

# Guidelines for Handling Transgenic Plants & Associated Organisms

Office of Biological Safety, University of Wisconsin-Madison

Guidance for handling potentially biohazardous plants and associated organisms lags behind that available for vertebrates and their infectious agents. The United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) regulates importation, interstate movement and environmental release of plant pests and transgenic plants but provides minimal guidance for management of facilities. The best available information at this time comes from the NIH Guidelines for Research Involving Recombinant DNA Molecules. While the NIH Guidelines specifically addresses recombinant DNA, the recommendations regarding effective containment are relevant equally to research using non-recombinant methods.

This document presents portions of the NIH Guidelines that pertain to containment of transgenic plants and associated organisms. The content is consistent with that of the Guidelines but the format has been rearranged to make it more readable. The current version of the NIH Guidelines can be accessed at [http://oba.od.nih.gov/rdna/nih\\_guidelines\\_oba.html](http://oba.od.nih.gov/rdna/nih_guidelines_oba.html).

For detailed information on Plant Biosafety Levels, refer to (Appendix P) of the NIH Guidelines

## Introduction to Containment

The principle purposes of containment are:

- To avoid unintentional transmission.
- To minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility.
- To avoid the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop.
- To avoid the unintentional introduction and establishment of an organism in a new ecosystem

Containment may be achieved by a combination of physical and biological means. Containment for transgenic plants and their associated plant pathogens relies more heavily on biological factors than is the norm for human and animal infectious agents. The goal is to protect the environment, not the researcher. The risk assessment considers the specific organism(s), geographic/ecological setting and the available mechanical barriers; the selected practices are tailored to the specific situation. It becomes especially difficult to prescribe containment when genetic modifications lead to uncertainty in characteristics like host range and competitiveness.

For research involving plants, four biosafety levels (BL1-P through BL4-P) are described. BL1-P is designed to provide a moderate level of containment for experiments for which there is convincing biological evidence that precludes the possibility of survival, transfer, or dissemination of recombinant DNA into the environment, or in which there is no recognizable and predictable risk to the environment in the event of accidental release. BL2-P is designed to provide a greater level of containment for experiments involving plants and certain associated organisms in which there is a recognized possibility of survival, transmission, or dissemination of recombinant DNA containing organisms, but the consequence of such an inadvertent release has a predictably minimal biological impact. BL3-P and BL4-P describe additional containment conditions for research with plants and certain pathogens and other organisms that require special containment because of their recognized potential for significant detrimental impact on managed or natural ecosystems. (Section II-B.)

BL1-P relies upon accepted scientific practices for conducting research in most ordinary greenhouse or growth chamber facilities and incorporates accepted procedures for good pest control and cultural practices. BL1-P facilities and procedures provide a modified and protected environment for the propagation of plants and microorganisms associated with the plants and a degree of containment that adequately controls the potential for release of biologically viable plants, plant parts, and microorganisms associated with them. BL2-P and BL3-P rely upon accepted scientific practices for conducting research in greenhouses with organisms infecting or infesting plants in a manner that minimizes or prevents inadvertent contamination of plants within or surrounding the greenhouse. BL4-P describes facilities and practices known to provide containment of certain exotic plant pathogens. (Section II-B.)

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## Determination of Containment Level (Section III)

Knowledge of the organisms and judgment based on accepted scientific practices should be used in all cases in selecting the appropriate level of containment. For example, if the genetic modification has the objective of increasing pathogenicity or converting a non-pathogenic organism into a pathogen, then a higher level of containment may be appropriate depending on the organism, its mode of dissemination, and its target organisms.

Experiments that fall under Section III-E require Institutional Biosafety Committee notice simultaneous with initiation. Those that fall under Section III-D require Institutional Biosafety Committee approval before initiation.

BL1-P is recommended for all experiments with recombinant DNA-containing plants and plant-associated microorganisms (excluding those covered below for Section III-D). Example: plant transformation using recombinant *Agrobacterium* where the genetic modification is not expected to increase adverse characteristics. [Section III-E]

Experiments requiring BL2-P or a higher level of containment involve plants that are noxious weeds, that can interbreed with noxious weeds in the immediate geographic area; or have recognized potential for rapid and widespread dissemination or for serious detrimental impact on managed or natural ecosystems.

