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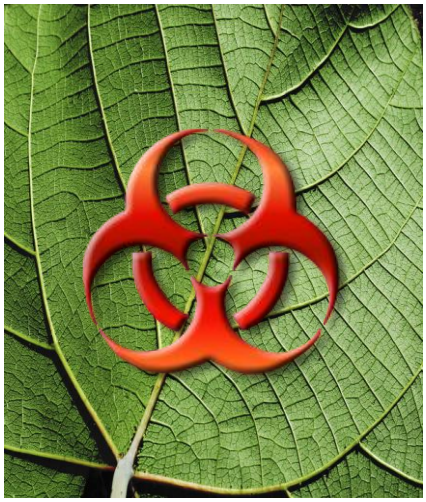

Plant Containment Manual
for Plant Studies involving Recombinant DNA in the UTIA Greenhouse Facilities

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APPROVALS PAGE

This manual summarizes the requirements for working with recombinant DNA-containing plants, plant-associated microorganisms (bacteria, fungi, protozoa, viruses, viroids) and plant-associated small animals (e.g. arthropods) in UTIA greenhouses. The content is derived from the *NIH Guidelines for Research Involving Recombinant DNA Molecules* and other applicable guidelines and regulatory standards. The Institutional Biosafety Committee of The University of Tennessee as well as the Administrative signatories below have reviewed and endorsed this document.

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Foreword:
This manual has been prepared to outline the concepts and procedures for greenhouse plant containment, which are to be used by all personnel working with recombinant DNA-containing plants, plant-associated microorganisms (bacteria, fungi, protozoa, viruses, viroids) and plant-associated small animals (e.g. arthropods) in the UTIA Greenhouse Facilities. Principal investigators (PI) and supervisors who have personnel conducting routine or research-related work in these facilities will be provided a copy of this manual. It is the responsibility of each PI, supervisor, or other end-user of the UTIA Greenhouse Facilities to become familiar with and follow all applicable procedures.

To clarify the terminology in this document, "recombinant", "transgenic" or "genetically modified (GM)" are equivalent terms, and all imply the intentional transfer of DNA (or its synthetic equivalent) to a host. "Greenhouse facility" or "facility" refers to the actual greenhouse bays including all connected hallways and the headhouse areas. "Greenhouse bay" refers to an individual greenhouse room within the greenhouse facility.

Section 1: Introduction
The use of genetic manipulation and other techniques to produce transgenic or genetically modified plants, recombinant plant pathogens (or pests), 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 as: (i) recombinant nucleic acid molecules that are constructed by joining nucleic acid molecules and that can replicate in a living cell, (ii) synthetic nucleic acid molecules that are chemically, or by other means, synthesized or amplified nucleic acid molecules that may wholly or partially contain functional equivalents of nucleotides, 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 in the institution, the public, or the environment.

When conducting transgenic plant, plant pathogen, and insect pest research within a greenhouse, following prescribed risk-based containment strategies and work practices is essential to preventing the accidental release of transgenic research materials to the environment.

The basic principles of greenhouse containment are:

- Prevention of interbreeding with native species (i.e. seed and pollen containment);
- Control of insect vectors (or other agents that may spread seed, pollen, or plant pathogens);
- Containment of plants, plant pathogens, or vectors (e.g., arthropods, nematodes) that could harm local flora; and
- Decontamination/inactivation of project-generated wastes.

Section 2: Regulations, Guidance, and Oversight

National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)
The NIH Guidelines specify practices for working with recombinant DNA (rDNA) molecules, synthetic nucleic acids, and organisms and viruses that contain recombinant

DNA. The NIH Guidelines also give information about determining risk assessment, containment, work practices, and facility design. Although the NIH Guidelines are advisory and provide guidance for institutions, compliance is a condition of funding for the *entire institution*. Finally, the NIH Guidelines indicate that the ultimate responsibility for safe handling of transgenic or GM plants and plant-associated organisms lies with the PI and other persons who manage any part of the research.

