Plant Containment Manual for Plant Studies involving Recombinant DNA in the MSU Greenhouse Facility
# Table of Contents

Certification and Approvals .......................................................... 3

Introduction .................................................................................. 4

Roles and Responsibilities............................................................... 4

  Institutional Biosafety Committee (IBC) ........................................ 4
  Biosafety Officer (BSO) ................................................................. 4
  Principal Investigator (PI) ............................................................. 4
  Laboratory Staff ........................................................................ 4

Recombinant DNA ........................................................................ 5

Regulations, Permitting Agencies, and Oversight ......................... 5

  NIH Guidelines ....................................................................... 5
  Permitting Agencies ................................................................. 5
    United States Department of Agriculture (USDA)/
    Animal and Plant Health Inspection Service (APHIS) ............... 5
    Environmental Protection Agency (EPA) ..................................... 6
    Food and Drug Administration (FDA) ....................................... 6
    Centers for Disease Control and Prevention (CDC) ................. 6

Plant Biosafety Levels (BL-P) ...................................................... 7

  BL1-P .................................................................................... 7
  BL2-P .................................................................................... 8
  BL3-P/BL4-P ........................................................................ 8

Containment .................................................................................. 8

  Access .................................................................................... 8
  Records ................................................................................ 8
  Structural Containment ............................................................ 9
  Signs and Labeling .................................................................. 9
  Proper Hygiene/Housekeeping ................................................ 9
  Pest Control .......................................................................... 10
  Transporting Transgenic Material ............................................. 10
  Biological Containment Techniques ........................................ 10
  Disposal of Materials ............................................................. 11

Containment Breach ...................................................................... 11

Standard Operating Procedures ................................................... 11

Appendix A: Physical and Biological Containment for Recombinant DNA Research Involving Plants (Appendix P of the NIH Guidelines) .................................................. 14
Certification and Approvals

This manual summarizes the requirements for working with recombinant DNA containing plants, plant-associated microorganisms and plant associated small animals in the MSU greenhouse. With the intent of being in compliance with the NIH Guidelines for Research Involving Recombinant DNA molecules and other applicable guidelines and regulatory standards. The Institutional Biosafety Committee at Montana State University have reviewed and endorsed this document. This manual is required to be reviewed annually, or updated when changes occur.

Signature of IBC Chair

__________________________  ________________________
Mike Babcock                Date

Print Name

Signature of Biosafety Officer

__________________________  ________________________
Kirk Lubick                 Date

Print Name

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Introduction

The purpose of this manual is to define the policies and procedures for greenhouse plant containment pertaining to research operations at the Montana State University (MSU) Greenhouse facility. These policies and procedures are designed to safeguard against the accidental release of recombinant DNA containing plants, plant-associated microorganisms and plant associated animals to the environment outside of the Greenhouse facility, as required by federal, state, and local regulatory policies. All MSU Principal Investigators (PIs), laboratory personal, and greenhouse staff must adhere to these policies and procedures in their research and the management of their laboratories.

Roles and Responsibilities

Institutional Biosafety Committee (IBC)
- The IBC reviews and approves all projects involving recombinant DNA containing plants, plant-associated microorganisms and plant-associated animals.

Biosafety Officer (BSO)
- Provides consultation in containment practices and containment breaches.
- Ensures that the laboratory and greenhouse policies and procedures are in compliance with all federal, state, and local regulations.
- Conducts inspections of laboratory and greenhouse facilities.
- Provides biosafety training to laboratory personnel, greenhouse staff, and PIs.
- Reports to the IBC and the institution any significant research-related accidents or containment breaches.
- Reviews and gives input on laboratory and greenhouse specific Standard Operating Procedures (SOPs) and policies.

Principal Investigator (PI)
- Obtains approval from the IBC for all proposed projects involving recombinant DNA containing plants, plant-associated microorganisms and plant-associated animals to be conducted in MSU laboratory and Greenhouse facilities prior to the initiation of the experiment.
- Ensures that laboratory staff are adequately trained in the practices and techniques required to ensure safety and appropriate containment.
- Ensures that laboratory staff working in MSU laboratories and greenhouse facilities are experienced and proficient in working with rDNA containing plants, plant-associated microorganisms and plant-associated animals.
- Makes available SOPs, policies and procedures that describe the practices used in containment of rDNA containing plants to all laboratory staff.
- Ensures that the required safety practices, techniques and PPE are provided and used.
- Notifies the Greenhouse Facility Manager and BSO of any problems or issues that may lead to a breach in containment.
- Complies with NIH Guidelines and APHIS reporting requirements.
- Completes an audit and properly disposes of all recombinant DNA containing plants, plant-associated microorganisms and plant-associated animals at the conclusion of the project.

