sorghum (2 pots)	No Growth	No Growth
Tobacco (4 pots)	No Growth	No Growth

Autoclaving: Bulk Soil

Soil Autoclave Results

BIs at Depths of:
0”, 4”, 8”, 12”



23.3”
Trash can
height

18”
Initial Soil
Depth

Fail

BIs at Depths of:
0” and 4”

9”
New Max
Soil Depth

Fail

Pass

Parameters	60min @ 121°C, 16psi	90min @ 121°C, 16psi	180min @ 121°C, 16psi

Complete Study Result Summary

Material	Inactivation Method	Validation Method
Mixed Plant and Soil	Autoclave	BI
Just Stems in Soil	Pasteurization	Planting to verify non-viability
Soil only	Autoclave	BI

Final Decontamination Parameters based on Study Results:

Autoclave Parameters: 121 °C and 16 PSI

Material	Contains or used with recombinant nucleic acids*	Contaminated with plant pathogens or pests	Not contaminated
Mixed Plant and Soil	Autoclave: 60min	Autoclave: 60min	Composted
Just Stems in Soil	Pasteurization: 3 hours	Autoclave: 9” max depth 90min, (2x)	Composted
Soil only	Autoclave: 9” max depth 90min, (2x)	Autoclave: 9” max depth 90min, (2x)	Composted

*Compostable after inactivation

Outcomes:

- Validate existing and modified decontamination procedures for all UNL greenhouses to use for disposal of plant material and soil
- Establish minimum standards of deactivation of plant material and soil
- Confidence that plant-associated waste is properly and effectively deactivated prior to disposal
- Verified procedures will be incorporated in a revised UNL greenhouse facilities manual established by the UNL Faculty Greenhouse Committee

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