Sections III-D-5 and III-E-2 of the NIH Guidelines refer to "Experiments Involving Whole Plants." These sections include guidelines for research with genetically modified whole plants and genetically modified microorganisms. Appendix P of the NIH Guidelines "Physical and Biological Containment for Recombinant DNA Research Involving Plants" specifies physical and biological containment practices suitable for greenhouse research at four different plant biosafety levels (BL-P), BL1-P through BL4-P (see **Appendix A** for BL1-P and BL2-P practices as defined by the NIH Guidelines) . As the BL-P increases, so does the stringency of physical and/or biological containment practices for GM plants and plant-associated organisms.

Permitting Agencies

If you plan to apply for any of the following permits, please contact the Biosafety Office (974-1938 or 974-9836) prior to your application submission for assistance with form preparation and implementation of specific biocontainment procedures that may apply to your work. Also, please notify the Biosafety Office if you currently possess any of the following permits.

- *United States Department of Agriculture (USDA)/Animal and Plant Health Inspection Service (APHIS)*

  APHIS is an integral part of the USDA's mission to protect agriculture in the United States from pests and diseases. Biological materials that may pose a risk to plants and/or animals or their environment are tightly regulated by APHIS. APHIS permits for working with certain plants, plant pests, and plant-associated organisms are granted by one of two agencies based on the biological material involved and the at-risk population (i.e. plants):

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

  PPQ (in cooperation with state agriculture regulatory agencies) safeguards 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 protects America's agriculture and environment using a regulatory framework 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 Biosafety

Office 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*, Bt) and novel microbes for commercial use (e.g., pollutant degrading bacteria). More information is available on these topics 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, and/or human and veterinary pharmaceuticals. However, the FDA's oversight does not apply to the research and development phases of the product(s).

- *Centers for Disease Control and Prevention (CDC)*
  Together the CDC and USDA APHIS regulate certain plant pathogens that are recognized as potential bioterrorism agents. The CDC and USDA APHIS created the National Select Agent Registry for permitting and tracking agents and toxins that may be a threat to public health and agriculture. Currently, there are eight plant pathogens listed as Select Agents (view the list at http://biosafety.utk.edu/agents). **Any person who would like to work with or store select agents will need to contact the Biosafety Office prior to attempting to obtain or work with the agents.**

Institutional Biosafety Committee (IBC)
The NIH Guidelines mandate the establishment of an IBC that reviews, approves and oversees projects involving rDNA. The University of Tennessee's IBC reviews the use of rDNA research; agents infectious to humans, animals, and plants (i.e. exotic, quarantined, and/or high priority plant pathogens, including Select Agents); venomous animals and poisonous plants (i.e. those plants causing deleterious effects to human health via dermatological exposure and/or accidental ingestion); and biological toxins. The IBC recommends policies to guide PIs in carrying out UT's Biosafety program. This committee will also evaluate personnel, facilities, and biological safety/containment procedures. Another responsibility of the IBC is to maintain documentation and communicate with the NIH, as well as other regulatory agencies.

Biosafety Office
The Biosafety Office acts as the liaison between the IBC and The University of Tennessee research community, providing training, consultation, and guidance to ensure safety for all faculty, staff, students, and volunteers working with biological materials. Additionally, the Biosafety Office is responsible for monitoring applicable federal, state, local, and institutional regulations, guidelines, and policies that impact research at The University of Tennessee.

Principal Investigator (PI)
The PI is ultimately responsible for the project and for ensuring compliance with all applicable biological safety/containment regulatory standards and guidelines. The PI (in cooperation with the Biosafety Office/IBC) determines the appropriate project-specific containment level and develops Standard Operating Procedures (SOPs). Training and oversight of personnel, and communication with the Greenhouse Coordinator and staff are

the responsibility of the PI. If the procedures involve the use of rDNA, the PI will need IBC approval before project initiation.