Laboratory Staff
- Participates in, and completes, all required training.
- Understands the associated risks and importance of containment when working with rDNA containing plants, plant-associated microorganisms and plant-associated animals.
- Follows all laboratory and greenhouse practices, protocols and complies with all applicable policies, procedures, and guidelines.
• Informs the PI of potential problems with SOP’s or equipment that may result in a potential breach of containment.
• Reports thefts, security incidents, accidents, spills, or breaches in containment to their PI and the BSO.

Recombinant DNA

The use of genetic manipulation and other techniques to produce transgenic or genetically modified plants, recombinant plant pathogens, and transgenic arthropods is common in plant science research. The definition of a transgenic plant is a plant that contains a gene(s) that has been artificially inserted instead of the plant acquiring them through pollination or other natural means. Recombinant and synthetic nucleic acid molecules are defined in the NIH Guidelines (Section I-B) as:

(i) Molecules that a) are constructed by joining nucleic acid molecules and that can replicate in a living cell, i.e., recombinant nucleic acids;
(ii) Nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or
(iii) Molecules that result from the replication of those described in (i) or (ii) above.

Research involving recombinant and synthetic nucleic acids must be conducted in a manner that does not pose a significant risk to the health or safety of laboratory workers, others at MSU, the community, or the environment.

Regulations, Permitting Agencies, and Oversight

NIH Guidelines

The National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) outlines practices for work involving recombinant DNA (rDNA) molecules, synthetic nucleic acids, and organisms and viruses that contain recombinant DNA. The NIH Guidelines also provides information about determining risk assessment, containment, work practices, and facility design. The NIH Guidelines indicate that the ultimate responsibility for handling of transgenic or genetically modified plants and plant-associated organisms lies with the PI and other persons who manage any aspect of the research. Compliance with the NIH Guidelines is a condition of funding for the entire institution.

Specific regulations for research involving modified whole plants can be found in sections III-D-5 and III-E-2 of the NIH Guidelines. Appendix P of the NIH Guidelines “Physical and Biological Containment for Recombinant DNA Research Involving Plants” outlines the physical and biological containment practices that must be employed for greenhouse research at the four plant biosafety levels (BL-P), BL1-P through BL4-P (Appendix A).

The use of genetic manipulation and other techniques to produce transgenic or genetically modified plants, recombinant plant pathogens, and transgenic arthropods is common in plant science research.

Permitting Agencies

Research involving transgenic or genetically modified plants, recombinant plant pathogens, or transgenic animals may require permits from a Federal agency. Prior to applying for a permit through any of the following agencies, PI’s should contact the MSU BSO for assistance to ensure that the appropriate biocontainment procedures are in place for the proposed research project.

United States Department of Agriculture (USDA)/Animal and Plant Health Inspection Service (APHIS)
Biological materials that may pose a risk to plants and/or animals or the environment are regulated by APHIS. APHIS permits for working with certain plants, plant pests, and plant-associated organisms are granted by agencies based on the biological material involved and the at-risk population (i.e. plants). These agencies are:

- Plant Protection and Quarantine (PPQ)
- Biotechnology Regulatory Services (BRS)

**PPQ**
Ensures that the appropriate protections are in place to protect agriculture and natural resources from the risks associated with the entry, establishment, or spread of plant pests, plant pathogens, and noxious weeds to ensure an abundant, high-quality, and varied food supply. Generally, PPQ purview includes only unmodified (i.e. non- transgenic) materials. For additional compliance considerations and applicability of institutional oversight to unmodified plant pests, plant pathogens, noxious weeds, see Appendix B.

**BRS**
BRS protects America’s agriculture and environment through regulatory oversight that allows for the safe development, transport, and use of genetically modified organisms (GMOs), including plants, plant pests, and arthropods. BRS also regulates and oversees environmental releases of these GMOs (i.e. transgenic field releases).

