NCCentral UNIVERSITY
Environmental Health and Safety

Plant Biosafety in Research Greenhouses

Version #1.0

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I. Purpose

This guide is intended as guidance to anyone at NCCU who works in a greenhouse with transgenic or other containment-eligible materials to provide information about the purpose of containmnet, the variety of methods to achieve it, and the facilities and practices that satisfy the requiremnets of established guidelines and regulations.

II. Definitions

Greenhouse – a structure with walls, a roof, and a floor designed an used principally for growing plants in a controlled and protected environment. The walls and roof are ussually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

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.

III. Regulations and Oversight

Transgenic plants and plant pests are subject to federal guidelines, regulations, and rules pertaining to their containment, movement, and release into the environment. As NCCU is federally funded institution that conducts biotechnology research, an Institutional Biosafety Committee (IBC) serves as the local authority. Ultimately, responsibility for the safe handling of these materials lies with the principal investigator (PI) and other individuals who manage any part of the plant research.

A. NIH Guidelines and Appendix L

Appendix L, Physical and biological containmnet for recombinant or synthetic nucleic acid molecules (rsNAM) research involving plants, of the NIH Guidelines specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving rsNAM-containing plants, plant-associated microorganisms, and small animals. If plants are grown in the laboratory in growth chambers, tissue culture rooms, or on open benches, they are regulated according to the guidelines contained in Appendix G, Physical Containment.
The plants covered in Appendix L 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.

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.

Greenhouse research is not generally subject to federal regulation.

B. U.S. Department of Agriculture – Animal and Plant Health Inspection Service (USDA-APHIS)

The USDA-APHIS has authority under the Federal Plant Protection Act to protect US agriculture from pests and disease. This authority was extentended to cover rsNAM-containing plants and other potential plant pests. APHIS also adheres to international standards created by the International Plant Protection Convention.

C. Environmental Protection Agency (EPA)

The EPA's Biopesticides and Pollution Prevention Division (BPPD) of the Office of Pesticide Programs (OPP) regulates two categories of Genetically Engineered Organisms (GEOs): Plant-incorporated protectants (PIPs, i.e., pesticidal substances produced within the plant) and genetically engineered (GE) microbes (i.e., genetically engineered microbial pesticides, that is, novel microorganisms, formed by deliberate combinations
of genetic material from different taxonomic genera, that contain or express new combinations of traits and are intended for commercial use as pesticides).

D. Food and Drug Administration (FDA)

Commercial products modified by genetic engineering for human and animal consumption, food additives, and human and veterinary drugs are subject to regulation by FDA.

E. Center for Disease and Control (CDC)

The CDC created the National Select Agent Registry program for permitting and tracking agents and toxins that may be a threat to the health of the public, animals, or plants, or to animal or plant products. The program is jointly administered by the CDC and APHIS. Currently, there are seven listed plant pathogens Select Agents and Toxins.

F. Institutional Biosafety Committee (IBC)

Any institution where research is conducted with transgenic organisms and that receives federal funding for research is required to appoint an Institutional Biosafety Committee (IBC). The Committee is responsible for maintaining and/or verifying documentation of reseach utilizing the rsNAM at the institution and acts as a point of contact for NIH and other agencies.

The IBC:

- Reviews the rsNAM research programs or proposals and evaluates the containment level designation for the proposed work.
- Ensures compliance with federal, state, and NIH guidelines by evaluating facilities, procedures, and expertise of personnel involved in the research.
- Is responsible for adopting the emergency plan for responding to breach of containment.
- Facilitates the timely disposal of experimental materials.

G. Principal Investigator (PI)

- Is ultimately responsible for the research project and for ensuring compliance with biosafety standards.
- Functions as project manager as well as researcher.
- Bears responsibility for training and supervising personnel.
- Communicates with the IBC, regulators, greenhouse manager and support staff, and corrects any operations that may result in a loss of containment.
- Recommends a containment level designation for the project and, in accordance with the NIH Guidelines and/or APHIS requirements, develops the necessary containment protocols.
- Is responsible for all APHIS-regulated materials.
- Must register all experiments using GEO material with the IBC (see Section III of the NIH Guidelines).

H. Greenhouse Staff

Regardless of individual duties, all staff should become familiar with the containment requirements of the ongoing research in greenhouse. Both, the greenhouse manager and the PI should work with the staff to ensure compliance with safety procedures and standards.

IV. Biosafety Levels

Biosafety levels provide a description of combination of administrative controls, work practices and procedures, equipment, and facility features required to achieve a designated level of containment. The purpose of containment is to avoid unintentional transmission of a rsNAM-containing plant genome, including nuclear or organelle hereditary material or release of rsNAM-derived organisms associated with plants from inside the greenhouse to receptive environments outside the greenhouse. The containment priciples are based on the recognition that the organisms that are used pose no health threat to humans or higher animals.

Section III of the NIH Guidelines describes the four physical containment levels for experiments involving

rsNAM. Appendix L of the NIH Guidelines categorize experiments for rsNAM research involving plants according to specific risk criteria. Based on this criterias, experiments may be assigned to one of four biosafety levels, BL1-P through BL4-P. The Guidelines also specify the physical and biological containment conditions and practices required for greenhouse experiments for each biosafety level.

To make a biosafety level assignment, consider the following:

- Source and nature of the introduced rsNAM
  - exotic infectious agent or pathogenic organism
  - fragment of DNA or complete genome
- Recipient organism
  - mode and ease of dissemination
  - invasivness
  - noxious weed or capable of interbreeding with noxious weeds
  - potential for outcrossing between recipient organisms and related species
  - potential for detrimental impact on natural and managed ecosystems
- Nature of expressed protein
  - vertebrate toxin or potential or known allergen
  - toxic to other organisms in local environment
- Local environment
  - nature and importance of nerby crops
  - presence of sexually compatible wild or weedy species
- Experimental procedures
  - transport to or from greenhouse
  - necessary containment measures

A brief comparison of criteria used to assign an appropriate biosafety level is shown in Table 1.

Table 1. Suggested criteria for assigning biosafety levels

CRITERIA	TRANSGENIC PLANTS	TRANSGENIC MICROBES		TRANSGENIC ARTHROPODES AND THEIR MICROBES
		Exotic	Non-Exotic	
Not a noxious weed or cannot outcross with one	BL1-P			
Not easily disseminated			BL1-P	
No detriment to environment		BL2-P or BL1-P+	BL1-P	BL2-P or BL1-P+
Noxious weed or can interbreed with weeds	BL2-P or BL1-P+			
Contain complete genome	BL2-P or			

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of non-EIA(1)	BL1-P+			
Contains genome of EIA	BL3-P or BL2-P+			
Detriment to environment			BL2-P or BL1-P+	BL3-P or BL2-P+
EIA with detriment to environment	BL3-P or BL2-P+			
May reconstitute genome of infectious agent *in planta*	BL3-P or BL2-P+			
Contains vertebrate toxin	BL3-P	BL3-P	BL3-P	
Select Agent plant pathogens	BL3-P	BL3-P	BL3-P	BL3-P+ or BL4-P

[1]EIA – Exotic infectious agent

A. Biosafety Level 1 for Plants (BL1-P)

BSL-P1 is recommended for all experiments with transgenic plants and associated agents in which there is no evidence that the modified organism would be able to survive and spread in the environment and, if accidentally released, would not pose an environmental risk. For example: transgenic plants that are not noxious weeds or agents that have no recognized potential for rapid dissemination. A BL1-P designation would be assigned, for example, to an experiment that uses a transgenic strain of *Rhizobium* containing *Agrobacterium* genes known to affect root colonization, or plants using *Agrobacterium* DNA segments as part of the transformation process.

Requirements at BL1-P include:

- Access to the laboratory and greenhouse is limited or restricted when experiments are in progress.
- Prior to entering the greenhouse, personnel are required to read and follow instructions on BL1-P greenhouse practices and procedures.
- All procedures must be performed in accordance with accepted greenhouse practices appropriate to the experimental organism.
- Records are kept of experiments currently in progress in the greenhouse facility.
- Experimental organisms are rendered biologically inactive by appropriate methods before disposal.
- 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.
- The greenhouse floor may be composed of gravel or other porous material. Impervious (e.g., concrete) walkways are recommended.
- Windows and other openings in the walls and roof of the laboratory and 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). Screens are recommended.

- Laboratories and greenhouses must be locked when unoccupied. All agents must be secured against accidental exposure, unauthorized use, and theft. All rsNAM must be stored in locked containers.

B. Biosafety Level 2 for Plants (BL2-P)

Recommended for transgenic plants that are noxious weeds, plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent, plants associated with transgenic non-exotic microbe that has a recognized potential for serious detrimental impact on managed or natural ecosystems, or plant pathogens that have a recognized potential for serious detrimental impact on managed or natural ecosystems. Examples of agents worked with at BL2-P include: *Meliodogyne incognita* (root-knot nematode), *Pepino mosaic virus* (PepMV), *Pectinophora gossypiella* (Pink bollworm), and *Pseudomonas syringae*.

Additionaly to BL1-P, requirements at BL2-P also include:

- A program 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.
- A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.
- If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their preence shall be indicated on a sign posted on the greenhouse access doors.
- If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.
- A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagates of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagates of experimental organisms are readily disseminated through soil.
- Materials containing experimental microorganisms must be transferred in a closed, leak proof, non-breakable container.
- An autoclave must be available for the treatment of contaminated plant material including soil.
- A greenhouse practices manual shall be prepared and adopted. The manual should (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.
- If part of greenhouse is compossed of gravel or similar material, appropriate treatments should be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel.

- 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.
- 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.
- Laboratories and greenhouses must be locked when unoccupied.
- All agents must be secured against accidental exposure, unauthorized use, and theft.
- All recombinant nucleic acids and BL2-P agents must be stored in locked containers.
- All material in the open bay or common use areas must be secured when not in use.
- PI needs to report any greenhouse accident involving the inadvertent release or spill of microorganism to the IBC, NIH OSP, and other appropriate authorities (if applicable) immediately.

V. Strategies of Containment

Almost any organism, from microbes to whole plants, can be easily transported into and out of a containment facility in a multiple ways:

- Transmission of organisms on personnel and their clothing, shoes, and personal items
- Poor adherence to prescribed protocols
- Air current created when passing through doorways
- Small animal intruders (birds, rodents, insects, etc.)
- Irrigation and waste water
- Ventilation air currents
- Material handling equipment and their maintenance
- Escape of research organisms and cross-contamination of other experiments within the facility

Appropriate selection of biological containment pratices need to be used to meet the containment requirements for given organism.

A. Biological Containment Practices (Plants)

Effective dissemination of cross-pollination plants (not self-pollination) by pollen or seed can be prevented by one or more of the following:

- Cover the reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity.
- Remove the reproductive structures by imploying male sterile strains, or harvest the plant material prior to the reproductive stage.

- Ensure that experimental plants flower at a time of year when cross-fertile plants are not flowering within the normal pollen dispersal range of the experimental plant.
- Ensure that cross-fertile plants are not growing within the pollen dispersal range of the experimental plant.

VI. Management Practices

Before entering the greenhouse, all staff working around the organisms of interest should be fully informed of containment measures applicable to a given research project. Prescribed procedures and practices should be appropriate for the assigned biosafety level.

A. Access

Routine access to facilities housing confined research material is restricted, regardless of the biosafety level to minimize the spread of pollen, seed, or other propagative material that could be carried by people moving between rooms or facilities.

- At BL1-P, access is limited or restricted at the discretion of the greenhouse manager or PI when experiments are in progress.
- At BL2-P, the manager is required to limit greenhouse access to individuals directly involved with the experiments.
- Discretionary access is generally reserved for maintenance personnel.
- Visitors who have a special interest in the research are escorted.

If the greenhouse consists of one large room access may need to be restricted; all authorized personnel should have access to a key or key card to enter. Signs must be posted at the entryways, indicating that access is restricted for the research program in progress. These signs may also contain access instructions.

B. Apparel and Hygiene

Personnel entering BL1-P and BL2-P facilities may generally wear their usual street or lab clothing. However, lab coats that remain at the facility are recommended and often required. It is important that no personal items such as backpacks, coats, or purses be brought into containment facilities without good reason, as they may allow pests to 'hitchhike' out. Special care should also be taken to ensure footwear do not convey organisms from the facility. Eating, drinking, and smoking should be prohibited. Wearing disposable gloves is encouraged upon entry to the facility or when handling live material. Hands should be washed carefully when leaving greenhouse.

C. Signage

- No special signs are required for BL1-P containment greenhouses.
- Entryways into BL2-P and higher facilities should be posted with signs indicating that access is limited to authorized personnel only. If the experiment uses organisms that pose a risk to the local ecosystem or agriculture, a sign so stating must be placed on the access doors to the greenhouse.
- The sign should state the name and contact number of the responsible individual(s), the plants in use, and any special requirements for using the area. It may include contact information for the greenhouse manager and others (e.g., EHS, University Police, Work related injuries) to be called in case of emergency.
- Universal biohazard symbol should be posted, if there is a risk to human health.
- Signage used to identify emergency exits is required as per standard building codes.
- Transgenic material in a greenhouse room must be marked to distinguish it from non-transgenic organisms, such as plants serving as experimental controls or not involved with the experiment.
- It is recommended that GEOs have a designated boundary on the bench, e.g., using color-coded markers. In addition, individual pots, bench sections, or entire benches can be marked with stakes or signs to identify the plant and the primary genetic modification.
- All organisms in the room must be treated in accordance with the highest level of containment required by any experimental material present.

D. Storage and Handling

- Plant parts, cultures, whole plants, and seeds are routinely stored and manipulated in containment facilities.
- Coolers, freezers, and growth chambers equipped with locks are recommended for storage.
- Transgenic seed should be stored in a locked cabinet located preferably in a greenhouse room to minimize handling in unconfined spaces, and should be clearly identified and labeled to distinguish it from other stored seeds or materials in the cabinet.
- Seed that is stored or handled outside the area of containment, such as in a cabinet or on a potting bench in a headhouse corridor, should be kept in a spill-proof container.
- Seed counters, and related equipment used to process seed should be easy to thoroughly clean. For some operations, dedicated equipment may be required to ensure that mixing between runs or trials does not occur.
- All waste material and unused seed should be decontaminated appropriately for the risk involved.

E. Transfer of Materials

The NIH Guidelines specify requirements for transporting experimental materials to and from greenhouses

for levels BL2-P – BL4-P.

- For facilities designated BL2-P and higher, transgenic material in the form of seeds or propagules, potted plants, trays of seedlings, etc. must be transferred in closed nonbreakable containers.
- Special consideration is given to opening and handling of incoming packages. The material is generally moved inside tissue culture equipment, growth chambers, or greenhouses after being opened in a biological safety cabinet or sleeved cage within the containment area.

F. Sterilization, Disinfection, and Disposal

To prevent the survival of organisms unintentionally transported outside the greenhouse environment, experimental materials must be rendered biologically inactive before disposal. PI is responsible for proper termination and disposal of all material.

Plants and associated organisms can be inactivated by several methods:

Autoclaving
An autoclave shall be available for the treatment of contaminated greenhouse materials for BL2-P level. Material from smaller experiments can be inactivated by autoclaving all plants, plant parts, containers, and potting media. The recommendation is to autoclave materials at 15-30 psi and 121 °C for 15 – 180 minutes, depending on the type and state of the material being sterilized. Liquids after being sterilized must be cooled before it enters the sewer.

Chemical treatment
Containment laboratories may use common disinfectants such as sodium hypochlorite, phenols, formaldehyde, glutaraldehyde, and alcohol. The gravel under benches in BL2-P facilities can be decontaminated by, for example, treatment with a 10% sodium hypochlorite (household bleach). Periodic cleaning of all growing area surfaces with standard cleaning solutions or plain soap and water is highly recommended.

Freezing
Freezing is a common method for killing adult arthropods but has limited use as a sterilant.

Composting
For large volumes, composting is an acceptable treatment for experimental plant and soil materials that pose no recognized harm to the environment.

Desiccation
Plants without seeds can be devitalized through desiccation simply by withholding water, or they can be chopped or minced into pieces unable to grow independently under natural conditions.

Incineration
Incineration may also be used to destroy easily combustible, dry plant material; however, incineration must

be used with caution since not all seeds are easily burned.

Regardless of the method, decontamination must be appropriate for the organisms of interest. Time and temperature criteria for the targeted organisms, autoclave test strips, and equipment maintenance and testing are needed for validating termination methods. Materials can be disposed with confidence once decontamination is validated.

G. Pest Control

The NIH, APHIS, and other guideline sources require a pest control program when working with contained organisms in a greenhouse setting.

- Screens are recommended for BL1-P and required for BL2-P to exclude pollinating insects and birds.
- BL2-P facilities must have louvers fitted on exhaust fans that are open only when fans are running.
- The perimeters of greenhouses of every containment level should be sealed to prevent rodents and other large pests from entering.
- Fumigation or spray application of pesticides can be used to control certain insect pests such as whiteflies.
- Routine cleaning between experimental run with hot water and detergent applied with a power washer is a very effective method for reducing pest populations.
- 'Baking out' greenhouse rooms by raising the room temperature to 40 – 45 ºC and holding for two to three days is a common practice to reduce pest loads.

Protocols should be instituted to avoid the transmission of microbial pathogens both within the greenhouse and to the outside environment. For example, Tobacco Mosaic Virus (TMV) can be spread easily by handling susceptible plants.

H. Training and Reference Manuals

The staff is required to read, comprehend, and agree to adhere to the instructions provided in the Lab-Specific Safety Plan or in the project document submited and approved by IBC before entering the greenhouse. Personnel training is best accomplished through interactive sessions that include the PI and greenhouse manager.
For BL2-P, PI is required to develop emergency and contingency plans, as well as documents pertaining to routine operations, which should be available to all involved in the research.

I. Monitoring Containment

Escaped organisms may be detected by placing susceptible host plants, insect traps, or spore/pollen-catching devices both inside and outside the containment area. Traps and sentinel bioindicator plants can be used to detect unintended virus transmission, insect migration, and pollen or spore spread.

J. Procedures for Loss of Containment

The integrity of the containment facility is susceptible to equipment malfunctions, acts of nature, such as fire, flood, and storm damage, and human error. A loss of BL1-P containment due to any of these factors would likely have only minor environmental consequences, if any, and would not require a response. At BL2-P or higher, such events would present larger concerns.

For BL-2P and higher facilities, both APHIS and the NIH Guidelines require contingency plans for handling emergency situations, including theft or vandalism. These plans, drawn up by the IBC in consultation with the PI, must include measures to contain the breach, a personnel notification sequence, and decontamination procedures. In addition, the plans should include names and contact information for repair personnel, researchers, relevant authorities, and greenhouse staff.

Should an inadvertent release of transgenic material at BL2-P occur, the Principal Investigator must immediately report the incident in writing to the EHS (Hazard and Incident Report Form; 919-530-7125), the greenhouse manager, the Institutional Biosafety Committee, the NIH Office of Biotechnology Activities, and/or other designated authorities.

K. Records

- Records of experiments in progress must be kept for all biosafety levels.
- At BL2-P and higher, additional records must be kept of all plants and plant-associated organisms entering or leaving the greenhouse.
- Greenhouse manager has responsibility for entry and exit logs when required.
- Records must be available to greenhouse staff and inspectors.

L. Inspections

- Greenhouses should be inspected periodically to ensure that containment measures appropriate for transgenic and other organisms are rigorously applied.
- Inspections should be conducted on a regular schedule and whenever new types of experimental materials are brought into the facility.
- Inspectors may include the greenhouse manager, EHS, IBC representative, or state agriculture officials.

See Appendix A for a Greenhouse Checklist.

M. Standard Operating Procedures (SOP)

SOPs need to be developed, annualy updated, modified, and implemented as needed. SOPs should include the following activities:

- Use, maintainance, and disinfection of the facility and its equipment

- Response to emergencies
- Maintainance of security
- Management of visitors
- Response and handling of a containment breach
- Replacement of glazing in glasshouse

VII. Green Tobacco Sickness

A. Health Hazards Associated with Tobacco Leaves Handling

Nicotine exposure from handling tobacco leaves may cause nicotine poisoning, also called Green Tobacco Sickness (GTS), with symptoms including nausea and vomiting. Although GTS symptoms normally do not last more than 24 hours after workers stop handling tobacco leaves, there are currently no comprehensive studies evaluating long-term effects. Risk of nicotine poisoning increases when the nicotine contained in the tobacco leaves mixes with rain, dew, or sweat, allowing nicotine to get onto the skin and pass into the bloodstream more easily. New workers may have a lower tolerance to nicotine exposure than previously exposed workers. They are also less likely to know about GTS and protection methods, emphasizing the need for their employer to provide adequate training.

B. Reducing Nicotine Exposure

- Provide training to each tobacco plant handler on how to use PPE. Training should include information about how clothing can be used as PPE and how some clothing may no longer provide adequate protection if it becomes wet.
- Train tobacco plant handler to recognize GTS signs and symptoms and to alert supervisors if they develop symptoms or notice any other workers exhibiting symptoms.
- Train supervisors to ensure that any handler with GTS symptoms immediately drinks water and rests in the shade, in addition to receiving medical attention if necessary.

C. Personnel Protective Equipment

Gloves, long sleeve shirts, long pants and water-resistant clothing are recommended to prevent exposure to nicotine from tobacco leaves.

Gloves will protect workers handling tobacco leaves from nicotine absorption through their hands. If gloves are wet or compromised, they become less protective.

Long Sleeve Shirts and Pants protect handlers from nicotine absorption if tobacco leaves come into contact with their arms or legs. However, once the clothing becomes wet, it no longer provides adequate protection and may increase absorption risk.

Water-Resistant Clothing (e.g., rain suits) keeps dew or rain, which had contact with tobacco leaves, from contact with skin and clothing to protect handlers from exposure to nicotine contained in the water.

Washing with soap and water immediately after tobacco leaves handling is recommended to reduce exposure to nicotine. Washing can reduce the amount of nicotine that is on skin by 96%.

D. Additional Resources for Green Tobacco Sickness

- Brochure on Green Tobacco Sickness – North Carolina Department of Labor.
- Health Education on Green Tobacco Sickness – North Carolina Department of Health and Human Services.
- NIOSH website on Green Tobacco Sickness, which includes links to materials for supervisors and workers.

VIII. Appendix A Greenhouse Checklist

Principal Investigator: Click or tap here to enter text.

Location/Building/Room: Click or tap here to enter text.

Greenhouse Checklist	Yes	No	N/A	Comments
Access to the greenhouse is limited or restricted at the discretion of the Principal Investigator or laboratory supervisor	☐	☐	☐	
Personnel receive appropriate training on standard operating procedures, potential hazards associated with the work, and the necessary precautions to prevent incidents	☐	☐	☐	
All procedures are performed in accordance with accepted greenhouse practices	☐	☐	☐	
All plant pathogens utilized in the greenhouse are of domestic origin	☐	☐	☐	
Experimental organisms (e.g., transgenic plants, etc.) are rendered biologically inactive by an appropriate method (e.g., autoclaving or chemical treatment) prior to disposal	☐	☐	☐	
Other materials (e.g., pots, soil, etc.) used to conduct research involving plant pathogens or recombinant materials are decontaminated upon completion of research	☐	☐	☐	
Materials decontaminated outside of the facility are transported in durable, leak-proof, closed containers (e.g., plastic bags transported in tray or pan with a leak-proof bottom)	☐	☐	☐	
A program which controls undesirable species (e.g., weeds, arthropod pests, and pathogens) is in effect	☐	☐	☐	
Greenhouse is located away from public spaces	☐	☐	☐	
Greenhouse has lockable doors for access control	☐	☐	☐	
Posted signage includes researcher's name and emergency contact information	☐	☐	☐	

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Spill clean-up procedures are developed and posted as applicable	☐	☐	☐	
Greenhouse has impervious walkways (e.g. concrete); greenhouse floor may be composed of gravel or other porous material	☐	☐	☐	
Open windows and other openings in the walls and roof of the facility are screened to prevent entrance of arthropod vectors as applicable	☐	☐	☐	