Greenhouse Coordinator and Support Staff
The Greenhouse Coordinator oversees day-to-day operations in the greenhouse. The Greenhouse Coordinator should be given the resources to recognize and communicate problems (or potential problems), such as loss of containment, unauthorized entry, improper work procedures, etc., to the PI and Biosafety Office/IBC.

Section 3: Plant Biosafety Levels (BL-P)
A plant biosafety level is a combination of physical and biological containment practices to reduce the public health threat to humans or animals, 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-Ps, BL1-P to BL4-P. As the BL-P increases, so does the level of protection and stringency of physical and/or biological containment practices used to minimize the impact on natural ecosystems outside the facility.

There are several issues to consider when selecting a 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 (recipient)?
  - 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? If so, 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 weedy species capable of sustaining and/or spreading the genetic modification(s) present in the area?
- 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 is a low level containment that is designated 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 and are not easily disseminated. Some examples include: plants that are not noxious weeds, plants with no potential for out-crossing 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 the 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 builds upon the practices, procedures, containment equipment, and facility requirements of BL1-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 the 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

The University of Tennessee Knoxville/ Institute of Agriculture does not have high containment facilities required for BL3-P and BL4-P work. Therefore, high containment guidance is not given in this manual. For more information on high containment greenhouse practices/procedures or facility requirements, contact the Biosafety Office.

Section 4: Containment

Facility protection is of utmost importance when attempting to maintain healthy research plants and in turn ensure that the data coming from these research plants is reliable. When planning an experiment, all the ways in which an organism can breach containment must be considered. Many factors, such as traffic flow of personnel, air flow within the facility, prevention of cross-contamination, proper labeling, and permit requirements are involved in this process.

Authorization

Applications for use of the South, Central, and North Greenhouse should be made directly to the UTIA Greenhouse Committee. The Greenhouse Committee makes recommendations directly to the UTIA Space Committee, which authorizes use. All other greenhouse space at the UTIA is managed at the department level. Please see **Appendix C** for required space request form.

Access

Access to BL1-P or BL2-P greenhouse facilities and/or bays should be limited or restricted by the PI to individuals directly involved with the experiments when experiments are in progress.

All support staff (e.g. UT Facilities Services) and external contractors must be approved by the PI and Greenhouse Coordinator. Additionally, they must be informed 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 UT 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 UT 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 detail 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 give record logs for BL1-P and BL2-P containment greenhouse bays to the Greenhouse Coordinator and the Biosafety Office at the time of decommissioning 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 Office to ensure compliance with all procedural and/or containment expectations indicated in the permit(s) (**see Appendix B**).

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 Office and/or Greenhouse Coordinator. 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 Office*. Items to look for include but are not limited to:

- 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 Office 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 will be posted at the entrance to the PIs individual greenhouse bay. The signs can be obtained from the Biosafety Office. The sign will indicate the following information: the 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 go a long way in preventing the accidental release and/or unintentional spread of plant pests and pathogens, both transgenic and non-transgenic. Basic practices and procedures include:

- Keeping greenhouse bay(s) clean and uncluttered;
- Avoiding eating and drinking in greenhouse bays with transgenic plants, recombinant plant pathogens or arthropods;
- Washing hands before leaving the greenhouse facility;
- Wearing disposable fluid-resistant gloves when handling transgenic plant material, recombinant plant pathogens, and arthropods;
- Wearing facility-dedicated or disposable lab coats/smocks when handling transgenic plant material, recombinant plant pathogens, and arthropods;
- Thoroughly inspecting street clothes/shoes for transgenic material (especially seed and/or pollen) prior to leaving the greenhouse bay;
- Observing all special containment measures such as footbaths, sticky mats, etc. when present;
- Changing 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);
- Eliminating any unnecessary equipment in greenhouse bays with transgenic plants, and recombinant plant pathogens or arthropods, especially at BL2-P (equipment in BL2-P containment greenhouse bays must be sanitized at the end of the project); and
- Prohibiting smoking throughout the UTIA Greenhouse Facilities.