### BL2-P or BL1-P+ Biological Containment is recommended for:

- Plants modified by rDNA that are noxious weeds or can interbreed with noxious weed in the immediate geographic area. [Section III-E-2-b-(1)]
- Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent. [Section III-E-2-b-(2)]
- Plants associated with recombinant DNA-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems. [Section III-E-2-b-(3)]
- Plants associated with recombinant DNA-modified exotic microorganisms that have no recognized potential for serious detrimental impact on managed or natural ecosystems. [Section III-E-2-b-(4)]
- Experiments with recombinant DNA-modified arthropods or small animals associated with plants, or with arthropods or small animals with recombinant DNA-modified microorganisms associated with them if the recombinant DNA-modified microorganisms have no recognized potential for serious detrimental impact on managed or natural ecosystems. (Section III-E-2-b-(5))

### BL3-P or BL2-P+ Biological Containment is recommended for:

- Experiments involving most exotic infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant DNA techniques are associated with whole plants. [Section III-D-5-a]
- Experiments involving plants containing cloned genomes of readily transmissible exotic infectious agents with recognized potential for serious detrimental effects on managed or natural ecosystems in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation in plants. [Section III-D-5-b]
- Experiments with microbial pathogens of insects or small animals associated with plants if the recombinant-DNA modified organism has a recognized potential for serious detrimental impact on managed or natural ecosystems. [Section III-D-5-e]

BL3-P is recommended for experiments involving sequences encoding potent vertebrate toxins introduced into plants or plant-associated organisms. [Section III-D-5-d]

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## Biological Containment in the Greenhouse (Appendix P)

The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth. The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.

Appendix P of the NIH Guidelines specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant DNA-containing plants, plant-associated microorganisms, and small animals. Appendix P applies when the research plants are of a size, number, or have growth requirements that preclude the use of laboratory containment conditions described in Appendix G, Physical Containment.

The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant DNA-containing plant genome, including nuclear or organelle hereditary material or release of recombinant DNA-derived organisms associated with plants.

The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility, e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem.

Four biosafety levels, referred to as Biosafety Level (BL) 1 - Plants (P), BL2-P, BL3-P, and BL4-P, are designed to provide differential levels of biosafety for plants in the absence or presence of other experimental organisms that contain recombinant DNA. These biosafety levels, in conjunction with biological containment conditions provide flexible approaches to ensure the safe conduct of research.

### **Biological Containment of Plants: Effective dissemination of plants by pollen or seed can be prevented by one or more of the following procedures:**

- Cover the reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity;
- Remove reproductive structures by employing male sterile strains, or harvest the plant material prior to the reproductive stage;
- Ensure that experimental plants flower at a time of year when cross-fertile plants are not flowering within the normal pollen dispersal range of the experimental plant; or (iv) ensure that cross-fertile plants are not growing within the known pollen dispersal range of the experimental plant.

### **Biological Containment of Microorganisms: Effective dissemination of microorganisms beyond the confines of the greenhouse can be prevented by one or more of the following procedures:**

- Confine all operations to injections of microorganisms or other biological procedures (including genetic manipulation) that limit replication or reproduction of viruses and microorganisms or sequences derived from microorganisms, and confine these injections to internal plant parts or adherent plant surfaces;
- Ensure that organisms, which can serve as hosts or promote the transmission of the virus or microorganism, are not present within the farthest distance that the airborne virus or microorganism may be expected to be effectively disseminated;
- Conduct experiments at a time of year when plants that can serve as hosts are either not growing or are not susceptible to productive infection; Use viruses and other microorganisms or their genomes that have known arthropod or animal vectors, in the absence of such vectors;
- Use microorganisms that have an obligate association with the plant; or

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- Use microorganisms that are genetically disabled to minimize survival outside of the research facility and whose natural mode of transmission requires injury of the target organism, or assures that inadvertent release is unlikely to initiate productive infection of organisms outside of the experimental facility.

### **Biological Containment of Macro-organisms: Effective dissemination of arthropods and other small animals can be prevented by using one or more of the following procedures: Use non-flying, flight-impaired, or sterile arthropods**

- Use non-motile or sterile strains of small animals;
- Conduct experiments at a time of year that precludes the survival of escaping organisms;
- Use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or
- Prevent the escape of organisms present in run-off water by chemical treatment or evaporation of run-off water.