

## Regulatory Requirements for Use of Transgenic Plants in the Greenhouse



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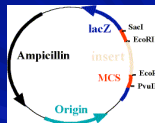
## Agenda

- Introduction
- Guidance and Oversight
- Biosafety Levels
- Containment

2

## Introduction

- NIH Guidelines specifies practices for:
  - rDNA molecules
  - Organisms and viruses that contain rDNA
- rDNA molecules are:
  - Molecules constructed outside cells by joining DNA segments to DNA molecules that replicate in a living cell
  - Molecules that result from replication of those above



3

## Introduction

- Transgenic or genetically modified organisms (GMO)
  - Plants
  - Plant-associated organisms
- Greenhouses
- Guidance is not abundant



4

## Guidance and Oversight

- NIH Guidelines
  - Risk assessment
  - Containment
  - Work practices
  - Facilities
- Although advisory, compliance=funding!!
- Sections III-E-2 and D-5 “Experiments Involving Whole Plants”
  - Genetically-modified whole plants
  - Genetically-modified microorganisms

5

## Guidance and Oversight

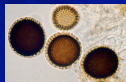
- Appendix P- “Physical and Biological Containment for Recombinant DNA Research Involving Plants”
- Specifies physical and biological containment, and practices suitable for greenhouse
- Biosafety levels
- Plants include:
  - Vascular plants including crops, ornamentals, and forest species
  - Mosses, liverworts, macroscopic algae



6

## Guidance and Oversight

- Plant-associated microorganisms
  - Fungi, bacteria, viruses
  - Benign, beneficial (mycorrhizae, *Rhizobium*), or pests
- Plant-associated animals or arthropods
  - Invertebrate vectors
  - Pests
  - Nematodes



7

## Guidance and Oversight

- Other Federal agencies
  - USDA/APHIS
    - Protect US agriculture
    - Any introduction of GMOs
  - EPA
    - Plants producing pesticidal substances (e.g., Bt)
    - Novel microbes for commercial use (e.g., pollutant degrading bacteria)
  - FDA
    - Engineered for human and animal consumption
    - Human and veterinary drugs



8

## Guidance and Oversight

- Institutional Biosafety Committee  
(formerly the rDNA Committee)
- Membership with NIH
- At least five members including two non-affiliated members
- CU Faculty and Staff with various expertise from different fields
- Currently, six members with plant biology or plant pathology experience



9

## Guidance and Oversight

- What does the IBC do?
  - Review rDNA research and use of biological agents and toxins
  - Evaluate personnel, facilities, and procedures
  - Recommend policies to guide principal investigators and EH&S in carrying out the University's Biosafety Program
  - Maintain documentation and communicate with NIH



10

## Guidance and Oversight

- Principal investigator
  - Ultimate responsibility
  - Submit Memorandum of Understanding and Agreement (MUA)
  - Determine appropriate containment and develop protocols (e.g., greenhouse practices manual)
  - Training and oversight of personnel
  - Communicate with the lab and greenhouse staff!!!!

11

## Guidance and Oversight

- Greenhouse staff
  - Become familiar with the project
  - What's transgenic and what's not
  - Know what's in the greenhouse practices manual
  - Management and disposal practices
  - Awareness and reporting

12

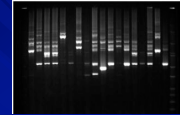
## Plant Biosafety Levels

- Combination of practices, physical, and biological containment conditions
- Increasing levels of environmental protection and containment
- Avoid unintentional transmission or release
- No threat to humans or animals
- Minimize ecosystem effects outside of facility
- BL1-P through BL4-P

13

## Plant Biosafety Levels

- Factors to consider
  - Recipient organism
    - Transmission, detrimental impact, outcrossing
  - Nature of introduced DNA
    - Pathogens, exotic agents
  - Compatible species in local environment
    - Wild or weedy species
  - Procedures and practices
    - Movement of materials, containment



14

## Plant Biosafety Levels

- BL1-P
  - Low level of containment
  - Low environmental risk (i.e., inability to survive and spread)
  - Plant-associated microorganisms not easily disseminated- minimal impact
  - e.g., not noxious weeds, cannot outcross, plant transformation with *Agrobacterium*

15

## Plant Biosafety Levels

- BL2-P
  - Higher level of containment
  - Recognized potential for rapid and widespread dissemination
  - Some environmental impact
  - Capable of interbreeding with weeds or related species

16

## Plant Biosafety Levels

- BL2-P
  - Complete genome of non-exotic infectious agent
  - Plant-associated microbes- manageable environmental harm
  - Exotic microbes- little potential for impact on ecosystems
  - Plant-associated insects- no serious ecosystem impact

17

## Plant Biosafety Levels

- BL3-P
  - Significant impact on environment
  - Exotic infectious agents detrimental to environment
  - Vertebrate toxins

18

## Containment

- Protect the environment, not the researcher
- Risk assessment
  - Organism
  - Geographic/ecologic setting
  - Mechanical barriers
  - Selected practices
  - Consequences and likelihood of release