APHIS permits are available as electronic permits (e-permits) through the APHIS website. Holders of APHIS permits assume all legal responsibility for the materials, their transport, and their security. Researchers are advised to contact the MSU BSO or the appropriate agency if they have questions about the permits required for their research.

**Environmental Protection Agency (EPA)**
The EPA regulates two categories of GMOs: plants producing toxins (e.g., *Bacillus thuringiensis*) and novel microbes for commercial use (e.g., pollutant degrading bacteria). Information is available on these two categories through the Biopesticides and Pollution Prevention Division of the EPA.

**Food and Drug Administration (FDA)**
The FDA regulates GMO-derived commercial products for human and animal consumption, as well as human and veterinary pharmaceuticals. FDA’s oversight does not apply to the research and development phases of the product(s).

**Centers for Disease Control and Prevention (CDC)**
The CDC and USDA APHIS jointly regulate certain plant pathogens that are recognized as potential bioterrorism agents as specified in the National Select Agent Registry. The National Select Agent Registry is charged with permitting and tracking agents and toxins that pose a threat to public health and agriculture. Currently, there are eight plant pathogens listed as Select Agents and MSU is not registered to work with any of these pathogens.
Plant Biosafety Levels (BL-P)

A plant biosafety level designation will dictate the physical and biological containment practices aimed at reducing the public health threat, and avoid an unintentional transmission or release of regulated plant material into the environment. The BL-P levels of containment were specifically devised to describe containment for transgenic plants. There are four BL-P, and as the level increases, the level of protection and physical and/or biological containment practices also increase.

There are several issues to consider when determining the appropriate BL-P:

- **What is the source and nature of the introduced genetic material?**
  - Is it from an exotic infectious agent or pathogenic organism?
  - Is it a fragment of DNA or a complete genome?

- **What is the nature of the host organism?**
  - Can the host readily disseminate the genetic material? By what mechanism(s)?
  - Is the recipient likely to be invasive to local ecosystems?
  - Is the recipient a USDA APHIS-listed noxious weed or capable of interbreeding with noxious weeds?
  - What is the potential for outcrossing between the recipient organism and related species?
  - What is the potential for detrimental impact on natural or managed ecosystems?

- **Are bioactive proteins expressed?**
  - What is the nature of expressed proteins?
    - Are the proteins vertebrate toxins or potential/known allergens?
    - Are the proteins toxic to other organisms in the local environment?

- **What is the profile of the local environment?**
  - Are potentially affected important crops located nearby?
  - Are sexually compatible wild or weed species capable of sustaining and/or spreading the genetic modification(s)?

- **What experimental procedures may impact containment?**
  - Will it be necessary to transport sensitive materials to/from the greenhouse?
  - Will arthropods or other potential vectors be used during the course of the project? How will these be contained to prevent or minimize the release of genetically modified materials?

**BL1-P**

BL1-P designation is for experiments that are deemed a low risk to the environment. This designation also applies to plant associated microorganisms that are considered to have a minimal impact on the environment. Some examples include: plants that are not noxious weeds, plants with no potential for outcrossing with related species, and *Agrobacterium*-mediated transfer of innocuous genetic material.

Work involving other organisms that require a containment level of BL1-P or lower may be conducted concurrently in a greenhouse bay as long as all work is conducted using BL1-P practices. See Appendix A for
specific BL1-P requirements.

**BL2-P**

BL2-P applies to experiments with transgenic plants and plant associated organisms that have the potential for rapid and widespread dissemination, and the capability of interbreeding with weeds or related species. However, these materials are not likely to have a serious detrimental impact on natural ecosystems.

Work involving other organisms that require a containment level of BL2-P or lower may be conducted concurrently in a greenhouse bay as long as all work is conducted using BL2-P practices. See Appendix A for specific BL2-P requirements.

**BL3-P/BL4-P**

Montana State University does not have containment facilities to conduct BL3-P or BL4-P designated research, and will not be presented in this document. For more information on these levels of containment, please see Appendix P of the NIH guidelines.

**Containment**

Greenhouse containment is essential in preventing the accidental release of transgenic research materials into the environment. When planning an experiment, all ways that an organism can breach containment must be considered. Traffic flow of personnel, air flow within the facility, prevention of cross-contamination, proper labeling, and permit requirements are important elements in this process.

