Regulatory Requirements for Use of Transgenic Plants in the Greenhouse

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Agenda

- Introduction
- Guidance and Oversight
- Biosafety Levels
- Containment

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

Introduction

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

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

Guidance and Oversight

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

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
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

• 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

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????

• 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
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

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

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

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

Plant Biosafety Levels

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

Containment

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

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
Biological Containment

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

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

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

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
### Physical Containment

<table>
<thead>
<tr>
<th>BL1-P</th>
<th>BL2-P</th>
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<tbody>
<tr>
<td>- Inactivate organisms before disposal</td>
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<td>- Control undesired species</td>
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<tr>
<td>- Contain arthropods and other motile organisms in appropriate cages, and minimize escape from greenhouse</td>
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*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

### Physical Containment

<table>
<thead>
<tr>
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<tr>
<td>- Principal investigator shall report inadvertent release or spill</td>
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<tr>
<td>- Decontamination of run-off water not necessarily required, but</td>
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<td>- Periodically treat gravel to eliminate trapped organisms</td>
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<tr>
<td>- Transfer transgenic material in closed, secondary containment</td>
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*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

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<td>- Experiments requiring lower containment may be conducted concurrently; all under BL2-P practices</td>
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<td>- Concrete floor recommended; gravel under benches acceptable</td>
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Physical Containment

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

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

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

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

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