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, as they are pollen vectors. In the UTIA Greenhouse Facilities, 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 require BL2-P containment, which are brought to or removed from the greenhouse facilities, must be transferred in a closed, leak proof, non-breakable container. The outside of the container must be sanitized prior to transport to ensure that transgenic pollen and seed are removed. A similar approach should be taken at BL1-P as well.

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

- 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; or
- Running the experiment when pollination will not occur outside (e.g. winter months).

Transgenic insects or mites, or unmodified insects or mites associated with transgenic plants, should be housed in appropriate containment caging systems (e.g. Bugdorms) to minimize escape from the greenhouse bay. A cheaper 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; and/or
- 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;
- Genetic attenuation of the microorganism;
- Limiting production of aerosols during inoculation;
- Ensuring adequate distance between infected and susceptible hosts;
- Chemically treating runoff water to kill microorganisms; and/or
- Using microorganisms that have an obligate association with the plant host.

Section 5: Disposal of Materials
BL1-P experimental plants and soil must be rendered biologically inactive before final disposal. These materials can be rendered inactive by desiccation, steam treatment, chemical treatment, freezing, or by a validated autoclave. 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.

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

Section 6: Containment Breach

A containment breach can occur many ways, such as weather related incidents, vandalism, and human error. There are a few steps to take if an accident results in the inadvertent release or spill of recombinant microorganisms, transgenic arthropods, and/or transgenic plants from physical containment:

- 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. 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 Coordinator, Biosafety Office, IBC, and other appropriate agencies within 24 hours, or as indicated by permit.

Section 7: 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); and
- 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 Coordinator and the Biosafety Office. A copy of the contingency plan must also be posted on (or near) the door to the affected 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.

Section 8: Abbreviations and Acronyms

APHIS: Animal Plant Health Inspection Agency
BL-P: Plant Biosafety Level
BRS: Biotechnology Regulatory Service
CDC: Centers for Disease Control
EPA: Environmental Protection Agency
FDA: Food and Drug Administration
GM: Genetically Modified

GMO: Genetically Modified Organism
IBC: Institutional Biosafety Committee
NIH: National Institute of Health
PI: Principal Investigator
PPQ: Plant Protection and Quarantine
rDNA: Recombinant DNA
SOP: Standard Operating Procedure
USDA: United Stated Department of Agriculture

Section 9: References

Animal and Plant Health Inspection Service: http://www.aphis.usda.gov/

A Practical Guide to Containment- Greenhouse Research with Transgenic Plants and Microbes http://www.isb.vt.edu/cfdocs/greenhouse_manual.cfm

Center for Disease Control National Select Agent Registry: http://www.cdc.gov/od/sap/

Environmental Protection Agency- Biopesticides and Pollution Prevention Division: http://www.epa.gov/pesticides/biopesticides/

Food and Drug Administration: www.fda.gov

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

Appendices

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. Standard Practices (BL1-P)

Appendix P-II-A-1-a. Greenhouse Access (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 BL2-P may be conducted in the greenhouse concurrently with experiments

that require BL2-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.

Appendix B: Compliance Considerations for the Use of Regulated Plant Pests, Plant Pathogens, and Noxious Weeds in UTIA Greenhouse Facilities

Plant pests, plant pathogens, and noxious weeds are responsible for significant losses in agriculture. As such, local, state, and/or federal agencies (e.g. USDA APHIS PPQ) regulate the transport, possession, and use of many of these organisms/agents. This authoritative oversight covers both genetically modified/transgenic and wild-type/unmodified organisms/agents. Institutionally, the IBC and Biosafety Office have taken the following stance regarding regulatory agency compliance:

- **It is the responsibility of the PI to ensure that all regulatory compliance standards are met**. **This includes, but is not necessarily limited to, obtaining all necessary permits for the transport, possession, and use of regulated materials.**
- If a regulatory agency has outlined specific compliance criteria that have biosafety and/or biocontainment implications for the research staff and/or UTIA Greenhouse Facilities, these must be communicated to the Biosafety Office (i.e. provide a copy of any permits or other regulatory documents to the Biosafety Office). This communication: 1) ensures that all compliance expectations are met, and 2) provides a central location for all compliance documents in the event of regulatory audits.
- Routine agency-permitted use of plant pests, plant pathogens, and/or noxious weeds will not need to be reviewed/approved by the IBC unless the research involves the use of exotic agents that could seriously damage local/domestic ecosystems (especially those listed as USDA APHIS Select Agents), organisms/agents under isolation/quarantine restrictions, or any organism/agent that could impact human health.
- The Biosafety Office may be contacted at any time if information is needed on the applicability of local, state, and/or federal regulatory requirements, the applicability of these requirements to the proposed research project(s), and general information/guidance on obtaining regulatory permits (i.e. applicable agencies, required application forms, methods of applying, etc).
- Regardless of the applicability of regulatory standards, any greenhouse experiment that will include the use of these plant pathogens, plant pests, and/or noxious weeds should be conducted in a manner that will eliminate the potential for these organisms to disseminate into the environment.

Appendix C: UTIA Space Committee Greenhouse Request Form

Please respond to all questions.

Return Electronically to Bonnie Ownley, UTIA Greenhouse Committee Chair, bownley@utk.edu

Active Research Project (Title):

Principal Investigator (Name):
UTIA FTE Appt: (%) TAES; (%) Extension; (%) CASNR; (%) Other

Co-PI(s) or collaborators:
UTIA FTE Appt:

***If assigned space*, provide individual(s) responsible for daily maintenance of greenhouse and headhouse space: name(s) & weekend & after-hours emergency contact info:**

1. Have you lost access to UTIA greenhouse space due to bridge or business incubator building construction? If yes, please indicate approximate square footage lost.

2. Do you currently use *any* UTIA greenhouse resources? Indicate square footage.

3. Do you intend to exchange your current sq ft for access to the new Greenhouse Facility?

4. Are you willing to attend a *PRIVA* climate control computer-training workshop?

5. Is this application dependent upon access to an entire greenhouse bay?

6. Are you willing to share space within a greenhouse bay? Indicate *minimum* **floor** space needed.

7. Do you anticipate investing your own research dollars to equip, modify, or enhance greenhouse spaces? If yes, explain how much, on what you plan to invest, and what proportion of that investment cannot be transported between greenhouse facilities.

8. For what duration are you requesting access to the new Greenhouse facilities?

9. Will you accept shorter access to these resources?

10. Will you be using transgenic or genetically-modified organisms? If yes, explain how you will contain GMOs and dispose of GMO material after use. Do you have an approved IBC protocol? If yes, indicate application number.

Research, Teaching and Extension Objectives & Justification*:*
RTE proportions supported by this proposal:* (%) *research;* (%) *Extension/outreach;* (%) *teaching

Please discuss the following in your submission (you may delete these lines):

- In which months do you anticipate your greatest greenhouse demand, and which months will have limited usage? What special climatic conditions do you anticipate needing?
- Explain how fulfilling the duties of your job description are contingent upon access to greenhouse facilities. Similarly, describe if access to greenhouse facilities was a negotiating point that affected your decision to accept your current job with UTIA.
- If using greenhouse space in conjunction with Teaching or Outreach objectives, explain how you intend to make use of the new Greenhouse facility.
- If necessary, explain how your research protocol(s) will require greater than average lighting and air temperature control or sequestration from arthropod pests, plant diseases, or viruses.
- Justify your Greenhouse space request for durations exceeding 1 year.
- Explain how access to Greenhouse space will improve your ability to attract competitive funding, generate new intellectual property, benefit UTIA clientele (TN, Southeastern U.S., and U.S.) or provide research advances to your field of scientific expertise.