**Access**

Access to MSU’s greenhouse facility including growth chambers bays is restricted to individuals directly involved with experiments that are in progress. All support staff and external contractors must be approved by the PI and Greenhouse Manager to have access to the facility. The Greenhouse Manager must be notified when the plants contained within that greenhouse bay or growth chamber are under restricted access. Additionally, all individuals with access to the experiments must be knowledgeable of any special containment strategies, required personal protective equipment, and entry/exit procedures.

**Records**

In BL1-P greenhouse bays, it is important that all users read the MSU Greenhouse Manual and follow applicable SOPs. Record logs of all in-process experiments in the greenhouse facility should be kept by the PI (IBC registrations or a simple list of ongoing experiments are suitable records for BL1-P).

Persons working in BL2-P greenhouse bays must read and follow the MSU Greenhouse Manual and applicable SOPs. Record logs of all in-process BL2-P experiments in the greenhouse facility must be kept by the PI. These records should include a detailed inventory of all experimental plants, microorganisms, arthropods, or small animals that are brought into or removed from the greenhouse facility, including the date, what was taken, where it was taken, name of person moving material, and how the container was sanitized.

It is the responsibility of the PI to provide the Greenhouse Manager and Biosafety Officer access to record logs at the time of decommissioning or in the event of an audit by regulatory authorities.

Additionally, any specific federal/state permits required for greenhouse projects must be on file with the
Biosafety Officer to ensure compliance with all procedural and containment expectations indicated in the permit(s).

**Structural Containment**
The greenhouse bay floors in BL1-P containment may be composed of gravel or other porous material, and concrete walkways are recommended.

BL2-P greenhouse bays are required to be composed of an impervious material (e.g. concrete). Screens are required in BL2-P greenhouse bays to exclude small arthropods and birds.

Regular inspections of the physical condition of the greenhouses are performed by the Biosafety Officer and/or Greenhouse Manager. All authorized greenhouse users are required to be vigilant for structural damage due to age related wear and tear, seasonal influences, extreme weather, vandalism, and other causes. Observations must be reported to the Greenhouse Coordinator, PI, and the Biosafety Officer. Items include, but are not limited to, the following:

- Doors that do not properly close;
- Damaged door sweeps;
- Cracks, breaks to glass;
- Damage to screens;
- Evidence of insects in the greenhouse; and/or
- Damaged or missing seals between structural components, around pipes and conduit.

*If it is suspected that structural damage has resulted in a loss in containment of transgenic plants or plant-associated organisms at BL2-P, the Biosafety Officer must be notified since this is reportable to the NIH Office of Biotechnology Activities, USDA APHIS, and/or other designated authorities.*

**Signs and Labeling**
In BL2-P greenhouse bays a “Caution-Experiment in Progress” sign must be posted at the entrance to the individual greenhouse bay. These signs can be obtained from the Biosafety Officer. The sign will indicate the following information: plant species and novel trait; microorganisms used; precautionary information (including if the organisms used have a recognized potential for causing detrimental impacts on the environment); and a responsible individual with a 24-hour emergency contact number. If there is a risk to human health, the universal biohazard symbol will be present on the sign, along with relevant safety information. Though not required for BL1-P, similar signage is highly encouraged.

All transgenic seeds, plants, and materials must be clearly labeled and identified to distinguish them from other non-transgenic materials.

**Proper Hygiene/Housekeeping**
Good basic hygiene/housekeeping practices are important in preventing the accidental release and/or unintentional spread of plant pests and pathogens. Basic practices and procedures include:

- Keep greenhouse bay(s) clean and uncluttered;
- Do not eat or drink in greenhouse bays;
- Wash hands before leaving the greenhouse facility;
- Wear disposable fluid-resistant gloves when handling transgenic plant material, recombinant plant pathogens, and arthropods;
- Wear facility-dedicated or disposable lab coats/smocks when handling transgenic plant material,
recombinant plant pathogens, and arthropods;
• Thoroughly inspect street clothes/shoes for transgenic material (especially seed and/or pollen) prior to leaving the greenhouse bay;
• Observe all special containment measures such as footbaths, sticky mats, etc. when present;
• Change clothes prior to entering greenhouse if there is an increased potential to introduce unwanted plant pests/pathogens (e.g. working in an insect rearing facility before entering the greenhouse facilities);
• Eliminate any unnecessary equipment in greenhouse bays with transgenic plants, and recombinant plant pathogens or arthropods, especially at BL2-P.
• Sanitize equipment in BL2-P containment greenhouse bays at the end of the project); and
• Prohibit smoking throughout the MSU Greenhouse Facility.