19

## Containment

- Basic Principles
  - Avoid transmission or release
  - Prevent introduction and establishment of organism in new ecosystem
  - Minimize impact on organisms and ecosystems outside of facility
  - Avoid inadvertent spread of serious pathogen
- Achieved through biological methods, physical barriers, and management practices

20

## Biological Containment

- Works in conjunction with Biosafety Levels
- Highly effective
- Can be used to lower physical containment or Biosafety Levels
- Reproductive, spatial, or temporal

21

## Biological Containment

- Plants- minimize dissemination of pollen or seed
  - Harvest material prior to reproductive stage
  - Cover reproductive structures
  - Use male sterile lines
  - Cross-fertile plants not growing or flowering
    - Time, distance of experimental plants
  - Localize engineered genes in non-reproductive parts



22

## Biological Containment

- Microorganisms- minimize dissemination
  - Genetic attenuation
  - Eliminate vectors
  - Limit production of aerosols during inoculations
  - Obligate association with the plant host
  - Distance between infected and susceptible hosts



23

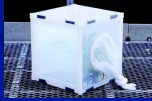
## Physical Containment

- BL1-P
  - Access at discretion of greenhouse director
  - Read and follow BL1-P practices and procedures; appropriate for organisms
  - Record of experiments currently in progress
- BL2-P
  - Access limited to individuals directly involved with experiments
  - Read and follow BL2-P practices and procedures; appropriate for organisms
  - Record of experiments currently in progress, and organisms brought into or out of facility

24

## Physical Containment

- BL1-P
  - Inactivate organisms before disposal
  - Control undesired species
  - Contain arthropods and other motile organisms in appropriate cages, and minimize escape from greenhouse
- BL2-P
  - Inactivate organisms before disposal
  - Control undesired species
  - Contain arthropods and other motile organisms in appropriate cages, and minimize escape from greenhouse



25

## Physical Containment

- BL1-P
  - Experiments requiring lower containment may be conducted concurrently; all under BL1-P practices
  - Floor may be composed of gravel or other porous material
  - Screens are recommended
  - No personal protective equipment required
- BL2-P
  - Experiments requiring lower containment may be conducted concurrently; all under BL2-P practices
  - Concrete floor recommended; gravel under benches acceptable
  - Screens are required
  - No personal protective equipment required

26

## Physical Containment

- BL2-P
  - Principal investigator shall report inadvertent release or spill
  - Decontamination of run-off water not necessarily required, but
  - Periodically treat gravel to eliminate trapped organisms
  - Transfer transgenic material in closed, secondary containment

27

## Physical Containment

- BL2-P
  - Appropriate signage
    - Name of responsible individual, plants in use, special requirements, GMO vs. non-GMO
  - If risk to human health- universal biohazard sign
  - Indicate presence of organisms that can adversely impact ecosystems



28

## Physical Containment

- BL2-P
  - Autoclave is available
  - Construct fans to minimize ingress of arthropods



29

## Physical Containment

- BL2-P
  - Prepare a greenhouse practices manual
    - Standard practices and facilities
    - Special practices
    - Contingency plans for release
      - Contact information
      - Personnel notification
      - Decontamination



30

## Research with Restricted Pathogens

- Agricultural Bioterrorism Protection Act of 2002: Possession, Use and Transfer of Biological Agents and Toxins; 7 CFR 331
- Select Agents- threats to plant health and plant products
- Registration of entities, e.g., universities, industries
  - ▲ DOJ/FBI approval for SA handlers
  - ▲ Biosecurity, safety, emergency response, etc.
  - ▲ Recordkeeping for inventories, access, etc.
  - ▲ Must contact EH&S!!!
- ▲ [http://www.aphis.usda.gov/ppq/permits/agr\\_bioterrorism/](http://www.aphis.usda.gov/ppq/permits/agr_bioterrorism/)

31

## Research with Restricted Pathogens

- *Liberobacter africanus*
- *Liberobacter asiaticus*
- *Peronosclerospora philippinensis*
- *Phakospora pachyrhizi*
- Plum pox potyvirus
- *Ralstonia solanacearum*, race 3, biovar 2
- *Sclerophthora rayssiae* var. *zeae*
- *Synchytrium endobioticum*
- *Xanthomonas oryzae* pv. *oryzicola*
- *Xylella fastidiosa* (citrus variegated chlorosis strain)



32



## Summary

- Transgenic plants and associated organisms are commonly used
- Environmental protection is the goal
- Guidelines and risk assessment direct appropriate Biosafety Levels
- Biological and physical containment

33

## Resources

NIH Guidelines, Appendix P- "Physical and Biological Containment for Recombinant DNA Research Involving Plants"

[http://www4.od.nih.gov/oba/rac/guidelines\\_02/Appendix\\_P.htm](http://www4.od.nih.gov/oba/rac/guidelines_02/Appendix_P.htm)

A Practical Guide to Containment- Greenhouse Research with Transgenic Plants and Microbes

[http://www.isb.vt.edu/cfdocs/greenhouse\\_manual.cfm](http://www.isb.vt.edu/cfdocs/greenhouse_manual.cfm)

Guidelines for Handling Transgenic Plants and Associated Organisms

<http://www2.fpm.wisc.edu/biosafety/Base/PlantContainment.htm>

34