Pest Control
The NIH Guidelines specify that a weed and pest control program must be in place for all levels of greenhouse containment. Additional precautions need to be taken in BL2-P containment if macroorganisms such as flying arthropods and nematodes are released in the greenhouse bay since they are pollen vectors. In the MSU Greenhouse Facility, PIs assigned to the bays are responsible for pest management. Plants must be regularly inspected for signs of insect infestation.

Transporting Transgenic Material
Experimental plants, seeds, and microorganisms that are transported to and from the greenhouse facility must be transported in a double walled, leak-proof, shatterproof container. The outside of the container must be sanitized prior to transport to ensure that transgenic pollen and seed are removed.

Biological Containment Techniques
Unless integral to the research project, the production/dissemination of transgenic pollen and seed should be eliminated. There are several special practices that can be used to prevent the spread of transgenic material that include, but are not limited to, the following:

• Removing flower heads or bagging plants prior to flowering;
• Harvesting material before the reproductive stage;
• Using male sterile lines;
• Localizing engineered genes in the non-reproductive parts of the plant by expressing the transgene transiently rather than in stably transformed plants; or
• Conducting the experiment when pollination will not occur outside (e.g. winter months).

Transgenic or unmodified insects or mites that are associated with transgenic plants should be housed in appropriate containment caging systems (e.g. BugDorms) to minimize escape from the greenhouse bay. A cost effective alternative can be constructed using plastic sheeting. Additional biocontainment techniques to be used when working with insects and mites include:

• Treatment or evaporation of runoff water to kill eggs and larvae;
• Destruction of pollinating insects in cages after pollen transfer.

Recombinant microbes such as bacteria, fungi, protozoa, viruses, and nematodes may be used during experiments. Additionally, unmodified microbes may be used in association with transgenic plants. In these cases, the goal of containment is to minimize dissemination of pollen and the microorganisms. Containment techniques that can be used when working with microorganisms include:

• Elimination of potential vectors;
Disposal of Materials

BL1-P experimental plants and soil must be rendered biologically inactive before final disposal. Viable non-seed plant tissue can be rendered inactive by desiccation, steam treatment, chemical treatment, freezing, or by a validated autoclave while seeds can be rendered inactive by steam or chemical treatment or by autoclaving. Transport of viable materials to the site of deactivation must be conducted in double walled containers (such as an autoclave bag in an autoclave tub). If viable BL1-P transgenic materials must be transferred to another facility for inactivation, a transportation containment SOP must be reviewed and approved by the IBC. After plant materials are inactivated using validated parameters, they may be disposed of in the regular trash.

BL2-P plant materials (including soil and pots) must be autoclaved using validated parameters prior to disposal, or as indicated by the permit. Appendix P of the NIH Guidelines states that BL2-P materials should be autoclaved. If an autoclave is not available, other suitable methods of inactivation may be used (e.g. chemicals, steam carts). However, the efficacy of these alternatives must be validated and documented. After plant materials are inactivated, they may be disposed of in the regular trash. If plant materials contain transgenic materials that may harm humans, a biohazard symbol must be present on the outside of the plastic bag prior to sterilization. After treating the plant materials using validated parameters, the biohazard symbol must be covered (i.e. place in non-see-through trash bag) prior to final disposal in the regular trash.

Containment Breach

Weather related incidents, vandalism, or human error can result in a containment breach. Seeds can become attached to clothing and/or shoes, especially if greenhouse containment practices are not rigorously followed. These seeds can be easily spread by the wind and could grow in the surrounding area, causing volunteers. Therefore, routine volunteer monitoring outside the greenhouse should be conducted. There are several steps that should be taken if an accident results in the inadvertent release or spill of recombinant microorganisms, transgenic arthropods, and/or transgenic plants from physical containment:

- If known, seed/pollen dissemination distances should be considered when determining the monitoring area. If a known breach of containment has occurred, volunteer monitoring should be enhanced by increasing the monitoring zone and/or frequency of monitoring.
- Determine if any transgenic material has been removed from the greenhouse bay/facility (or other containment vessels within the greenhouse bay/facility) or is otherwise unaccounted for.
- Contain and recover all transgenic materials as best as possible.
- The PI must report the containment breach to the Greenhouse Manager, Biosafety Office, IBC chair, and other appropriate agencies within 24 hours, or as indicated by permit.

Standard Operating Procedures

SOPs need to be prepared for all transgenic experiments that will be conducted in the greenhouse facilities. SOPs should be stored in a notebook inside the greenhouse bay. For projects that require BL2-P containment, SOPs must include:

- Growth and management practices for the transgenic materials;
• Biocontainment techniques;
• Methods of inactivation of transgenic materials (including soil and pots);
• A written contingency plan to be implemented in the event of the unintentional release of transgenic material.

Copies of all BL2-P SOPs as well as the contingency plan must be made available to the Greenhouse Manager and the Biosafety Officer. A copy of the contingency plan must also be posted on or near the door to the relevant greenhouse bay.

Copies of any permits/performance standards associated with the greenhouse work must be included in the SOP notebook.

If applicable, copies of Material Safety Data Sheets (MSDS) for chemicals and suitable descriptions of biological agents should also be accessible in the notebook.
Appendix A: Physical and Biological Containment for Recombinant DNA Research Involving Plants (Appendix P of the NIH Guidelines)

Appendix P 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. All provisions of the NIH Guidelines apply to plant research activities with the following modifications:

Appendix P shall supersede Appendix G (Physical Containment) when the research plants are of a size, number, or have growth requirements that preclude the use of containment conditions described in Appendix G. The plants covered in Appendix P include but are not limited to mosses, liverworts, macroscopic algae, and vascular plants including terrestrial crops, forest, and ornamental species.

Plant-associated microorganisms include viroids, virusoids, viruses, bacteria, fungi, protozoans, certain small algae, and microorganisms that have a benign or beneficial association with plants, such as certain Rhizobium species, and microorganisms known to cause plant diseases. The appendix applies to microorganisms which are being modified with the objective of fostering an association with plants.

Plant-associated small animals include those arthropods that: (i) are in obligate association with plants, (ii) are plant pests, (iii) are plant pollinators, or (iv) transmit plant disease agents, as well as other small animals such as nematodes for which tests of biological properties necessitate the use of plants. Microorganisms associated with such small animals (e.g., pathogens or symbionts) are included.

The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P require prior approval by the Institutional Biosafety Committee.

Appendix P-I. General Plant Biosafety Levels

Appendix P-I-A. 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.

Appendix P-I-B. 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.

Appendix P-I-C. Four biosafety levels, referred to as Biosafety Level (BL) 1 - Plants (P), BL2-P, BL3-P, and BL4-P, are established in Appendix P-II, Physical Containment Levels. The selection of containment levels required for research involving recombinant DNA molecules in plants or associated with plants is specified in Appendix P-III, Biological
Containment Practices. These biosafety levels are described in Appendix P-II, Physical Containment Levels. This appendix describes greenhouse practices and special greenhouse facilities for physical containment.

Appendix P-I-D. BL1-P through 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 described in Appendix P-III, Biological Containment Practices, provide flexible approaches to ensure the safe conduct of research.

Appendix P-I-E. For experiments in which plants are grown at the BL1 through BL4 laboratory settings, containment practices shall be followed as described in Appendix G, Physical Containment. These containment practices include the use of plant tissue culture rooms, growth chambers within laboratory facilities, or experiments performed on open benches. Additional biological containment practices should be added by the Greenhouse Director or Institutional Biosafety Committee as necessary (see Appendix P-III, Biological Containment Practices), if botanical reproductive structures are produced that have the potential of being released.

Appendix P-II. Physical Containment Levels

Appendix P-II-A. Biosafety Level 1 - Plants (BL1-P)


Appendix P-II-A-1-a-(1). Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress.

Appendix P-II-A-1-a-(2). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL1-P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.

Appendix P-II-A-1-b. Records (BL1-P)

Appendix P-II-A-1-b-(1). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-A-1-c. Decontamination and Inactivation (BL1-P)

Appendix P-II-A-1-c-(1). Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Appendix P-II-A-1-d. Control of Undesired Species and Motile Macroorganisms (BL1-P)

Appendix P-II-A-1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P-II-A-1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.
Appendix P-II-A-1-e. Concurrent Experiments Conducted in the Greenhouse (BL1-P) Appendix P-II-A-1-e-
(1). Experiments involving other organisms that require a containment
level lower than BL1-P may be conducted in the greenhouse concurrently with experiments that
require BL1-P containment, provided that all work is conducted in accordance with BL1-P
greenhouse practices.

Appendix P-II-A-2. Facilities (BL1-P)
Appendix P-II-A-2-a. Definitions (BL1-P)
Appendix P-II-A-2-a-(1). 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.

Appendix P-II-A-2-a-(2). 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-II-A-2-b. Greenhouse Design (BL1-P)
Appendix P-II-A-2-b-(1). The greenhouse floor may be composed of gravel or other porous material. At a
minimum, impervious (e.g., concrete) walkways are recommended.

Appendix P-II-A-2-b-(2). Windows and other openings in the walls and roof of the greenhouse facility may
be open for ventilation as needed for proper operation and do not require any special barrier to contain or
exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are
recommended.

Appendix P-II-B. Biosafety Level 2 - Plants (BL2-P)
Appendix P-II-B-1. Standard Practices (BL2-P)
Appendix P-II-B-1-a. Greenhouse Access (BL2-P)
Appendix P-II-B-1-a-(1). Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse
Director, to individuals directly involved with the experiments when they are in progress.

Appendix P-II-B-1-a-(2). Personnel shall be required to read and follow instructions on BL2-P practices and
procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are
appropriate to the experimental organisms.

Appendix P-II-B-1-b. Records (BL2-P)
Appendix P-II-B-1-b-(1). A record shall be kept of experimental plants, microorganisms, or small animals
that are brought into or removed from the greenhouse facility.

Appendix P-II-B-1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse
facility.
Appendix P-II-B-1-b-(3). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety Committee, NIH/OBA and other appropriate authorities immediately (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Documentation of any such accident shall be prepared and maintained.

Appendix P-II-B-1-c. Decontamination and Inactivation (BL2-P)

Appendix P-II-B-1-c-(1). Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Appendix P-II-B-1-c-(2). Decontamination of run-off water is not necessarily required. If part of the greenhouse is composed of gravel or similar material, appropriate treatments should be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel.

Appendix P-II-B-1-d. Control of Undesired Species and Motile Macroorganisms (BL2-P)

Appendix P-II-B-1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P-II-B-1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Appendix P-II-B-1-e. Concurrent Experiments Conducted in the Greenhouse (BL2-P)

Appendix P-II-B-1-e-(1). Experiments involving other organisms that require a containment level lower than BL-p may be conducted in the greenhouse concurrently with experiments that require BL-p containment provided that all work is conducted in accordance with BL2-P greenhouse practices.

Appendix P-II-B-1-f. Signs (BL2-P)

Appendix P-II-B-1-f-(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

Appendix P-II-B-1-f-(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.

Appendix P-II-B-1-f-(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P-II-B-1-g. Transfer of Materials (BL2-P)
Appendix P-II-B-1-g-(1). Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container.

Appendix P-II-B-1-h. Greenhouse Practices Manual (BL2-P)

Appendix P-II-B-1-h-(1). A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms.

Appendix P-II-B-2. Facilities (BL2-P)

Appendix P-II-B-2-a. Definitions (BL2-P)

Appendix P-II-B-2-a-(1). 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.

Appendix P-II-B-2-a-(2). 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-II-B-2-b. Greenhouse Design (BL2-P)

Appendix P-II-B-2-b-(1). A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil.

Appendix P-II-B-2-b-(2). Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms; however, screens are required to exclude small flying animals (e.g., arthropods and birds).

Appendix P-II-B-2-c. Autoclaves (BL2-P)

Appendix P-II-B-2-c-(1). An autoclave shall be available for the treatment of contaminated greenhouse materials.

Appendix P-II-B-2-d. Supply and Exhaust Air Ventilation Systems (BL2-P)

Appendix P-II-B-2-d-(1). If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.

Appendix P-II-B-2-e. Other (BL2-P)

Appendix P-II-B-2-e-(1). BL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses.