Plant Pathology Series No. 137

Working with Permitted Plant Pathogens, Insect Vectors, Nematodes, Human Pathogens and Recombinant Microorganisms in Plant Pathology Selby Hall APHIS Facility 2135

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II. INTRODUCTION

The objective of the Animal and Plant Health Inspection Service (APHIS) is to protect the health and value of American agriculture and natural resources. APHIS uses its umbrella of protection to assure its customers and stakeholders that it is rigorously monitoring against the introduction or re-emergence of both animal and plant pests and diseases that could limit production and damage export markets. Plant pests and pathogens are monitored and regulated by APHIS through the use of Plant Protection and Quarantine (PPQ) permits and facility inspections. Any organism that is under regulatory authority of the USDA-APHIS for the importation, transit, and/or domestic movement of the organism will be referred to as a "permitted organism" in this document.

Over the past 20 years the Department of Plant Pathology's (Wooster) need to work with pathogens that are restricted has increased significantly. In 2010, the Department of Plant Pathology (Wooster) was selected by USDA-APHIS to participate in a pilot program for the establishment of USDA-APHIS-PPQ Super Containment facilities. The objective of this document is to provide both a departmental general operating procedure (GOP) and standard operating procedure (SOP) for each pathogen type (viruses, bacteria, fungi, oomycetes and insect vectors) that describe how APHIS-permitted organisms will be handled by all members of the department. A SOP has also been included describing how plants infected with biosecurity level 2 (BSL-2) bacteria and viruses will be handled. The pathogen SOPs provided in this document are not specific to any species. Therefore, SOPs may need to be modified by the permittee to accommodate conditions requested by APHIS for specific pathogen species. This document also includes information about the facilities in Selby Hall and pertinent APHIS and Ohio State University – Institutional Bio-safety Committee (OSU-IBC) resources.

III. FACILITIES

Selby Hall is listed by USDA, APHIS as Facility 2135 (previously 324). Selby Hall is a concrete block, brick-faced building located on the campus of the Ohio Agricultural Research and Development Center, The Ohio State University, 1716 Wilson Road (Appendix XIV, plate 1). Floors in Selby Hall are tile-covered cement. There is an attached head house area extending north from the ground floor, with four greenhouse ranges accessed from a central corridor (Appendix I.D). The building houses the Department of Plant Pathology, part of the USDA-ARS Corn, Soybean and Wheat Quality Unit and the Molecular and Cellular Imaging Center (MCIC). The campus is located at 1680 Madison Avenue, Wooster and is one mile south of Wooster on State Route 83. The building is open to OARDC employees and the public during operating hours (M-F 8:00 am - 5:00 pm; 7:30 am - 4: 30 pm in the summer). Individual rooms, laboratories and the containment greenhouse are accessed through lockable doors. External doors are locked at all times outside of working hours.

A. Selby Hall Laboratories and Rooms Used for Permitted Organisms

1. Floor Plans

   Floor plans for ground, first and second levels of Selby Hall are shown in Appendix I.A-C. Grey shading indicates rooms where permitted organisms are received, cultured, manipulated or stored. The individual Principal Investigator (PI) or personnel responsible for each room and the equipment available in each room and activites performed are listed in the table in Appendix III.

2. Signage

   a. All rooms where permitted organisms are received, cultured, manipulated or stored will have OSU biohazard signs (Appendix XII) with emergency contact information posted on the outside door. Restricted entry signs will also be posted on all entry doors where permitted organisms are received, cultured, manipulated or stored.

   b. All equipment used to handle permitted organisms will have OSU biohazard sticker attached to it. The sticker will be visible to the handler. Stickers can be obtained from the Containment Director (CD).

B. Selby Hall Phytotron

1. Growth chambers

   a. Lockable growth chambers (GC) are located near the Selby Hall head house in room 035 (Appendix I.E; Appendix XIV, plates 2-5).

b. Access to 035 is from one exterior door and three interior doors connecting to the head house. (Appendix I.E; Appendix XIV, plate 3).

c. The phytotron has twenty growth chambers of various sizes.

d. Two growth chambers (GC14 and GC19) have been authorized by the OSU Safety Committee for use with human pathogens in biohazardous risk group 2 (BSL-2). The growth chambers are clearly marked with OARDC approved biohazard signage (Appendix XIV, plate 4).

e. Growth chambers are locked at all times and used according to PPQ permit conditions and OSU-IBC requirements.

f. Insects are controlled within individual chambers as needed or as specified by the PPQ permit or OSU-IBC requirements.

2. Insect rearing growth chamber

a. Growth chambers used for insect rearing contain sealed light fixtures to prevent the accidental escape of insects and yellow sticky insect traps to monitor for insect escape.

b. Yellow sticky insect traps hang from the two interior doors of the Phytotron area (035) to trap any insects that may escape.

c. Growth chambers containing permitted organisms are locked at all times.

C. Selby Hall General Use Greenhouses (pathogens and plants not requiring permits)

1. The greenhouses are located off the north side of Selby Hall. Access can be made from the Selby Hall head house (Appendix XIV, plate 5), an exterior garage door (Appendix XIV, plate 6) or seven side door entrances (Appendix XIV, plate 7).

2. The exterior garage door and the entrance from the Selby Hall head house are unlocked during operating hours, when greenhouse personnel are present.

3. All side door entrances remain locked during operating hours and are only accessible to authorized personnel carrying a departmental key.

D. Selby Hall Containment Greenhouse

1. Floor plan

a. A schematic of the floor plan is shown in Appendix II. Mechanical plans and blueprints are not available.

b. The Containment Greenhouse (CG) contains two units: G1 and CG2. Access to both units is through a lockable door (marked as X on the floor plan, Appendix II). Access to G1 is through a locked screened door (Appendix XIV, plate 8) and contains greenhouse (G) rooms G101, G102, G103, G104, G105, G106, G107, G108 and G109. Each room has its own separate entrance (Appendix XIV, plate 9). Access to CG2 is from G1 via a locked door (Appendix XIV, plate 10) and contains rooms CG110, CG111, CG112, CG113, CG114, CG115, CG116, CG117, CG118, CG119, CG120, CG121 and CG122. Each room has a separate entrance. An air lock entry separates G1 and CG2. Unless otherwise noted, permitted organisms are limited to CG2. There is an emergency exit exterior door in CG2 (Appendix XIV, plates 12 and 13), which cannot be opened from the outside to prevent unauthorized entry.

2. Physical containment standards

a. The location is at 1716 Wilson Road on the campus of the Ohio Agricultural Research and Development Center. It is several hundred yards from state routes 83 and 302, one mile from US highways 30 and 250, and 14 miles from interstate highway 71 (via State Route 83).

b. The location is more than 500 yards from commercial production fields.

c. The location is more than 300 yards from OARDC research plots.

d. The location is more than 40 miles from a commercial international airport.

e. The north, east and west sides are open (no barrier) and the south side is the greenhouse corridor (Appendix, plate 14).

3. Structural description

a. Exterior walls are poured concrete approximately 3 ft. high on the top of which are mounted panes of translucent 0.5 in.-thick polycarbonate panels (Appendix XIV, plate 15).

b. Ceilings consist of 0.5 in.-thick polycarbonate panels.

c. Corridor and greenhouse room floors are poured concrete. (Appendix XIV, plate 16).

d. Exterior and corridor walls are translucent, 0.5 in.-thick polycarbonate panes or panels.

e. Heating is provided by fin-tube radiators located on the outer walls of individual rooms (Appendix XIV, plate 17).

f. Ventilation is provided by an outside air intake through a north-facing ridge vent in the central corridor (Appendix XIV, plate 18).

g. Incoming air passes through two layers of antivirus screen (0.009 in., 1:1 ratio, 0.0105 in. x 0.0502 in.) (Appendix XIV, plate 19). Air is drawn into the individual rooms by circulation/exhaust fans located on the outside walls of the rooms (Appendix XIV, plate 20). Air entering the room passes through a thick fiber water percolation pad that usually has water flowing through it during the growing season (Appendix XIV, plate 21). Exit plenums for air exhausted from the greenhouse compartments are attached to the circulation/exhausted fans and are covered with a single layer of antivirus screen for CG2 compartments (Appendix XIV, plate 22). No screens are present for the exit plenums of G1 (Appendix XIV, plate 23). The CG does not operate under negative pressure.

h. Electricity is provided to each room to operate room lights, plant growth lights and fans for the air circulation and exhaust system.

i. Hydrants are available in each room to provide water for plants. Open drains to city sewer system are indicated as open circles (O; Appendix II). Closed circles (●) are blocked (inactive) drains (Appendix II; Appendix XIV, plate 24). Unless described otherwise in specific permits, water draining from pots moves to floor drains and the city sewer system (Appendix XIV, plate 25).

j. All benches in the G and CG are constructed of a thick aluminum metal mesh attached by aluminum legs and supports. (Appendix XIV, plate 26).

k. A record of maintenance and repairs within G1 and CG2 is maintained by the greenhouse manager and can be provided upon request.

4. Signage

a. "Authorized Personnel Only" is posted on the entry door to G1 and CG2.

b. An illuminated exit sign is posted on the CG2 emergency exit.

c. The PI and the name and extension number of the contact person for the existing project are posted on the room doors.

d. Special instructions for the existing project are posted on the room doors.

5. Disposal of Contaminated CG2 Materials

a. Whether used under permit or not, all soil, plant material and miscellaneous lab materials (gloves, stakes etc.) leaving CG2 will be contained within labeled (project PI and date) clear autoclave bags and steam-sterilized in an A.K. Robins 240EF autoclave at (15-18 psi for a minimum of four hours) prior to disposal.

b. Used pots and collection trays are soaked in a 27% sodium hypochlorite solution for five minutes. Pots will be completely dried prior to use.

6. Disinfestation of CG2 Rooms

a. Upon completion of a project or expiration of the pathogen permit, materials in each room are disposed of according to the methods described in III.D.5.

b. The greenhouse manager will disinfest all rooms as soon as all materials are removed from the room.

c. Benches, interior and exterior doors, door handles, and garden hoses and nozzles are sanitized with a Clorox bleach (1:1) treatment. Rooms may be re-occupied after a minimum of four hours drying time.

IV. GENERAL OPERATING PROCEDURES

A. Standard Operating Procedures (SOP)

1. A standard operating procedure will be made for each permitted organism or recombinant microorganism.

2. Guidelines for developing the SOPs for each pathogen group can be found in section V of this document.

3. The PPQ permit number will be included on the first page of the SOP of the permitted culture.

4. OSU-IBC protocols will be attached to the SOP.

B. Inventory

1. All permitted or recombinant cultures will be entered into the permittee's laboratory culture inventory upon receipt.

2. A description of the location where the cultures are stored and maintained will be attached to the SOP of the permitted culture.

3. All cultures will be removed from the laboratory culture inventory upon culture disposal.

C. Labeling and tagging

1. Stored cultures will be clearly labeled with the pathogen identity, strain or isolate number and the date of storage.

2. Plant material infected with a permitted culture will be clearly labeled with the date of infection, the pathogen identity and the strain or isolate number.

3. Growing permitted cultures will be clearly labeled with the pathogen identity, strain or isolate number and the date of culturing.

D. Autoclaving waste

1. There are five autoclaves within the department. Two are located in room 135 (A and B), two in room 222 (A and B) and one in the head house (Appendix XIII, plates 37-40). Autoclaves housed in rooms 135 and 222 are maintained by the departmental building coordinator. The greenhouse manager maintains the autoclave located in the head house.

2. The internal temperature of both autoclaves in room 135 and autoclave A in room 222 is checked at least two times per year under the direction of the Building Coordinator and once per year by a Preventive Maintenance Medical, Inc. (307 Harcourt Road, Mount Vernon, Ohio 43050; Phone: 1-800-295-3030; Fax: 1-740-397-9793; PMmedical@core.com; www.preventivemaintenancemedical.com; Service Representative: Bob Baker: 740-501-2800). ***Only these autoclaves should be used to destroy permitted materials, and headhouse autoclave to destroy plant material inoculated with permitted materials.*** Autoclaves that are not to be used for permitted cultures are clearly signed (Appendix XIV, plate 39).

3. The internal temperature of the A.K. Robins 240EF autoclave in the head house is checked at least two times per year with a biological indicator test under the direction of the Building Coordinator. ***This autoclave is used for destruction of plants inoculated with permitted materials, in conjunction with autoclave log.***

4. Waste must be autoclaved in clear autoclave bags at 121°C (250 °F) for a minimum of 30 minutes or according to the conditions described in the PPQ permit or OSU-IBC protocols.

5. *Plant material will be steam-sterilized in the A.K. Robins 240EF autoclave located in the head house only.* Plant material will be autoclaved for a minimum of 4 hours (15-18 psi) or according to the conditions described in the PPQ permit or OSU-IBC protocols.

6. All clear autoclave bags will be closed using sturdy twine and clearly marked with the date and name of the PI.

7. Autoclave tape or other indicator must be placed on each bag or sharps container prior to treatment. The autoclave tape or other indicator on each container must be checked to verify color change before disposal into the dumpster for sanitary landfill disposal. If the autoclave does not attain the minimum time and/or temperature or the autoclave tape does not change color, a notation must be made in the comment section of the autoclave log. The load must then be re-autoclaved after placing new tape or indicator on the material. If minimum time and temperature is not attained on the second cycle, users must contact the person responsible for maintaining the unit to initiate repairs. Waste must then be treated at the alternate autoclave facility.

8. All employees must complete the autoclave log form (Appendix IX) for all permitted materials. All parameters must be noted as listed on the log for each autoclave load.

E. Culture storage and disposal

1. Cultures or plant material initially received will be opened in containment Class II BSC A/B3 laminar flow hoods available in rooms 029 and 232 or Class II A2 laminar flow hood in room 134 (Appendix XIV, plates 41 and 42). The hoods are certified

annually by Laboratory Certification Services, Inc. (1171 Chesapeake Ave., Columbus, OH 43212; 1-800-800-7105).

2. Disposable packaging will be destroyed by autoclaving and all workspace will be surface sterilized with 70% ethanol.

3. All cultures or plant material will be stored long-term in either -80 ºC freezers located in rooms 028B, 134, and 232 (Appendix XIV, plate 43) or in liquid nitrogen storage located in room 005 (Appendix XIV, plate 44) within the MCIC facility.

4. Storage rooms and/or freezers will remain locked at all times and signs are posted indicating that doors should remain closed and locked at all times.

5. After completion of experiments or when the PPQ permit expires, all cultures or plant material will be destroyed by autoclaving or by the method indicated in the PPQ permit.

6. Autoclaved cultures and materials ***not*** containing human pathogens will be disposed of in the OARDC dumpster located outside of the Selby Hall soil room (Appendix II.D, position marked with a star).

7. Cultures and materials ***containing human pathogens*** will placed into a burn box containing two red plastic infectious waste bags. The boxes must be sealed with at least two layers of packing tape (NOT masking tape) and requested to be picked up by EHS at the Ohio State University.

8. All employees must complete the autoclave log form (Appendix IX) or all permitted materials. All parameters must be noted as listed on the log for each autoclave load. Filled autoclaved log forms will be retained by the BC for one year.

F. Personnel training

Prior to working with permitted or OSU-IBC regulated organisms:

1. All personnel working within laboratories using permitted pathogens will be trained by the PI or by the person named on the PPQ permit and/or OSU-IBC protocols.

2. Personnel working with permitted pathogens will read the conditions attached to the PPQ permits and/or OSU-IBC protocols used.

3. All laboratory personnel will read the SOP for the PPQ permit and/or OSU-IBC regulated organisms located in the PI's laboratory and sign a form (Appendix VII) indicating that they read and understand the bio-safety regulations for of the permitted pathogen and the conditions of the PPQ permit and/or OSU-IBC protocols.

4. Only trained personnel or those persons under the direct supervision of trained personnel will enter the long-term storage rooms, CG2 or the insect rearing/transfer facilities.

G. Containment Director (CD)

1. The CD is responsible for monitoring the containment rooms where the permitted organisms in Selby Hall are stored.

2. The authors of this document, in collaboration with the CD, will maintain and update the department SOP manual.

3. The CD will provide a copy of the department SOP manual to all employees working with PPQ permitted organisms and/or OSU-IBC regulated organisms.

4. The CD will maintain copies of all active PPQ permits and/or OSU-IBC protocols held by permitees in Selby Hall. *Permitees will be responsible for providing copies of all current permits to the CD.*

5. The CD will maintain contact information for all permitees within Selby Hall.

6. Contact information for the current CD can be found in Appendix IV.

H. In the case of emergency or unintentional Release

1. In the event of an unintentional release of permitted organisms CG2 and G1 will be immediately secured. The first course of action will be to prevent any further release of the organisms. See Appendix VI for more details of the procedures to follow.

2. In case of fire or wind damage in the containment greenhouses, remaining plants will be placed in double sealed containers and moved to another secured part of Selby Hall. The CD and permit holder will be contacted immediately.

3. In the case of vandalism in the CG or storage rooms, the OARDC police and the CD and permit holder will be contacted immediately. Missing items will be recorded in the SOP and noted in the culture inventory. Every attempt will be made to recover plant material or cultures that are lost and the areas will be decontaminated.

4. In the event of equipment failure in laboratory and storage areas, the pathogen cultures will remain in those rooms to prevent potential release. Non-viable cultures will then be destroyed according to the PPQ permits.

5. Reports will be filed with USDA-APHIS-PPQ (according to the procedures described in the PPQ permit), OARDC administration, Department of Plant Pathology Chair, the Department of Plant Pathology greenhouse committee (Appendix IV) and the CD.

V. PATHOGEN GROUP-SPECIFIC STANDARD OPERATING PROCEDURES FOR APHIS PERMITTED ORGANISMS

A. Standard operating procedure for plant pathogenic fungi

1. Fungal manipulations

   All laboratory manipulations including culture transfer, DNA extractions and plant inoculations using regulated fungal isolates will be performed within the Class II BSC A/B3 laminar flow safety cabinet available in laboratory 232 or the Labconco Purifier Logic Class II/A2 safety cabinet in laboratory 134. Work in these laboratories, which will be dedicated to permitted pathogens, is limited to trained personnel; surfaces are regularly decontaminated with 70% ethanol; food, drinks and smoking are strictly prohibited. Additional precautions are taken to confine the organisms including washing hands after working with pathogens and use of gloves while working with the pathogens. In addition, sterilizing UV lights in room 232 are present inside the safety cabinet and can be used to decontaminate the cabinet. All contaminated material including gloves, tips, tubes, Petri plates and infected plant tissue will be placed in clear autoclave containers and autoclaved before re-use or disposal. Cultures will be stored or grown in laboratory 134 or 232 (Appendix XIV, plates 35-36). Molecular biology work will be performed in laboratory 134 or the assigned laboratory of the PI.

2. Fungal growth

   Fungal cultures will be placed in closed, unbreakable plastic containers in laboratory 134 or 232 and transferred to a designated bench within the PI's laboratory or to an incubator in rooms 133 or 134 or 232 (Appendix XIV, plates 35-36, 45). Benches and/or laboratory entrances will be labeled with appropriate biohazard signs and the laboratories will be kept locked when not in use. Benches will be surface decontaminated with 70% ethanol after disposal of the cultures.

3. Fungal-inoculated plant growth

   Plants, seedlings, and/or leaflets inoculated in laboratory 134 or 232 will be placed in plastic trays or cups and fitted with plastic covers or contained in an appropriate size covered container to be transferred to rooms 133, 134 or 232, the containment greenhouses or the growth chambers. The plastic trays and covers and/or the closed containers will be surface decontaminated using 5% sodium hypochlorite. Pots containing inoculated plants will be placed in plastic trays to avoid water run-off (Appendix XIV, plate 28). Designated disposable laboratory coats, gloves, and pipette tips will be used and stored in the greenhouse compartment (Appendix XIV, plate 27). All contaminated material will be placed in clear autoclave containers, transported within closed non-breakable containers and autoclaved before disposal. Plant material and soil will be steam-sterilized twice before disposal.

4. Culture storage and disposal

Cultures initially received will be opened in containment Class II BSC A/B3 laminar flow safety cabinets located in laboratories 232. Disposable packaging will be destroyed by autoclaving and all containers and surfaces will be sterilized. All cultures should be stored long-term in either a -80 ºC freezer located in laboratory 134 and 232 or in liquid nitrogen storage located in the MCIC facility (005). Storage rooms will remain locked at all times. After completion of experiments or when the PPQ permit expires, all cultures will be destroyed by autoclaving or by the method indicated in the pertaining PPQ permit. Autoclaved cultures and materials not containing human pathogens, will be disposed of in the OARDC dumpster located outside of the Selby Hall soil room.

5. Personnel training

The PI will ensure that the personnel involved in the handling of the biohazardous agents are adequately trained. They will be taught about the hazard of biological agents, how to handle them and how to dispose of them. In accordance to departmental policy and NIH guidelines, a SOP for each PPQ permitted viral plant pathogen will be prepared and distributed to relevant personnel. A copy of the PPQ permit contract will be provided to personnel for their review. The personnel will be encouraged to self-review their work habits and correct deficiencies on a regular basis. Good hygiene will be practiced to minimize the exposure to such agents. The PPQ permit and SOP will be kept in the laboratory and personnel will be encouraged to use and review it. The trained personnel will sign a laboratory training log available in the PI's laboratory indicating that they have been informed of the hazards of working with biohazards and the proper procedures for working with PPQ permitted plant pathogenic viruses. All personnel involved in handling biohazardous agents will be required to participate in an annual review of Selby Hall standard operating procedures.

6. Spills

In case of accidental release of the plant fungal pathogen via breakage of containers or accidental spillage of cultures, the following procedure will be followed:

a. Liquid spills will be wiped with absorbent paper or cloth and the contaminated material will be placed in a clear autoclave bag, sterilized twice and disposed of according to the procedures described in section IV.D-E. of this document. After the liquid has been removed, the area in contact with the liquid will be thoroughly cleaned with a solution of 5.25% sodium hypochlorite. Materials used to clean the area will be disposed of as described above.

b. Solid material will be carefully removed from the surface using gloved hands, enfolded in absorbent paper or cloth placed in a clear autoclave bag, sterilized twice and disposed of according to the procedures described in section IV.D-E of

this document. After the solid has been removed, the area in contact with the solid should be thoroughly cleaned with a solution of 5.25% sodium hypochlorite. Materials used to clean the area will be disposed of as described above.

c. The spill and the completed clean-up will be reported to the PI and CD.

7. Ingestion, inhalation or self-inoculation

Except in the rare instances of immunocompromised humans, non-mycotoxin producing plant fungal pathogens used in these studies are not infectious to humans if inhaled or ingested. Plant pathogenic fungi that produce mycotoxins can be harmful to mammals if consumed. ***In case of self-inoculation (e.g. with an infected needle) or accidental ingestion of plant pathogenic fungi that produce harmful mycotoxins emergency professional health care will be sought immediately.*** The PI and CD will be contacted and The Ohio State University Employee Accident Report completed (Appendix XI).

B. Standard operating procedure for plant pathogenic bacteria

1. Bacterial manipulations

   All laboratory manipulations including culture transfer, DNA extractions and plant inoculations using regulated bacterial isolates will be performed within the Class II BSC A/B3 laminar flow safety cabinet available in laboratory 232 or the Labconco Purifier Logic Class II/A2 safety cabinet in laboratory 134. Work in these laboratories will be dedicated to permitted pathogens and is limited to trained personnel; surfaces are regularly decontaminated with 70% ethanol; food, drinks and smoking are strictly prohibited. Additional precautions will be taken to confine the organisms including washing hands after working with pathogens and use of gloves while working with the pathogens. In addition, sterilizing UV lights in room 232 are present inside the safety cabinet and will be used to decontaminate the cabinet. All contaminated material including gloves, tips, tubes, Petri plates and infected plant tissue will be placed in clear autoclave containers and autoclaved before re-use or disposal. Cultures will be stored or grown in laboratory 134 or 232 (Appendix XIII, plates 35-36. Molecular biology work will be performed in laboratory 134 or 232.

2. Bacterial growth

   Bacterial culture plates will be placed in closed, unbreakable containers in laboratory 232 and transferred to a designated bench within the PI's laboratory or to laboratory 134 or all work will be completed in laboratory 134 or 232. Benches and/or laboratory entrances will be labeled with appropriate biohazard signs and the laboratories will be kept locked when not in use. Benches will be surface decontaminated with 70% ethanol after disposal of the cultures.

3. Bacteria-inoculated plant growth

   Plants, seedlings, and/or leaflets inoculated in laboratories 134 or 232 will be placed in plastic trays or cups and fitted with plastic covers or contained in an appropriate sized covered container to be transferred to the containment greenhouses or growth chambers. The plastic trays and covers and/or the closed containers will be surface decontaminated using 5% sodium hypochlorite. Pots containing inoculated plants will be placed in plastic trays to avoid water run-off (Appendix XIII, plate 28). Designated disposable laboratory coats, gloves, and pipette tips will be used and stored in the greenhouse compartment (Appendix XIII, plate 27). All contaminated material will be placed in clear autoclave containers, transported within closed non-breakable containers and autoclaved before disposal. All plant material and soil from the greenhouse will be steam-sterilized **twice** before disposal.

4. Culture storage and disposal

   Cultures initially received will be opened in containment Class II BSC A/B3 laminar flow safety cabinets located in laboratory 232 or the Labconco Purifier Logic Class

II/A2 safety cabinet in laboratory 134. Disposable packaging will be destroyed by autoclaving and all containers will be surface sterilized. All cultures should be stored long-term in either a -80 ºC freezer located in laboratory 134 and 232 or in liquid nitrogen storage located in room 005 within the MCIC facility. Storage rooms will remain locked at all times. After completion of experiments or when the PPQ permit expires, all cultures will be destroyed by autoclaving or by the method indicated in the pertaining PPQ permit. Autoclaved cultures and materials not containing human pathogens, will be disposed of in the dumpster located outside of the Selby Hall soil room.

5. Personnel training

The PI will ensure that the personnel involved in the handling of the biohazardous agents are adequately trained. They will be taught about the hazard of biological agents, how to handle them and how to dispose of them. In accordance to departmental policy and NIH guidelines, a SOP for each PPQ permitted bacterial plant pathogen will be prepared and distributed to relevant personnel. A copy of the PPQ permit conditions will be provided to personnel for their review. Personnel will be encouraged to self-review their work habits and correct deficiencies on a regular basis. Good hygiene will be practiced to minimize the exposure to said agents. The PPQ permit and SOP will be kept in the laboratory and personnel will be encouraged to use and review it. The trained personnel will sign a laboratory training log available in the PI's laboratory indicating that they have been informed of the hazards of working with biohazards and the proper procedures for working with PPQ permitted plant pathogenic bacteria. All personnel involved in handling biohazardous agents will be required to participate in an annual review of Selby Hall standard operating procedures.

6. Spills

In case of accidental release of the plant bacterial pathogen via breakage of containers or accidental spillage of cultures, the following procedure will be followed:

a. Liquid spills will be wiped with absorbent paper or cloth and the contaminated material will be placed in a clear autoclave bag, sterilized twice and disposed of according to the procedures described in section IV.D-E. of this document. After the liquid has been removed, the area in contact with the liquid will be thoroughly cleaned with a solution of 5.25% sodium hypochlorite. Materials used to clean the area will be disposed of as described above.

b. Solid material will be carefully removed from the surface using gloved hands, enfolded in absorbent paper or cloth placed in a clear autoclave bag, sterilized twice and disposed of according to the procedures described in section IV.D-E. of this document. After the solid has been removed, the area in contact with the solid should be thoroughly cleaned with a solution of 5.25% sodium hypochlorite. Materials used to clean the area will be disposed of as described above.

c. The spill and the completed clean-up will be reported to the PI and CD.

9. Ingestion, inhalation or self-inoculation

Except in the rare instances of immunocompromised humans, plant bacterial pathogens used in these studies are not infectious to humans if inhaled or ingested. In case of self-inoculation (e.g. with an infected needle) or accidental ingestion of plant pathogenic bacteria the PI and Containment Director will be contacted and The Ohio State University Employee Accident Report completed (Appendix XI). Professional health care will be sought if necessary.

C. Standard operating procedure for plant pathogenic Oomycetes

1. Oomycete manipulations

   Cultures or infected plant materials initially received will be opened in Class II BSC A/B3 (laboratory 232) or in a Labconco Purifier Logic Class II/A2 (laboratory 134) laminar flow safety cabinets. Work in this laboratory will be dedicated to permitted pathogens and is limited to trained personnel; surfaces are regularly decontaminated with 70% ethanol; food, drinks and smoking are strictly prohibited. Additional precautions will be taken to confine the organisms including washing hands after working with pathogens and use of gloves while working with the pathogens. Routine culture transfers of regulated oomycetes will be performed within the Class II BSC A/B3 laminar flow safety cabinet in laboratory 232 or in the Class II/A2 laminar flow safety cabinet in laboratory 134. Following culture transfer, all laboratory manipulations of cultures, including growth and short-term storage, will be conducted in laboratory 134 and 232 or in the laboratory indicated in the pertaining PPQ permit. Plant inoculations using permitted oomycetes isolates will be done in laboratory 134, 232 or in the laboratory indicated in the pertaining PPQ permit. All contaminated material including gloves, tips, tubes, petri plates, paper towels and infected plant tissue will be placed in clear autoclave containers and autoclaved before disposal. All labware used during inoculation and molecular biology work will be surface decontaminated using 5% sodium hypochlorite prior to washing.

2. Oomycete growth

   Ooomycete cultures in liquid or on solid media will be placed in closed plastic containers and transferred from laboratory 232 to an incubator in laboratory 134 or completely done within laboratory 134 or 232. The incubator will be labeled with appropriate biohazard signs and the room will remained locked when not in use.

3. Oomycete-inoculated plant growth

   Plants, seedlings, and/or leaflets inoculated in laboratory 134 or 232 will be placed in plastic trays or cups or germination paper towels and fitted with plastic covers or contained in an appropriate sized covered container to be transferred to designated growth chambers, greenhouses or kept in laboratory 133, 134, or 232. The plastic trays and covers and/or the closed containers and other labware used during inoculation will be surface decontaminated using 5% sodium hypochlorite. Pots containing inoculated plants will be placed in plastic trays to avoid water run-off (Appendix XIV, plate 28). Designated disposable laboratory coats, gloves, and pipette tips will be used and stored in the greenhouse compartment (Appendix XIV, color plate 27). All contaminated material will be placed in clear autoclave containers, transported within closed non-breakable containers and autoclaved before disposal. All plant material and soil from the greenhouse will be steam-sterilized twice before disposal.

4. Culture storage and disposal

Cultures initially received will be opened in containment Class II BSC A/B3 laminar flow safety cabinets located in laboratory 232 or in Labconco Purifier Logic Class II/A2 safety cabinet located in laboratory 134. Disposable packaging will be destroyed by autoclaving and all containers will be surface sterilized. All cultures will be stored for the short-term at 15ºC in room 134 or 232. Cultures for long-term storage will be stored in liquid nitrogen in room 005 within the MCIC facility or on hemp seed in room 133. Storage rooms will remain locked at all times. After completion of experiments or when the PPQ permit expires, all cultures will be destroyed by autoclaving or by the method indicated in the pertaining PPQ permit. Autoclaved cultures and materials will be disposed of in the OARDC dumpster located outside of the Selby Hall head house.

5. Personnel training

The PI will ensure that the personnel involved in the handling of the biohazardous agents are adequately trained. They will be taught about the hazard of biological agents, how to handle them and how to dispose of them. In accordance to departmental policy and NIH guidelines, a SOP for each PPQ permitted fungal plant pathogen will be prepared and distributed to relevant personnel. A copy of the PPQ permit conditions will be provided to personnel for their review. Personnel will be encouraged to self-review their work habits and correct deficiencies on a regular basis. Good hygiene will be practiced to minimize the exposure to said agents. The PPQ permit and SOP will be kept in the laboratory and personnel will be encouraged to use and review it. The trained personnel will sign a laboratory training log available in the PIs laboratory indicating that they have been informed of the hazards of working with biohazards and the proper procedures for working with PPQ permitted plant pathogenic oomycetes. All personnel involved in handling biohazardous agents will be required to participate in an annual review of Selby Hall standard operating procedures.

6. Spills

In case of accidental release of the plant oomycete pathogen via breakage of containers or accidental spillage of cultures, the following procedure will be followed:

a. Liquid spills will be wiped with absorbent paper or cloth and the contaminated material will be placed in a clear autoclave bag, sterilized twice and disposed of according to the procedures described in section IV.D-E. of this document. After the liquid has been removed, the area in contact with the liquid will be thoroughly cleaned with a solution of 5.25% sodium hypochlorite. Materials used to clean the area will be disposed of as described above.

b. Solid material will be carefully removed from the surface using gloved hands, enfolded in absorbent paper or cloth placed in a clear autoclave bag, sterilized twice and disposed of according to the procedures described in section IV.D-E. of this document. After the solid has been removed, the area in contact with the solid should be thoroughly cleaned with a solution of 5.25% sodium hypochlorite. Materials used to clean the area will be disposed of as described above.

c. The spill and the completed clean-up will be reported to the PI and CD.

7. Ingestion, inhalation or self-inoculation

None of the plant pathogenic oomycetes used are infectious to humans. In case of self-inoculation (e.g. with an infected needle) or accidental ingestion the PI and CD will be contacted immediately and The Ohio State University Employee Accident Report (Appendix XI) will be completed. Professional health care will be sought if necessary.

D. Standard operating procedure for plant pathogenic viruses

1. Virus-infected plant material inoculations, growth and manipulations

   Manipulations of virus-infected plant material including seed and rub- inoculations and scoring will be performed in the phytotron (room 035), laboratories 026, 029, 031, 033, 039, 130, CG2 or designated growth chambers. Plant material inoculated with permitted viruses will be inoculated and grown only in designated, locked growth chambers or CG2. Inoculated materials will be transported within closed, non-breakable plastic containers or on solid-surfaced laboratory carts covered with a fine-meshed cage. All disposable contaminated materials including gloves, pipette tips, tubes, plant tissue and soil will be placed in clear autoclaved bags and autoclaved prior to disposal. All plant material and soil from the greenhouse will be steam-sterilized before removal to the dumpster for disposal in a sanitary landfill. Before being released for other use, greenhouse rooms will be disinfected according to the procedure described in section III.D.6. Contaminated glassware, pots and surfaces will be treated with sodium hypochlorite (5.25%) and rinsed with soap and water to inactivate any infectious residues. Liquid virus preparations will be treated with sodium hypochlorite (5.25%) for 30 minutes prior to disposal in laboratory drains in rooms 026, 029, 031, 033 or 130. Molecular biology work will be performed in the assigned laboratory of the PI.

2. Virus storage and disposal

   Infected material initially received will be opened in the containment Class II BSC A/B3 laminar flow safety cabinet in laboratory 029. Disposable packaging will be destroyed by autoclaving and all containers will be surface sterilized with sodium hypochlorite (5.25%). Received plant material will be transferred to growth chambers or CG2 within closed, unbreakable plastic containers. All infected plant material will be stored long-term in designated locked -20 ºC or -80 ºC freezers in laboratories 028B, 031, 033 or 130, or in liquid nitrogen storage located in room 005 within the MCIC facility. Storage rooms and -80 freezers will remain locked at all times and signs are posted indicating that doors should remain closed and locked at all times. After completion of experiments or when the PPQ permit expires, all infected plant material will be destroyed by autoclaving or by the method indicated in the pertaining PPQ permit. Autoclaved materials will be removed the dumpster prior to disposal in a sanitary landfill.

3. Personnel training

   The PI will ensure that the personnel involved in the handling of the biohazardous agents are adequately trained. They will be taught about the hazard of biological agents, how to handle them and how to dispose of them. In accordance to departmental policy and NIH guidelines, a SOP for each PPQ permitted viral plant pathogen will be prepared and distributed to relevant personnel. A copy of the PPQ permit contract will be provided to personnel for their review. The personnel will be

encouraged to self-review their work habits and correct deficiencies on a regular basis. Good hygiene will be practiced to minimize the exposure to such agents. The PPQ permit and SOP will be kept in the laboratory and personnel will be encouraged to use and review it. The trained personnel will sign a laboratory training log available in the PI's laboratory indicating that they have been informed of the hazards of working with biohazards and the proper procedures for working with PPQ permitted plant pathogenic viruses. All personnel involved in handling biohazardous agents will be required to participate in an annual review of Selby Hall standard operating procedures.

4. Arthropod infestations

Active surveillance will be in effect to prevent arthropod infestation within growth chambers used with viral pathogens and CG2. Specific measures for controlling arthropods will depend on the species present.

a. A species-specific contact pesticide or biocontrol agent will be applied according to the manufacturers label to all plants in CG2.

b. Plants will be inspected regularly and the pesticide will be re-applied until the infestation is eliminated or effectively managed by biocontrol.

c. All arthropod infestations will be reported to the PI and the greenhouse manager.

5. Ingestion, inhalation or self-inoculation

Plant pathogenic viruses used in these experiments are not infectious to humans. In case of injury or accidental ingestion the PI and CD will be contacted immediately. Professional health care will be sought if necessary.

E. Standard operating procedure for insect vectors

1. Insect rearing and live insect handling

   Insects reared on plants are confined within insect-escape-proof rearing cages in room 058A (Appendix XIV, plates 29 and 30), in growth chamber 7 (GC7), other chambers as outlined on specific permits, the phytotron (035), or as outlined in a specific permit. Insects are transported within these cages to the insect transfer room 036 (Appendix I.F.; Appendix XIV, plate 32) for handling and manipulation. Insects are released, manipulated, captured and transferred to other cages within insect transfer cabinets (Appendix XIV, plate 34) located in room 036. Room 036 is equipped with yellow sticky traps and insect zappers (Appendix XIV, plates 33 and 34) to trap any insects that escape from the transfer cabinet. Caged insects and plants are returned to room 058A, GC7 or areas outlined in specific permits. Insects in the system are tracked using an insect container log form (Appendix VIII).

2. Pathogen-infected plant growth

   Male insects are selected for virus maintenance transmission and most other experiments. If females are required for transmission in a specific protocol, the plants will remain in growth chambers. Insect-exposed plants that need to be transported to CG2 will be inspected for insects inside the transfer cabinets, then placed into large plastic containers with sealable lids and sprayed with a pyrethroid insecticide using manufacturers labeled rates in the 036 vestibule (Appendix XIV, plate 31). The plants will be transported to room 113, 115, 117, 119, 120, 121 or 122 of CG2. The rooms are sprayed as needed with abamectin (1.9%) and pyrethrum according to the manufacturers labeled rate to kill any new insects that may have hatched from pre-existing eggs on the plants.

3. Dead insect and plant material handling

   Insect and plant material for molecular research and microscopic analyses will be frozen in liquid nitrogen or chemically fixed before transport out of growth chambers or 036. For freezing, vials containing the insects or plant material will be dropped into liquid nitrogen for 5 min. Frozen samples are stored within locked -80C freezers. For fixation, insects will be paralyzed using ice or CO_2 until immobile in room 036, then transported in closed tubes and/or vials within small covered containers to the chemical fume hood in lab 031 for transfer to fixative. Molecular studies or microscopy is performed on killed insects and plant material within the laboratory of the PI or the MCIC (rooms 003-006).

4. Culture storage and disposal

   Prior to analysis, killed insects and plant material are stored long-term in either -80C freezers (028B) or in liquid nitrogen storage (room 005) within the MCIC facility. Freezers and room 005 will remain locked at all times, and signs are posted indicating

this. Any unused insects or plant material will be frozen in -20C freezer (room 036), then placed in clear autoclave bags and steam sterilized. Sterilized materials will be moved to the dumpster prior to disposal in a sanitary landfill.

5. Personnel training

The PI will ensure that the personnel involved in the handling of the biohazardous agents are adequately trained. They will be taught about the hazard of biological agents, how to handle them and how to dispose of them. In accordance to departmental policy and NIH guidelines, a SOP for each PPQ permitted insect vector will be prepared and distributed to relevant personnel. A copy of the PPQ permit conditions will be provided to personnel for their review. The personnel will be encouraged to self-review their work habits and correct deficiencies on a regular basis. Good hygiene will be practiced to minimize the exposure to such agents. The PPQ permit and SOP will be kept in the laboratory and personnel will be encouraged to use and review it. The trained personnel will sign a laboratory training log available in the PIs laboratory indicating that they have been informed of the hazards of working with biohazards and the proper procedures for working with insect vectors. All personnel involved in handling biohazardous agents will be required to participate in an annual review of Selby Hall standard operating procedures.

F. Standard operating procedure for plant pathogenic nematodes

1. Nematode rearing and handling

   Nematodes reared on plants will be confined to a non-containment greenhouse (G029). Nematode-infection experiments will be conducted in the non-containment greenhouses G026, G027 and G028. Nematode infested plants will be grown in sand/Turface mix in 8-12 in. pots. Each nematode species/strain will be kept on greenhouse benches within individual isolation trays. Plants will be watered daily, fertilized weekly with Peters 20-20-20, and pests will be controlled by regularly applying insect predators. Insecticides will be sprayed by the greenhouse manager as necessary to control insect pests. Insecticides will be applied following manufacturer product usage labels. A list of insecticidal sprays compatible with nematode rearing is available in laboratory 202 and with the greenhouse manager. Sterile nematode cultures will be maintained in tissue culture in Percival Scientific incubators located in laboratories 133 and 212.

2. Nematode and egg harvesting

   Pots with soil and infested roots will be placed in plastic containers with lids and transported to the head house (054) for nematode isolation. Shoots of infected/symptomatic plants will be removed prior to transport and discarded by placing tissue into clear plastic autoclave bags, and sterilized in the steam sterilizer located 054 as described in Section IV.D-E. Sterile nematode cultures will be processed in laboratories 202 and 212.

   Species of root-knot nematode (*Meloidogyne* spp.) will be isolated in the head house (054) by removing sand bound to the roots and washing the roots with sterile water. Egg masses and released eggs will be dissolved with sodium hypochlorite (5.25%), then captured using sucrose flotation in a high-speed centrifuge or by filtering through a 25-micron sieve. Isolated eggs will be added to disposable centrifuge tubes, secured by capping and transported to G029 for re-inoculation of healthy plants.

   Species of cyst nematodes (*Heterodera* spp.) will be isolated in the head house (054) by washing the soil and infested roots in autoclave tubs containing clean water. Cysts will captured using sucrose flotation in a high-speed centrifuge or by filtering through an 80-micron sieve. Isolated eggs will be released by crushing the cysts using a rubber stopper and washing with water onto a 25-micron sieve. Isolated eggs will be added to disposable centrifuge tubes, secured by capping and transported to G029 for re-inoculation of healthy plants.

3. Nematode and infected plant material disposal

   All disposable materials that come in contact with nematodes, eggs, infested soil or plant material will be steam sterilized according to the procedure described in IV.D-

E. Glassware will be rinsed and washed using hot water and detergent in laboratory 110. Sieves will be rinsed with water and placed into a drying oven at >80C located in 039 for 24 hours to kill any remaining eggs or nematodes (juvenile stage). After completion of the experiments any unused nematodes, eggs or plant material will be destroyed by autoclaving as described in IV.D-E. After completion of experiments or when the PPQ permit expires (if applicable), all infected plant material will be destroyed by autoclaving or by the method indicated in the pertaining PPQ permit (if applicable). Autoclaved materials will be moved to the dumpster located outside of the head house and prior to disposal in a sanitary landfill. All liquids used in the isolation of nematodes will be autoclaved and disposed of in the city sewer.

4. Personnel training

The PI will ensure that the personnel involved in the handling of the biohazardous agents are adequately trained. They will be taught about the hazard of biological agents, how to handle them and how to dispose of them. In accordance to departmental policy and NIH guidelines, a SOP for each PPQ permitted plant pathogenic nematode will be prepared and distributed to relevant personnel. A copy of the PPQ permit contract will be provided to personnel for their review. The personnel will be encouraged to self-review their work habits and correct deficiencies on a regular basis. Good hygiene will be practiced to minimize the exposure to such agents. The PPQ permit and SOP will be kept in the laboratory and personnel will be encouraged to use and review it. The trained personnel will sign a laboratory training log available in the PI's laboratory indicating that they have been informed of the hazards of working with biohazards and the proper procedures for working with PPQ permitted plant pathogenic nematode. All personnel involved in handling biohazardous agents will be required to participate in an annual review of Selby Hall standard operating procedures.

5. Ingestion, inhalation or self-inoculation

Plant pathogenic nematodes used in these experiments are not infectious to humans. In case of self-inoculation or accidental ingestion the PI and CD will be contacted immediately and The Ohio State University Employee Accident Report completed (Appendix XI). Professional health care will be sought if necessary.

G. Standard operating procedure for BSL-2 human pathogenic bacteria

1. Bacterial manipulations and growth

   All laboratory manipulations including culture transfer, bacterial growth and molecular applications using regulated human bacterial isolates will be performed within a containment Class II Type A2 laminar flow safety cabinet available in laboratories 139 or 232 in the Selby building on the OARDC Wooster campus. Work in this laboratory, which will be dedicated to these types of manipulations, is limited to trained personnel; surfaces must be decontaminated with 70% ethanol before and after completion of work; food, drinks and smoking are strictly prohibited in the laboratory. Contact lenses will not be worn while working with infectious agents. Lab coats and gloves are to be worn at all times while working with infectious agents. Coats will be removed before leaving the laboratory. Contaminated clothing will be decontaminated with 70% ethanol or 10% chlorine bleach and then laundered. Personnel will wash hands (soap, scrub for 20 sec., rinse with hot water, dry with single use paper towel) after working with infectious agents, after removing gloves and before leaving the laboratory.

2. Bacteria-inoculated plant growth

10. Plants, seedlings, and/or leaflets will be inoculated within the BLS-2 growth chambers (GC14 or GC19) located in room 035 of Selby Hall. Plants will be inoculated using drenches, dips or swabs. ***No spray (aerosol) inoculations will be performed.*** Pots containing inoculated plants will be placed in plastic trays to prevent run-off of potentially contaminated water. The trays will be decontaminated using 10% bleach prior to re-use. Designated disposable gloves, spill kits, bleach and other laboratory supplies required for the research will be stored in closed non-breakable labeled containers beside the growth chambers. All contaminated material will placed into a burn box containing two red plastic infectious waste bags. The boxes must be sealed with at least two layers of packing tape (NOT masking tape) and requested to be picked up by EHS at the Ohio State University. The growth chamber, plastic trays and covers and/or the closed containers will be surface decontaminated using 10% bleach. Personnel should wash hands (soap, scrub for 20 sec., rinse with water, dry with single use paper towels) after removing gloves working with infectious agents.

3. Signage

   A hazard warning sign incorporating the universal biohazard symbol will be posted on all growth chambers containing BSL-2 bacterial pathogens. The hazard sign will identify the hazardous agent and list the names and telephone numbers of the PIs and any other responsible personnel. A copy of the hazard warning sign can be found in Appendix XII.

4. Culture storage, transport and disposal

11. Bacteria initially received will be opened in a Class II Type A2 biosafety cabinet available in laboratories 139 or 232. Disposable materials and cultures ***containing human pathogens*** will placed into a burn box containing two red plastic infectious waste bags. The boxes must be sealed with at least two layers of packing tape (NOT masking tape) and requested to be picked up by EHS at the Ohio State University.

5. Personnel training

The PI will ensure that the personnel involved in the handling of the biohazardous agents are adequately trained. Prior to working with BSL-2 human bacterial pathogens the personnel will be required to read The Ohio State Institutional Laboratory Bio-safety Manual. Personnel must have a current certificate of completion for BSL-2 training through Ohio State University Environmental Health and Safety. Personnel will be taught about the hazard of biological agents, how to handle them and how to dispose of them. The personnel will be encouraged to self-review their work habits and correct deficiencies on a regular basis. Good hygiene will be practiced to minimize the exposure to such agents. A copy of the Standard Operating Procedure (SOP) and the OSU-IBC protocols will be kept in the laboratory and personnel will be encouraged to use and review it. The trained personnel will sign a laboratory training log available in the PIs laboratory indicating that they have been informed of the hazards of working with biohazards and the proper procedures for working with BSL-2 human and animal pathogenic viruses. Certificates of completion of the required training will be kept in the lab personnel-training notebook and in the HR office in Selby Hall. All personnel involved in handling biohazardous agents will be required to ***participate in an annual review of Selby Hall*** standard operating procedures.

6. Spills

Spills or accidents involving biohazardous BSL-2 human bacterial pathogen materials must be immediately reported to the PI or the laboratory supervisor if the PI is not available. Personnel not exposed to the spill will be immediately evacuated from the spill area and the entrance to the contaminated area will be restricted until clean-up is completed. The following procedures will be followed for decontamination:

a. Liquid spills will be wiped with absorbent paper or cloth and the contaminated material placed in a labeled autoclave bag, sterilized twice and disposed of according to the procedures described in IV.D-E. of this document. After the liquid has been removed, the area in contact with the liquid will be exposed to 10% sodium hypochlorite or 70% ethanol for 30 min. Materials used to clean the area will be disposed of as described above.

b. Solid material will be carefully removed from the surface using gloved hands, enfolded in absorbent paper or cloth placed in a labeled autoclave bag, sterilized

twice and disposed of according to the procedures described in Section V.G.4. of this document. After the solid has been removed, the area in contact with the solid will be exposed to 10% sodium hypochlorite or 70% ethanol for 30 min. Materials used to clean the area will be disposed of as described above.

c. If laboratory coats or personnel clothing were contaminated they will be removed, placed in a labeled autoclave bag and autoclaved for 60 minutes (121 °C, 15 psi) prior to laundering. Contaminated skin will be washed thoroughly (soap, scrub for 20 sec., rinse with hot water, dry with single use paper towel).

d. Completion of clean-up will be reported to the PI.

7. Ingestion, inhalation or self-inoculation

In the case of accidental self-contamination the PI will be contacted immediately. ***Professional medical evaluation, surveillance and treatment will be sought immediately*** and The Ohio State University Employee Accident Report completed (Appendix XI).

I. Standard operating procedure for BSL-2 animal and human pathogenic viruses in plants

1. Virus manipulations and growth

   All laboratory manipulations including virus propagation, virus transfer and molecular applications using regulated human and animal viral pathogens will be performed within a Class II Type A2 biosafety cabinet available in laboratories 123, 125 or 137 in the Food Animal Health Research Program (FAHRP) building on the OARDC Wooster campus. Work in these laboratories, which will be dedicated to these types of manipulations, is limited to trained and BSL-2 certified personnel; surfaces must be decontaminated with 10% bleach to inactivate infectious residues followed by cleaning with 70% ethanol after completion of work; food, drinks and cosmetics are strictly prohibited in the laboratory. Personal protective equipment appropriate for BSL-2 (lab coats, gloves and safety glasses) is worn when exposure to infectious agents or biohazardous waste. Contaminated glassware and pots will either be autoclaved for 60 minutes or treated with 10% bleach for a minimum of 30 minutes for decontamination. All disposable contaminated materials including gloves, pipette tips, tubes, plant tissue and soil will be autoclaved for 60 minutes prior to placement into biohazard burn boxes for pick up for incineration. Lab coats and gloves are to be worn at all times while working with infectious agents. Lab coats will be removed before leaving the laboratory and will be laundered with 10% chlorine bleach. Personnel should wash hands (soap, scrub for 20 sec., rinse with water, dry with single use paper towels) after removing gloves.

2. Virus-inoculated plant growth

   Plants, seedlings, and/or leaflets will be inoculated in a Class II Type A2 biosafety cabinet in FAHRP laboratories 123, 125 or 137, transported in sealed non-breakable containers to Selby Hall, and placed within the BLS-2 growth chambers (GC19 or GC14) located in room 035 in Selby Hall. ***No additional inoculations will be conducted within Selby Hall***. Pots containing inoculated plants will be placed in plastic trays to prevent run-off of potentially contaminated water. The trays will be decontaminated using 10% bleach. Designated disposable gloves, spill kits, bleach and other laboratory supplies required for the research will be stored in closed non-breakable labeled containers beside the growth chambers. All contaminated material will be placed in marked autoclave bags and transported within closed non-breakable containers to the FAHRP building. Contaminated materials will be autoclaved and placed in burn boxes in FAHRP room 135. ***No contaminated materials will remain in Selby Hall***. The growth chamber, plastic trays and covers and/or the closed containers will be surface decontaminated using 10% bleach. Personnel should wash hands (soap, scrub for 20 sec., rinse with water, dry with single use paper towels) after removing gloves working with infectious agents.

3. Signage

A hazard warning sign incorporating the universal biohazard symbol will be posted on all growth chambers containing BSL-2 viral pathogens. The hazard sign will identify the hazardous agent and list the names and telephone numbers of the PIs and any other responsible personnel. A copy of the hazard warning sign can be found in Appendix XII.

4. Virus storage, transport and disposal

Virus initially received will be opened in a Class II Type A2 Biosafety cabinet available in laboratories 123, 125 or 137 in FAHRP building. Disposable packaging will be destroyed by autoclaving (FAHRP room 135) and placed in burn boxes for incineration. All biohazard samples will be transported between FAHRP and Selby Hall in sealed non-breakable containers. Biohazard waste will be double bagged and transported in sealed non-breakable containers. Short term and long term storage of all cultures will occur in FAHRP. ***No animal or human pathogenic virus will be stored in Selby Hall***. After completion of the experiments or when the permits expire, all viruses will be destroyed by autoclaving (121°C, 15 psi for 60 min.) and placed in burn boxes for incineration or by the method indicated in the permit.

5. Personnel training

The PI will ensure that the personnel involved in the handling of the biohazardous agents are adequately trained. Prior to working with BSL-2 human viral pathogens the personnel will be required to read The Ohio State Institutional Laboratory Bio-safety Manual. Personnel must have a current certificate of completion for BSL-2 training through Ohio State University Environmental Health and Safety. Personnel will be taught about the hazard of biological agents, how to handle them and how to dispose of them. The personnel will be encouraged to self-review their work habits and correct deficiencies on a regular basis. Good hygiene will be practiced to minimize the exposure to such agents. A copy of the Standard Operating Procedure (SOP) and the OSU-IBC protocols will be kept in the laboratory and personnel will be encouraged to use and review it. The trained personnel will sign a laboratory training log available in the PIs laboratory indicating that they have been informed of the hazards of working with biohazards and the proper procedures for working with BSL-2 human and animal pathogenic viruses. Certificates of completion of the required training will be kept in the lab personnel-training notebook and in the HR office in FAHRP. All personnel involved in handling biohazardous agents will be required to ***participate in an annual review of Selby Hall and FAHRP*** standard operating procedures.

6. Spills

Spills or accidents involving biohazardous BSL-2 animal or human viral pathogen material must be immediately reported to the PI or the laboratory supervisor if the PI

is not available. Personnel not exposed to the spill will be immediately evacuated from the spill area and the entrance to the contaminated area will be restricted until clean-up is completed. The following procedures will be followed for decontamination:

a. Liquid spills will be wiped with absorbent paper or cloth and the contaminated material placed in a biohazard bag. The area in contact with the liquid will be exposed to 10% bleach for 30 minutes. Materials used to clean the area will be disposed of as described above and autoclaved for 60 minutes and placed in a burn box to be sent for incineration.

b. Solid material will be carefully removed from the surface using gloved hands, enfolded in absorbent paper or cloth placed in a biohazard bag, autoclaved for 60 minutes and placed in burn boxes in the Food Animal Health Research Program building. After the solid material has been removed, the area in contact with the solid will be exposed to 10% bleach 30 minutes. Materials used to clean the area will be disposed of as described above.

c. If laboratory coats or personnel clothing were contaminated they will be removed, placed in a biohazard bag and autoclaved for 60 minutes (121°C, 15 psi) prior to laundering or laundered with 10% bleach. Contaminated skin will be washed thoroughly (soap, scrub for 20 sec., rinsed with water, dry with single use paper towel).

d. Completion of clean-up will be reported to the PI.

7. Ingestion, inhalation or self-inoculation

In the case of accidental self-contamination the PI will be contacted immediately. ***Professional medical evaluation, surveillance and treatment will be sought immediately*** and The Ohio State University Employee Accident Report completed (Appendix XI).

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VI. STANDARD OPERATING PROCEDURES FOR RECOMBINANT MICROORGANISMS.

Most research involving recombinant DNA molecules at The Ohio State University is subject to the National Institutes of Health "NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules" *(http://osp.od.nih.gov/sites/default/files/resources/NIH_Guidelines.pdf)*. These guidelines address the safe conduct of research that involves construction and handling of recombinant DNA molecules and organisms containing them. Compliance with these guidelines is a condition of the contractual agreement that the NIH has with any institution that receives NIH funding. All researchers who are using recombinant DNA molecules as part of their research must file a registration document with The Ohio State University-Institutional Biosafety Committee (OSU-IBC), regardless of the funding source (see Appendix VII).

NIH's working definition of Recombinant DNA Molecules

(i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or

(ii) molecules that result from the replication of those described in (i) above.

A. Risk assessment.

The standard operating procedure for recombinant microorganisms will be developed based on the risk group of the agent being studied and the containment conditions required for that agent. Agents are classified into four risk groups and four physical containment levels according to their relative pathogenicity for healthy adult humans. The basis for the classification by risk is as follows:

- **Risk Group 1**: agents are not associated with disease in healthy adult humans.
- **Risk Group 2**: agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.
- **Risk Group 3**: agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available.
- **Risk Group 4**: agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

B. Standard microbiological practices

Standard molecular practices should be developed based on both the agents risk group and containment level and the host of the recombinant DNA (ie. microorganism, plant or animal). For the purposes of this document standard microbiological guidelines, as described by the NIH Guidelines, will only be described for ***risk group 1 and 2 agents***

(also referred to as biosafety levels 1 (BSL-I) and 2 (BSL-II). ***In all cases specific details in the standard operating procedure should be as described in the pathogen-specific SOPs outlined in Section IV.***

1. Biosafety Level I

    a. Access to the laboratory will be limited or restricted at the discretion of the PI when experiments are in progress.

    b. Work surfaces will be decontaminated once a day and after any spill of viable material.

    c. All contaminated liquid or solid wastes will be decontaminated before disposal.

    d. Mechanical pipetting devices will be used; mouth pipetting is prohibited.

    e. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area.

    f. Personnel will wash their hands:

        - after handling materials involving microorganisms containing recombinant DNA molecules
        - after handling plants or animals exposed to microorganisms containing recombinant DNA molecules and,
        - before exiting the laboratory.

    g. Spray inoculations of plants and animals are not permitted (unless otherwise stated by the IBC accepted agreement).

    h. In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower and changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules.

    i. Contaminated materials will be placed in sealable, durable leak-proof containers before being removed from the laboratory.

    j. Special containment equipment is not required for manipulations of agents assigned to BSL-I.

2. Biosafety Level II

a. All microbiological practices described for microorganisms in risk group BSL-I (Section V) apply to microorganisms in risk group BSL-II.

b. A biosafety manual/SOP will be prepared and adopted by all personnel working with BL-II hazardous agents. Personnel will be advised of all special hazards and will be required to read and follow instructions on practices and procedures. All personnel will be trained by the PI or assigned supervisor about the potential hazards of working with BSL-II hazardous agents.

c. All laboratories where microorganisms and plants containing recombinant DNA molecules in will be manipulated will have a hazard warning sign incorporating the universal biosafety symbol posted on access doors.

d. Laboratory coats, gowns, smocks, or uniforms will be worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing will be removed and left in the laboratory or covered with a clean coat not used in the laboratory.

e. Gloves should be worn when handling experimental plants and when skin contact with the agent is unavoidable.

f. Spills and accidents that result in overt exposures to microorganisms containing recombinant DNA molecules will be immediately reported to the Institutional Biosafety Committee and NIH/OBA. Reports to 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). Medical evaluation, surveillance, and treatment will be provided as appropriate and written records will be maintained.

g. Biological safety cabinets (Class I or II) or other appropriate personal protective or physical containment devices are used whenever:

- Procedures with a high potential for creating aerosols are conducted including but not limited to centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures or inoculations of plant material.
- High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

3. Biosafety Level I-Plants

The microbiological practices below will be used and *will supersede those in Section V, when conducting experiments in the greenhouse or growth chamber involving* ***recombinant DNA-containing plants, plant-associated microorganisms, and small animals***.

- Plant-associated microorganisms include:
    - viroids, virusoids, viruses
    - bacteria, fungi, protozoans
    - certain small algae
    - 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 are:
    - in an obligate association with plants
    - plant pests
    - plant pollinators
    - capable of transmitting plant disease agents
    - nematodes for which tests of biological properties necessitate the use of plants

a. Access to the greenhouse will be limited or restricted, at the discretion of the PI, when experiments are in progress.

b. Prior to entering the greenhouse, personnel will be required to read and follow instructions on BSL1-P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are outlined in Sections I-IV.

c. A record shall be kept of experiments currently in progress in the greenhouse facility.

d. Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

e. 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.

4. Biosafety Level II-Plants

a. All microbiological practices described for experiments in the greenhouse or growth chamber involving ***recombinant DNA-containing plants, plant-associated microorganisms, and insects in Section V apply to those in BSLII-P.***

b. All work involving ***recombinant DNA-containing plants, plant-associated microorganisms, and insects BSL-II*** will be conducted in the containment greenhouse facility.

c. A record will be kept of experimental plants, microorganisms, or small animals that are be brought into or removed from the greenhouse facility.

d. The PI will report any greenhouse accident involving the inadvertent release or spill of microorganisms to the greenhouse manager, Containment 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 985, 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.

e. 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 (***see appropriate pathogen-specific SOP in Section V***).

f. Arthropods and other motile macroorganisms (e.g., flying arthropods or nematodes) will be housed in appropriate cages.

C. Shipping

Detailed instructions on how to ship microorganisms or plant material containing recombinant DNA can be found at the Center for Disease Control and Prevention-Biosafety web page (http://www.cdc.gov/biosafety/publications/index.htm).

VII. APPLYING FOR PLANT HEALTH PERMITS

A. The Animal and Plant Health Inspection Service (APHIS)

APHIS is a multi-faceted Agency with a broad mission area that includes protecting and promoting U.S. agricultural health, regulating genetically engineered organisms, administering the Animal Welfare Act and carrying out wildlife damage management activities. More information about APHIS can be found at: http://www.aphis.usda.gov/plant_health/index.shtml.

B. Plant Protection and Quarantine (PPQ)

The Plant Protection and Quarantine (PPQ) is a program within APHIS set up to safeguard agriculture and natural resources from the risks associated with the entry, establishment, or spread of animal and plant pests and noxious weeds to ensure an abundant, high-quality, and varied food supply. More information about PPQ can be found at: http://www.aphis.usda.gov/plant_health/index.shtml.

Permits are required for the importation, transit, domestic movement and environmental release of organisms that impact plants, and the importation and transit of plants and plant products under authority of the Plant Protection and Honeybee Acts. Examples of permit types are listed below.

1. PPQ 526 Permits

   These are used for importation, interstate movement, possession, and/or release into the environment of infested or infective microorganisms, plant materials, and soils including:
   - Insects and mites (other than Bees, Butterflies and Moths, and Biocontrol Organisms)
   - Bees
   - Butterflies and moths
   - Biocontrol organisms
   - Plant pathogenic bacteria, viruses, fungi, mycoplasms, oomycetes and nematodes
   - Snails and slugs
   - Federal noxious weeds and parasitic plants
   - Earthworms
   - Soil (Use PPQ Form 525-A, Application for Permit to Receive Soil)
   - Widely prevalent regulated organisms

2. PPQ 587

   These permits are required for plants or plant products that are not infested with a pathogen or infective agent.

   - Plants for planting (including seeds)

- Fruit and vegetable information
- Rice and rice related information
- Indian corn or maize, broomcorn, and related plants
- Miscellaneous products associated with Khapra beetle
- Sugarcane products and by-products information
- Foreign cotton and covers information
- Cut flowers information

3. PPQ 588 Permits

   These permits are used to **import** prohibited plants or plant products for experimental purposes.

4. PPQ 586 Permits

   These permits are for transit of plants and/or plant products through the U.S (**interstate movement**).

C. How to apply for a PPQ permit.

1. PPQ permits are applied for on-line using the ePermits system (www.aphis.usda.gov/aphis/resources/permits). The ePermits system can be used by USDA Federal employees or by non-USDA employees. ***If you are not an USDA employee you will need to register for a Level 2 eAuthentication account.*** Register for an eAuthentication account at https://www.aphis.usda.gov/aphis/resources/permits/ct_eauth_epermits.
2. Once you are registered and have a user id and password, you can login to the ePermits page and follow the directions posted to complete a PPQ permit application.

3. Time line for PPQ permits

   Applications for permits are processed in the order received. ***It can take from 3 weeks to more than three months to obtain a permit.*** Many factors contribute to the time it will take to complete and receive an APHIS permit and the processing time ultimately depends on the complexity of the request. The time frames provided below are based on past experiences within the department of plant pathology and estimates provided by APHIS-PPQ.

   a. Registering for a Level 2 eAuthentication account will take 2-3 days.

   b. Completing a permit application form will take one to several hours.

   c. APHIS-PPQ review of application for completeness and evaluation of pest risk will take 2-8 weeks. Permits for select agents require additional paper work and can take up to one year to receive.

d. Containment facility inspection and mitigation of risk will take 1-5 months. However, because we are what APHIS calls a 'super containment facility', Selby Hall is inspected every 3 years. This shortens this step considerably.

e. State Department of Agriculture consolation, inspection and response will take 1-4 weeks. This time frame will be less if the facility has been recently inspected for use with a similar permitted organism.

f. Development of permit conditions and agreement of permit conditions by permittee will take 1-3 days.

g. Issue of final permit will take 1-2 days. An additional week is required for delivery of red and white labels for importation. Red and white labels can be requested from your ePermit account or by sending an email to redandwhitelabelrequest@aphis.usda.gov.

4. Containment Facility inspections

A facility inspection may be required before a PPQ 526 permit can be issued. A PPQ inspector will contact the applicant and arrange an inspection time if the facility must be inspected. The inspector will document aspects of the facility to determine if the facility and equipment are adequate for containment of the organism(s). A PPQ containment specialist will then evaluate the documentation and determine if the facility is adequate. Inspection guidelines can be downloaded at: https://www.aphis.usda.gov/animal_welfare/downloads/Inspection_Requirements.PDF

5. Importation and Shipping Requirements for Importing Regulated Organisms

a. A PPQ 526 Permit is required for the importation into the United States and territories of a plant pest or noxious weed. Permits are required despite the distribution of the organism with the United States.

b. Regulated organisms must be shipped in ***sturdy, leak proof containers by a bonded courier***. Imported shipments must have an original, Red and White shipping label on the outside of the package. These labels are addressed to the Plant Inspection Station. Each label is individually numbered and has a corresponding permit number. ***Detailed instructions for using the labels are given on the back of the label and in the permit.*** Red and White shipping labels are only required for importation, not domestic movement. ***The permit holder is required to account for the use and disposition of each label***, unless the labels are barcoded. For labels that are not barcoded, a label-tracking sheet is provided to the permittee with the label package. The tracking sheet is used to document label use and is returned to the Permit Unit when new labels are requested or when the permit expires. No tracking sheet is needed for barcoded labels.

c. The shipment must be addressed to the Inspection Station listed on the Red and White shipping label and the Red and White shipping label must be attached to the outside of the box.

d. Each shipment must contain a copy of the permit corresponding to the original Red and White shipping label.

e. Permitted articles may be hand carried into the United States only if certain requirements are met. First, ***hand carrying must be requested in the application and authorized in the permit conditions.*** The permit conditions will list the specific steps that must taken in order to hand-carry the articles into the U.S. An authorization to hand-carry shall only be issued to Citizens or permanent United States residents with a valid passport or permanent visa. Hand-carry authorizations shall not be issued to foreign nationals or individuals with temporary visa. A copy of the Hand Carry Policy can be found in Appendix X.

6. Permit conditions and enforcement

   a. Each permit comes with a clearly defined set of conditions.

   b. Upon receipt of the permit all personnel who will be working with the permitted organism will review the permit conditions.

   c. Failure to comply with the conditions of the permit may result in the immediate ***cancellation of one or all permits held by the permitee and the denial of future permits***.

   d. Failure to comply with the conditions of the permit may result in civil penalties and/or criminal prosecution (Federal Plant Protection Act, 2000).

   e. Additional conditions may be placed on the permit for special requests by the permitee (ie. hand carrying of permitted organisms). These conditions will be read and followed carefully. ***Additional civil penalties and/or criminal prosecution may also apply.***

7. General Contact Information

   a. For questions pertaining to **pest permits, soil and noxious weeds** contact permit services:

      Telephone: 301-851-2046 or 866-524-5421
      Email: Pest.Permits@aphis.usda.gov

   b. For questions pertaining to **plant and plant product permits** contact permit services:

Telephone 301-851-2046 or 877-770-5990
Email: plantproducts.permits@aphis.usda.gov

D. Biotechnology Regulatory Services (BRS)

APHIS regulates the introduction (importation, interstate movement, or environmental release) of certain genetically engineered (GE) organisms. All regulated introductions of GE organisms must be authorized by APHIS under either its permitting or notification procedures.

A person may petition the agency that a particular regulated article is unlikely to pose a plant pest risk, and, therefore, is no longer regulated under the plant pest provisions of the Plant Protection Act. More information on notifications and petition requests is at: http://www.aphis.usda.gov/biotechnology/submissions.shtml.epermits.

E. ***Plant Inspection Stations***

Federal regulations require that most imported plants and seeds enter the United States through certain ports of entry. USDA-APHIS-PPQ has 16 plant inspection stations in the United States, all of which are located at or near major international airports and seaports. The inspection stations in Georgia and Maryland are most commonly used to receive imports in Ohio. Contact information for these inspections stations are listed below. A complete list of inspections stations can be found on the APHIS Plant Inspections Stations webpage.

MARYLAND
USDA, APHIS, PPQ
National Plant Germplasm Inspection Station
Building 580, BARC-East
Beltsville, MD 20705
Phone: (301) 313-9327 - Fax: (301) 313-9232

GEORGIA
USDA, APHIS, PPQ
Plant Inspection Station
1220 Toffie Terrace
Atlanta, GA 30354
Phone (404) 260-7830 – Fax: (404) 260-7744

F. Agriculture Select Agent Program:

APHIS Agriculture Select Agent Program:

4700 River Road , Unit 2
Riverdale , MD 20737
Email: AgSAS@aphis.usda.gov
Phone:301-851-3300 (select option 3)
Fax: 301-734-3652

CDC Select Agent Program:

Email: lrsat@cdc.gov
Phone: 404-718-2000, 404-488-7100 (after hours)
Fax: 404-718-2096

VIII. THE INSTITUTIONAL BIOSAEFTY COMMITTEE

A. About The Institutional Biosafety Committee (IBC)

The Institutional Biosafety Committee (IBC) is a peer review process for research involving recombinant DNA and biohazards. Reviews are conducted to assure compliance with federal mandates, institutional policy, accreditation agencies, and / or sponsored program requirements. IBC maintains a "reasonable" balance between institutional research risk compliance, the conduct of research, and providing a safe and healthful work environment.

B. When is an IBC required?

"An IBC is required at all institutions that receive funding from the National Institutes of Health (NIH) for research involving recombinant DNA molecules. ***All recombinant DNA research at The Ohio State University (OSU), regardless of funding source, must be conducted in accordance with the NIH Guidelines for Research Involving Recombinant DNA Molecules and must be registered with the OSU IBC***.

The OSU IBC is further charged with reviewing and approving research conducted with microorganisms pathogenic to humans, plants, or animals. This review is conducted pursuant to the Centers for Disease Control and Prevention (CDC)/NIH publication, Biosafety in Microbiological and Biomedical Laboratories (currently in 5th edition, February 2007).

The OSU IBC in conjunction with the Office of Environmental Health and Safety will also provide guidance to the OSU research community regarding proper acquisition, handling, transfer, and disposal of potentially hazardous or regulated biological materials. The Office of Responsible Research Practices (ORRP) will also provide assistance with IBC registrations and applications." (http://orrp.osu.edu/about/)

C. Where can I find the regulations and guidelines?

Links to Regulations and Guidance for biosafety guidelines at OSU can be found at http://orrp.osu.edu/ibc/ibcregulations/.

D. Who is required to register with the IBC?

Investigators must register with the IBC if they are:

1. Creating recombinant DNA/RNA constructs (vector plus gene).
2. Inserting recombinant DNA/RNA constructs into cell lines/tissue cultures, whole animals, humans, or plants.
3. Using a microorganism that is pathogenic to humans (including immunocompromised individuals), plants, or animals (based on wild-type organism).
4. Using organisms deemed as select agents by APHIS or the CDC.

5. Using or collecting human or non-human primate materials (body fluids, tissues, cells, etc.).

E. Applying for an IBC application

Investigators will first register by submitting an application via the electronic system, eProtocol: orrp.osu.edu/ibc/e-protocolforms/. Using e-IBC you can submit protocols, view your existing IBC protocol records, check the progress of your protocols under review, create new protocol submissions for committee review, and submit reports or annual reviews to IBC.

F. Time line for application submission and review

1. Registering for an IBC profile/account will take 2-3 minutes. You must have an OSU email to register for an IBC profile/account.

2. New applications, responses to deferrals, and any amendments calling for significant changes to approved research require convened committee review and will be discussed at the subsequent monthly meeting. Investigators will receive a response to these submissions within 10 business days of the meeting. All other submissions including exempt applications may be reviewed administratively; approval typically occurs within five business days.

3. All protocols and/or amendments must be submitted for review at the end of business day on the Tuesday the week before the monthly meeting.

4. Reviewers meet monthly at 10 am at the Research Administration Building, 1960 Kenny Road, 1st Floor. Monthly meeting dates are listed on the IBC website:orrp.osu.edu/ibc/ibcmeetings/.

G. Contact Information

For general assistance with IBC submissions you can contact ORRP at 614-688-8457.

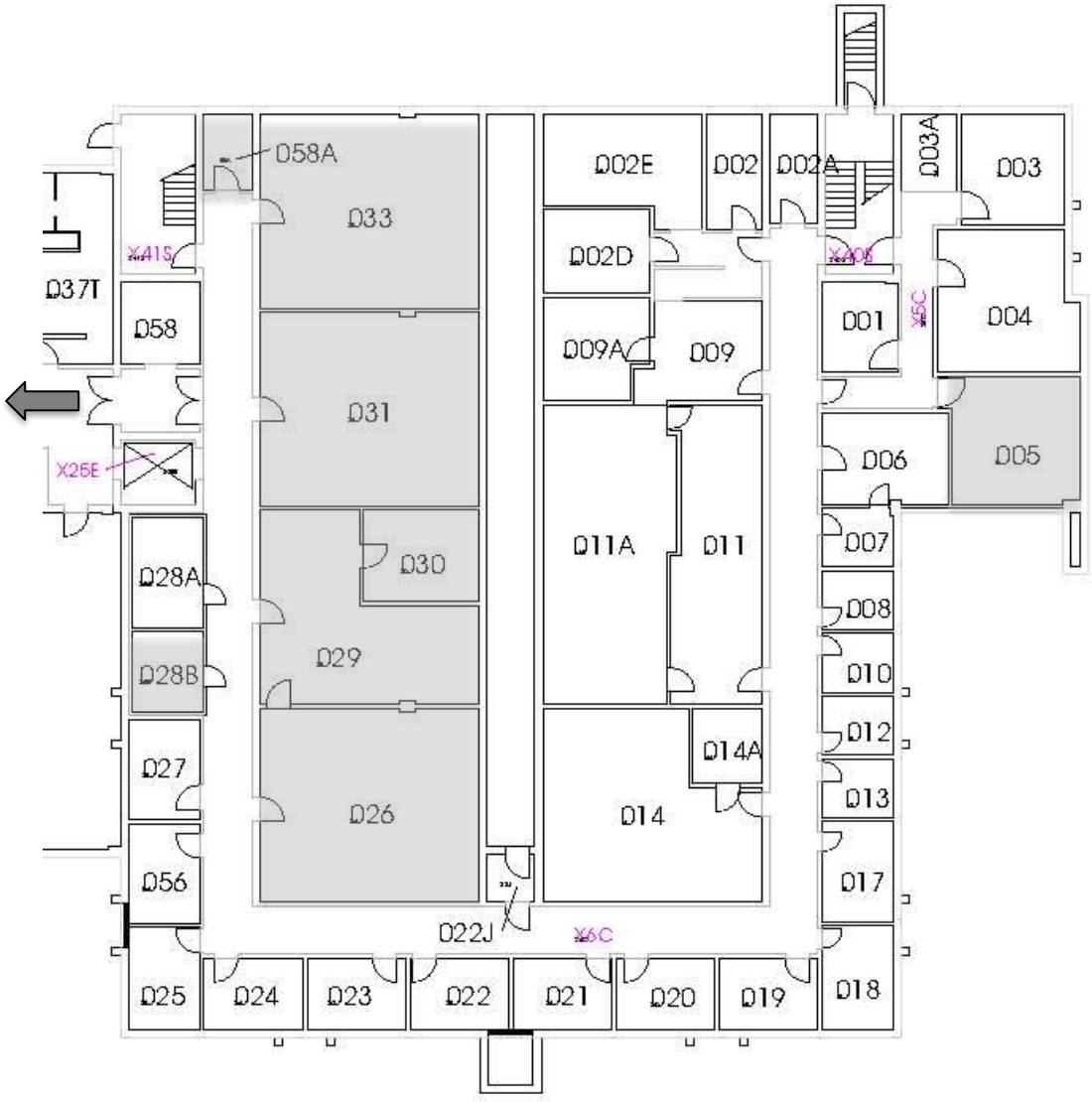
- For assistance with **Biosafety** contact Helen O'Meara, 614-292-0830.
- For assistance with **Human Subjects-General and Regulatory** contact Sandra Meadows, 614-688-8641.

APPENDICIES

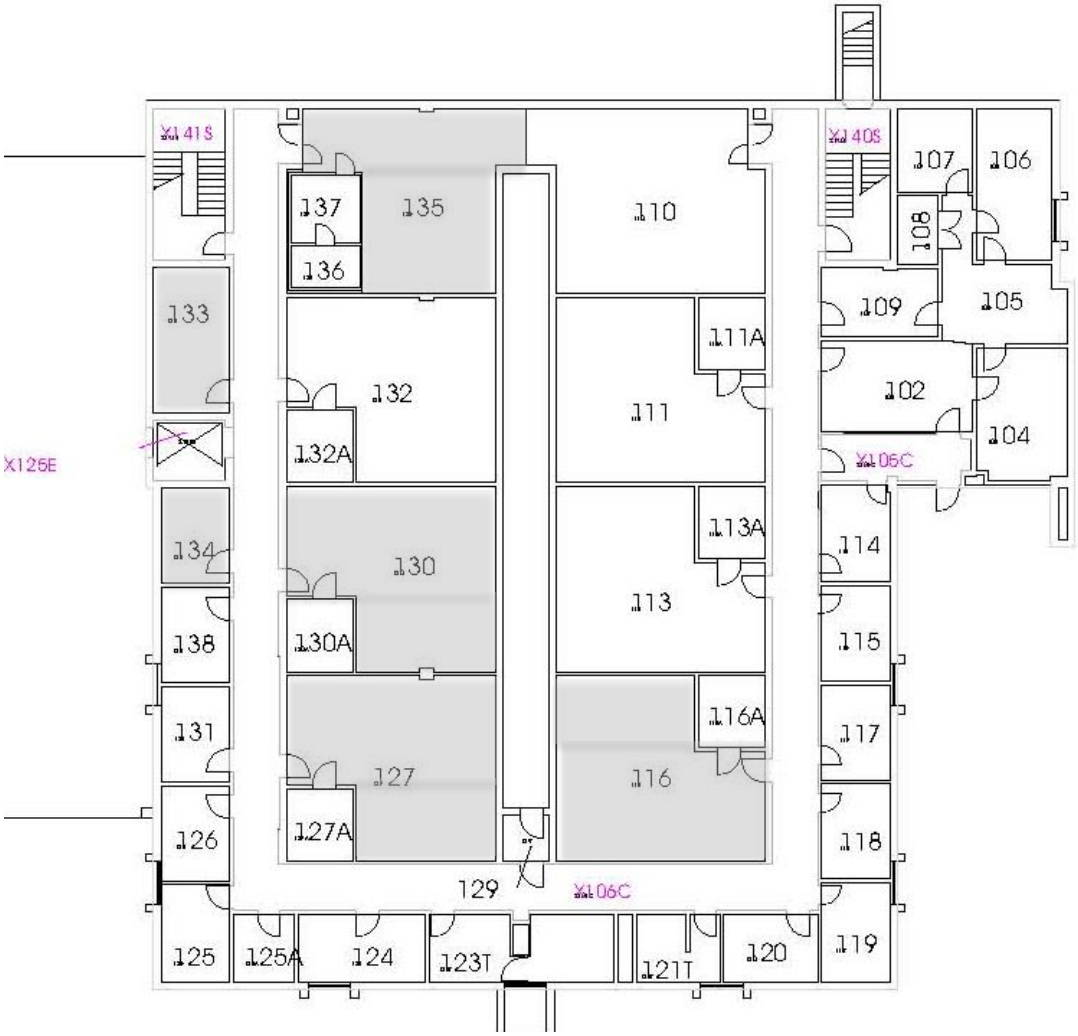
APPENDIX I

Selby Hall floor plans.

A. Floor plan for the ground level of Selby Hall. Grey shading indicates rooms where permitted organisms are cultured, manipulated and/or stored. The greenhouses extend North of the ground level as indicated by the grey arrow.



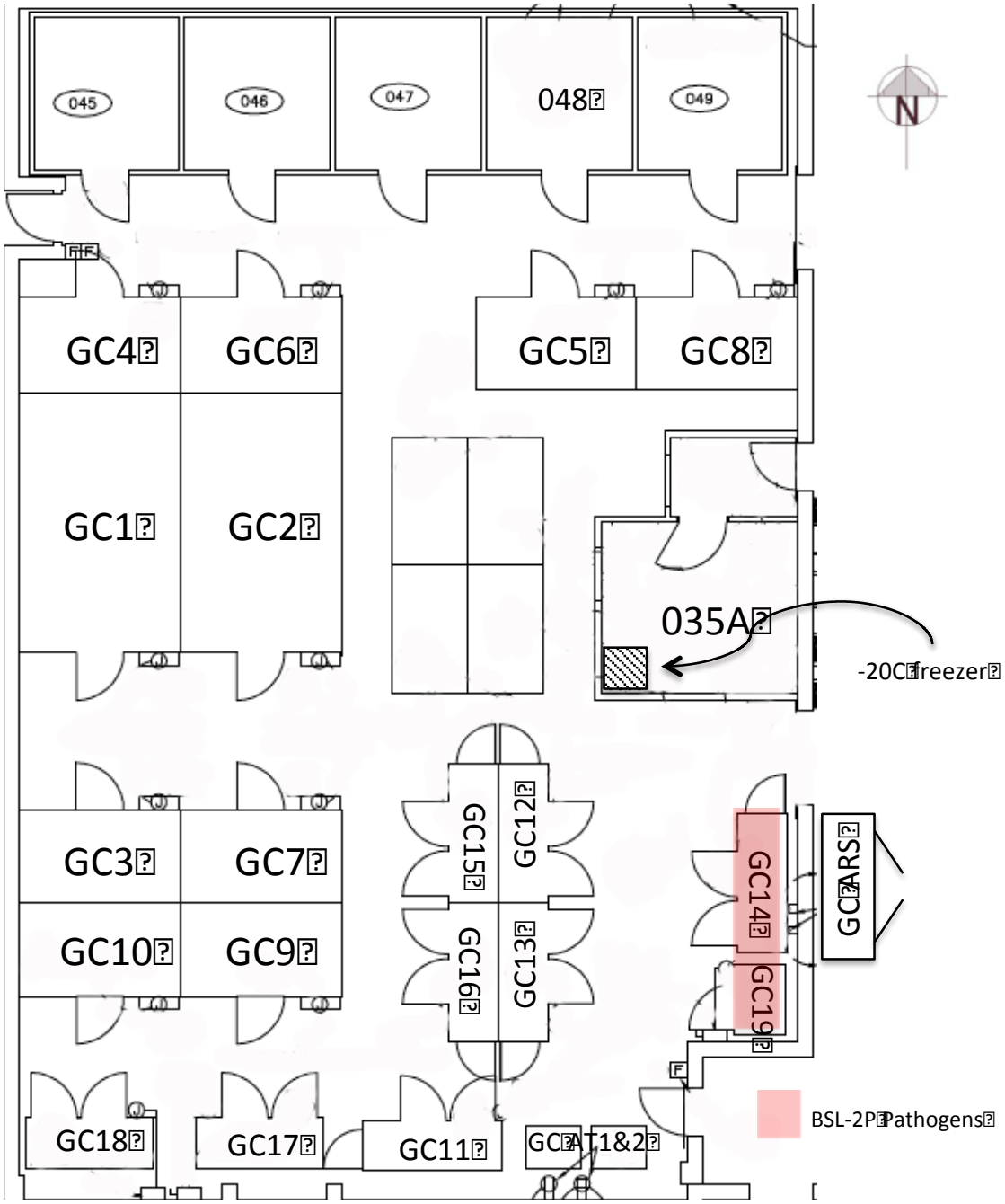
B. Floor plan for the first floor of Selby Hall. Grey shading indicates rooms where permitted organisms are cultured, manipulated and/or stored.



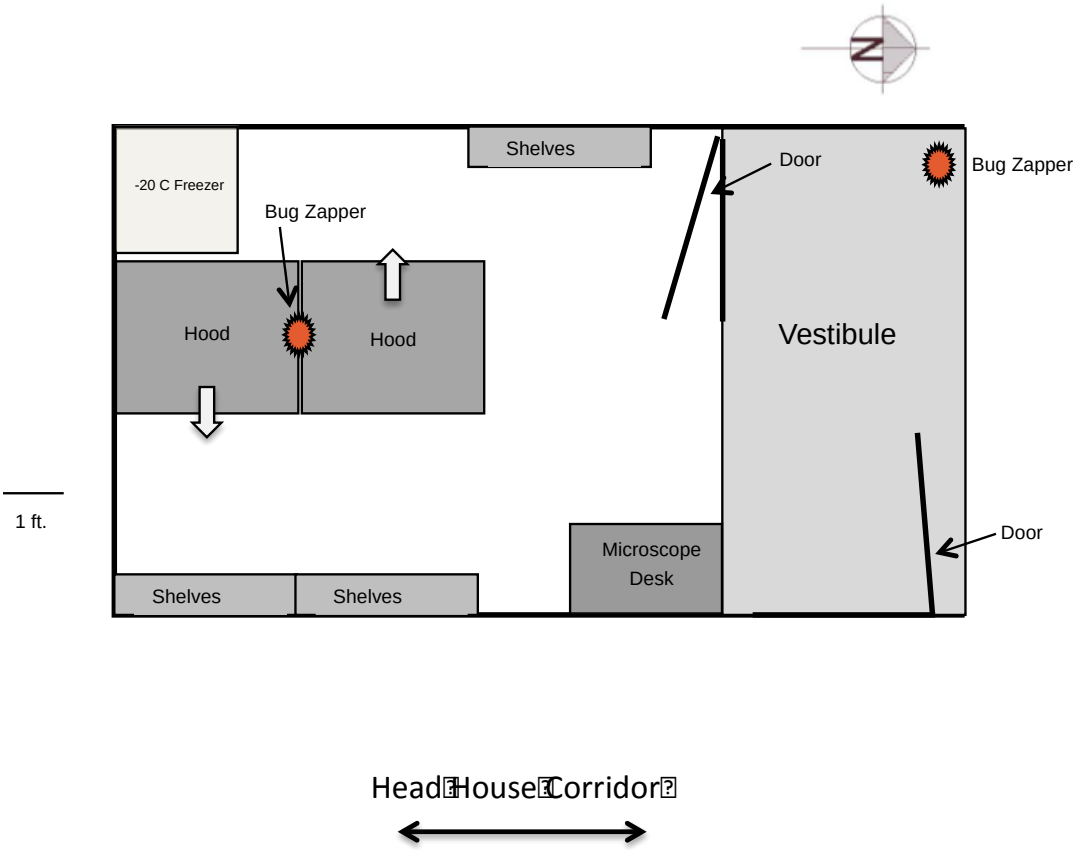
C. Floor plan for the second floor of Selby Hall. Grey shading indicates rooms where permitted organisms are cultured, manipulated and/or stored.

D. Plan for the phytotron, head house and greenhouse complex of Selby Hall. Grey shading indicates rooms where permitted organisms are cultured, manipulated and/or stored. Containment greenhouse G1 and CG2 are indicated. Location of dumpster used to dispose of autoclaved permitted materials (excluding human pathogens), is indicated by a star. The ground level extends South of the phytotron, head house and greenhouse complex as indicated by the grey arrow.

E. Detailed floor plan for the Phytotron (035) in Selby Hall.
All growth chambers (GC) are lockable, and may be requested for work with permitted microorganisms. Chambers indicated by red shading may be used for work with BSL-2 bacterial and viral pathogens. Rooms 045-049 are walk-in coolers and freezers that are not used for permitted microorganisms.

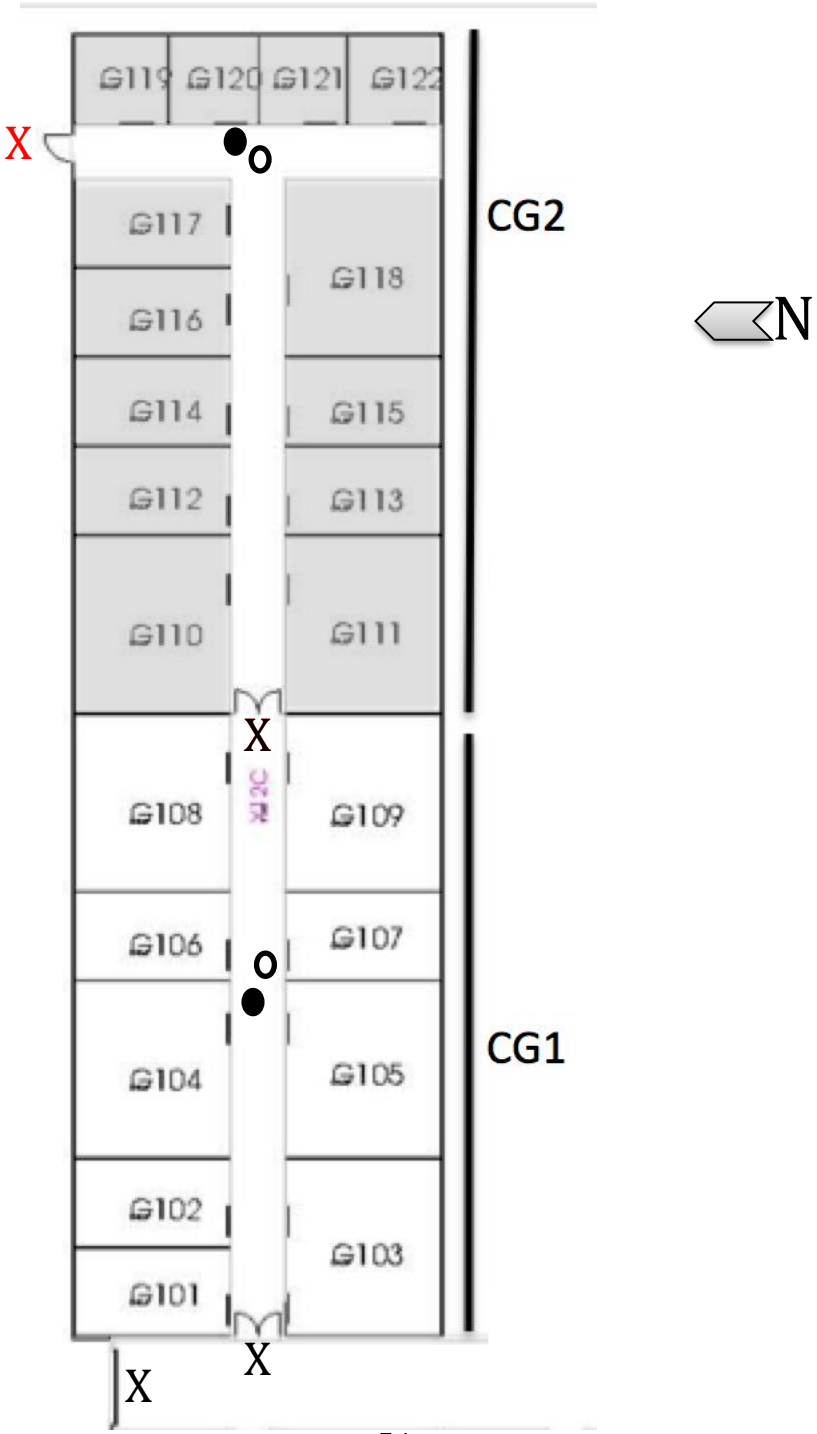


F. Detailed floor plan for the Insect Transfer Room (036) in Selby Hall.



APPENDIX II

The Containment Greenhouse (CG) Complex in Selby Hall.
The facility contains two units: G1 and CG2. Permitted organisms are limited to CG2. Lockable doors are marked with an **X**. Emergency exit is marked with an X (locked from exterior only). Open **O** and blocked drains to city sewer system are marked. Each room has a center drain to the city sewer system. An in-ground, covered pump in each room supplies the cooling pads.



APPENDIX III

Listing of rooms in Selby Hall used for permitted organisms.
Current listing of the rooms in Selby Hall that are used for permitted organisms including the personnel responsible for the room, the activities performed with permitted organisms in the room and any equipment that may be used to work with permitted organisms. GR, ground level; 1, first floor; 2, second level.

Level	Rm	Responsible Individual	Activities	Special Equipment
GR	054	Bob James, Greenhouse Manager	Sterilization Soil preparation and storage	• Autoclave #1
GR, 1	033, 130	Lucy Stewart, ARS Mol. Biol.	Virus inoculations Virus purification and characterization Seed storage	• Inoculators • Locked storage cabinets
GR	005	Tea Meulia, Dir. MCIC	Culture and virus storage	• Liquid N tanks
GR	026	Feng Qu, Assoc. Prof.	Virus purification and characterization	• Fume hood
GR	028B	Lucy Stewart, ARS Mol. Biol.	Virus storage	• Freezers (-80°C)
GR	029	Feng Qu, Assoc. Prof. Peg Redinbaugh, ARS Mol. Biol. Lucy Stewart, ARS Mol. Biol.	Transgenic pathogen transfers and analysis Virus storage	• Class II BSC A/B3 #1.
GR	030, 031	Peg Redinbaugh, ARS Mol. Biol. Lucy Stewart, ARS Mol. Biol.	Virus purification and characterization	• Fume hood
GR	035, 035A	Lee Wilson, Cont. Dir. Melanie Lewis Ivey, Asst. Prof. Peg Redinbaugh, ARS Mol. Biol.	Plant-pathogen interactions	• BSL-2P Growth chambers • Refrigerator (locked)
GR	058A	Peg Redinbaugh, ARS Mol. Biol.	Insect vector rearing	• Insect cages
GR	039	Peg Redinbaugh, ARS Mol. Biol. Anne Dorrance, Prof. Pierce Paul, Prof.	Fungal and oomycetes purification and characterization	
GR	040	Peg Redinbaugh, ARS Mol. Biol. Anne Dorrance, Prof. Chris Taylor, Assoc. Prof.	Nematode purification and characterization	• Microscopes
1	116, 127	Anne Dorrance, Prof.	Fungal and oomycetes purification and characterization	
1	133	Anne Dorrance, Prof.	Tissue and culture growth and storage	• incubation and storage shelves
1	134	Melanie Lewis Ivey, Asst. Prof. Lee Wilson, Cont. Dir.	Experimental analysis of all pathogen groups (excluding human pathogens) Culture transfer, growth and storage	• Class II A2 hood • Incubator • Refrigerator • Storage shelves
1	135	Lee Wilson, Cont. Dir.	Sterilization	• Autoclaves #2
2	202, 202A, 212, 212A	Chris Taylor, Assoc. Prof.	Nematode purification and characterization	
2	219 219A	M. Soledad Benitez Ponce, Asst. Prof.	Plant pathogen purification and characterization. Culture storage	• Laminar flow hood • Fume hood • -80°C freezer
2	222	Lee Wilson, Cont. Dir.	Sterilization	• Autoclaves
2	225	Lee Wilson, Cont. Dir.	Culture storage	• -80°C freezer • Refrigerator

2	229 229A	Melanie Lewis Ivey, Asst. Prof.	Plant pathogen purification and characterization	• Laminar flow hood • Fume hood • Refrigerator
2	232 232A	Melanie Lewis Ivey, Asst. Prof.	Plant and human pathogenic bacteria purification and characterization	• Class II BSC A/B3 #2
2	234 234A	Sally Miller, Prof.	Plant pathogen purification and characterization	• Laminar flow hood • Fume hood

APPENDIX IV

Selby Hall Containment Director and Greenhouse/Phytotron Committee

A. Containment Director

Lee Wilson, Research Associate, Department Safety Officer and Building Coordinator
Telephone: 330-202-3555 ext. 2865
Cell: 330-466-5559
Email: wilson.40@osu.edu

B. Selby Hall Greenhouse and Phytotron Committee (July 2016)

C. Taylor, Chair; 330-263-3847
A. Dorrance; 330-263-3560
M. Redinbaugh; 330-263-3965
L. Madden; (*ex officio*) 330-263-3839

APPENDIX V

Annual review of standard operating procedures.

A. The PI will ensure that personnel involved in the handling of the biohazardous agents are adequately trained as described in the pathogen specific standard operating procedures.

B. All personnel within the department including faculty, staff, students, visiting scientists, post-doctoral associates and interns working with or handling biohazardous agents will be required to partake in at least ***one department-organized annual review workshop per year***. The authors of this document or building coordinator will be responsible for organizing the annual review workshop, including but not limited to organizing the day, time, location and speaker/presenter of the workshop.

C. A faculty member or senior staff person familiar with the rules and regulations of handling biohazardous agents will be responsible for organizing and defining topics covered in the workshop.

APPENDIX VI

Line of communication in an event of a natural or man-made disaster.

A. In the event of a natural or human-made disaster the Ohio State University Disaster Preparedness and University State of Emergency Policy 6.17 (Policy 6.17) guidelines must be strictly followed.

B. If any containment facility becomes compromised as a result of the disastrous event, resulting in the potential accidental release of a permitted pathogen or insect vector into the environment, ***APHIS, PPQ must be immediately notified (301-734-6343***).

C. Following initial contact with APHIS, PPQ a written report (APHIS, PPQ, 4700 River Rd., Unit 133; Riverdale, MD 20737) of the incident must be submitted identifying: (a) the name of the permit holder (responsible person), (b) the permit number, (c) all released organisms identified to at least the species level, (d) the country or State of origin of the organism, (e) the nature of the release, and (f) measures already taken to contain, reduce or limit the effects of the accidentally released organism. Any plans prepared to contain, reduce or limit the effects of the accidentally released organism may be submitted as developed.

D. The USDA-APHIS-PPQ plant health safeguarding specialist in the state of Ohio will be contacted and informed of the disastrous event and of any accidentally released organism. Contact information for the current (as of July 2016) USDA-APHIS-PPQ plant health safeguarding specialist is below.

David L. Lentz, Jr.
Plant Health Safeguarding Specialist
USDA-APHIS-PPQ
Reynoldsburg, OH
419-525-3500 office
419-525-3502 fax
614-546-7059 cell
david.l.lentz@aphis.usda.gov

APPENDIX VII

Personnel training log form.

i. The training log form will be used to record all biosafety trainings completed by personnel handling biohazardous agents.

Training Log

By signing this log you are agreeing that you read and will follow the standard operating procedures and permit conditions of the indicated PPQ permit.

Permit Number	DATE	Personnel	Signature	Supervisor initials

APPENDIX VIII

Insect container log form.

An example of the insect container log form used to monitor the number of cages and locations of permitted insects and vectors with permitted pathogens. The log is maintained with online Corn and Soybean Virus Research USDA APHIS permit records in BuckeyeBox (https://osu.app.box.com/files).

# Cages	Species	Started	Purpose	Ended	Location	Comments
3	*D. maidis*	1/1/16	Maintenance		058	
4	*D. maidis*	1/1/16	Build-up	3/25/26	058	
3	*P. maidis*	1/1/16	Maintenance		058	
1	*G. nigrifrons*	6/20/16	MFSV vector selection		GC7	1 rack tubes
3	*G. nigrifrons*	1/1/16	MFSV maintenance		GC7	
1	*P. maidis*	1/1/16	MMV maintenance		GC7	
4	*D. maidis*	1/1/16	MRFV maintenance		GC15, 16	

APPENDIX IX

Autoclave log form.

i. The autoclave log form will be used to monitor the disposal of permitted materials.

Date	Name	Time Out	Room No.	APHIS Permit (Y or N)	Name of Permitted Organism

APPENDIX X

PPQ-APHIS Hand Carry Policy.

United States Department of Agriculture

Animal and Plant Health Inspection Service

Plant Protection and Quarantine

Plant Health Programs

4700 River Road
Riverdale, MD 20737

Subject: Policy and Procedures for Hand-Carrying Living Organisms under a USDA APHIS PPQ Permit

A specific authorization on a valid USDA APHIS PPQ permit (PPQ Form 526) is required for persons to move any of the following living organisms in personal baggage (i.e. "hand-carry") into the United States or any of its Territories or possessions from any place outside thereof, or from any Territory or possession into any other Territory or possession or the Continental United States: plant pests, Federally listed noxious weeds, parasitic plants, bees, earthworms or biological control agents.

The following considerations apply:

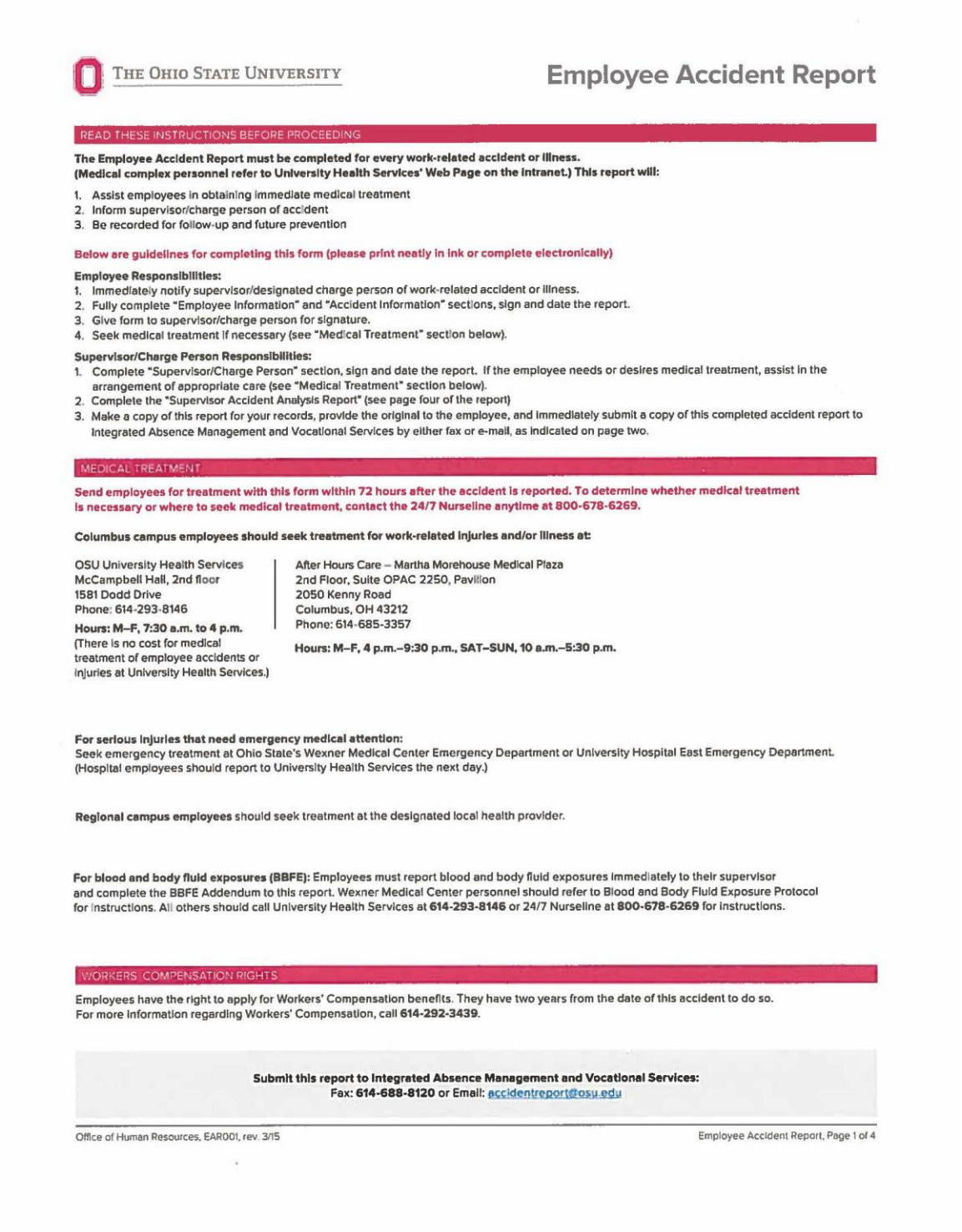
1. A new permit or an amendment to an existing permit authorizing hand-carrying will only be issued to persons transporting organisms to facilities currently approved to receive and contain them, or following an inspection of a newly proposed receiving facility by PPQ personnel and a determination that the facility is physically and operationally adequate to contain the permitted organisms.
2. An authorization to hand-carry shall only be issued to citizens or permanent United States residents with a valid Passport or Permanent Visa. Hand-carry authorizations shall not be issued to foreign nationals or individuals with temporary Visas.
3. Applications must specifically request a hand-carry option, and identify all individuals the applicant seeks to have authorized to hand-carry. Applications must include current address, telephone number, fax number, and email addresses of individuals not associated with the receiving facility who the permit holder seeks to have authorized to hand-carry.
4. An authorization to hand-carry is not transferable and cannot be assigned to other individuals or organizations not identified in the permit.
5. Requests to hand-carry are authorized based on factors including: risk of the organisms to United States agriculture and the environment, country of origin of the shipment, and source of the organisms (e.g., field collected or laboratory reared/processed).
6. An authorization to hand-carry shall be denied for good cause when the desired organisms are deemed to pose exceptional risk, when the facility's containment capabilities are likely to be exceeded, or when there is substantial risk of diversion of organisms from reaching containment. Other factors used in the determination include applicant history in complying with the terms and conditions of prior permits and information provided by the applicant supporting the need to hand-carry.
7. An authorization to hand-carry includes only the organism identified in the permit. Presence of unauthorized organisms in any packages on an individual authorized to hand-carry is a permit violation. Presence of unauthorized organism at the receiving containment facility at any time is also evidence of a permit violation.
8. Denial of a request to hand-carry shall not prejudice the issuance of a permit for receipt of the organism by other means.

Safeguarding American Agriculture
APHIS is an agency of USDA's Marketing and Regulatory Programs

An Equal Opportunity Provider and Employer

APPENDIX XI

The Ohio State University Employee Accident Report is a fillable pdf form available at: https://hr.osu.edu/wp-content/uploads/form-accident-report.pdf.



THE OHIO STATE UNIVERSITY

Employee Accident Report

READ THESE INSTRUCTIONS BEFORE PROCEEDING

The Employee Accident Report must be completed for every work-related accident or illness.
(Medical complex personnel refer to University Health Services' Web Page on the Intranet.) This report will:

1. Assist employees in obtaining immediate medical treatment
2. Inform supervisor/charge person of accident
3. Be recorded for follow-up and future prevention

Below are guidelines for completing this form (please print neatly in ink or complete electronically)

Employee Responsibilities:
1. Immediately notify supervisor/designated charge person of work-related accident or illness.
2. Fully complete "Employee Information" and "Accident Information" sections, sign and date the report.
3. Give form to supervisor/charge person for signature.
4. Seek medical treatment if necessary (see "Medical Treatment" section below).

Supervisor/Charge Person Responsibilities:
1. Complete "Supervisor/Charge Person" section, sign and date the report. If the employee needs or desires medical treatment, assist in the arrangement of appropriate care (see "Medical Treatment" section below).
2. Complete the "Supervisor Accident Analysis Report" (see page four of the report)
3. Make a copy of this report for your records, provide the original to the employee, and immediately submit a copy of this completed accident report to Integrated Absence Management and Vocational Services by either fax or e-mail, as indicated on page two.

MEDICAL TREATMENT

Send employees for treatment with this form within 72 hours after the accident is reported. To determine whether medical treatment is necessary or where to seek medical treatment, contact the 24/7 Nurseline anytime at 800-678-6269.

Columbus campus employees should seek treatment for work-related injuries and/or illness at:

OSU University Health Services
McCampbell Hall, 2nd floor
1581 Dodd Drive
Phone: 614-293-8146
Hours: M–F, 7:30 a.m. to 4 p.m.
(There is no cost for medical treatment of employee accidents or injuries at University Health Services.)

After Hours Care – Martha Morehouse Medical Plaza
2nd Floor, Suite OPAC 2250, Pavilion
2050 Kenny Road
Columbus, OH 43212
Phone: 614-685-3357
Hours: M–F, 4 p.m.–9:30 p.m., SAT–SUN, 10 a.m.–5:30 p.m.

For serious injuries that need emergency medical attention:
Seek emergency treatment at Ohio State's Wexner Medical Center Emergency Department or University Hospital East Emergency Department. (Hospital employees should report to University Health Services the next day.)

Regional campus employees should seek treatment at the designated local health provider.

For blood and body fluid exposures (BBFE): Employees must report blood and body fluid exposures immediately to their supervisor and complete the BBFE Addendum to this report. Wexner Medical Center personnel should refer to Blood and Body Fluid Exposure Protocol for instructions. All others should call University Health Services at **614-293-8146** or 24/7 Nurseline at **800-678-6269** for instructions.

WORKERS' COMPENSATION RIGHTS

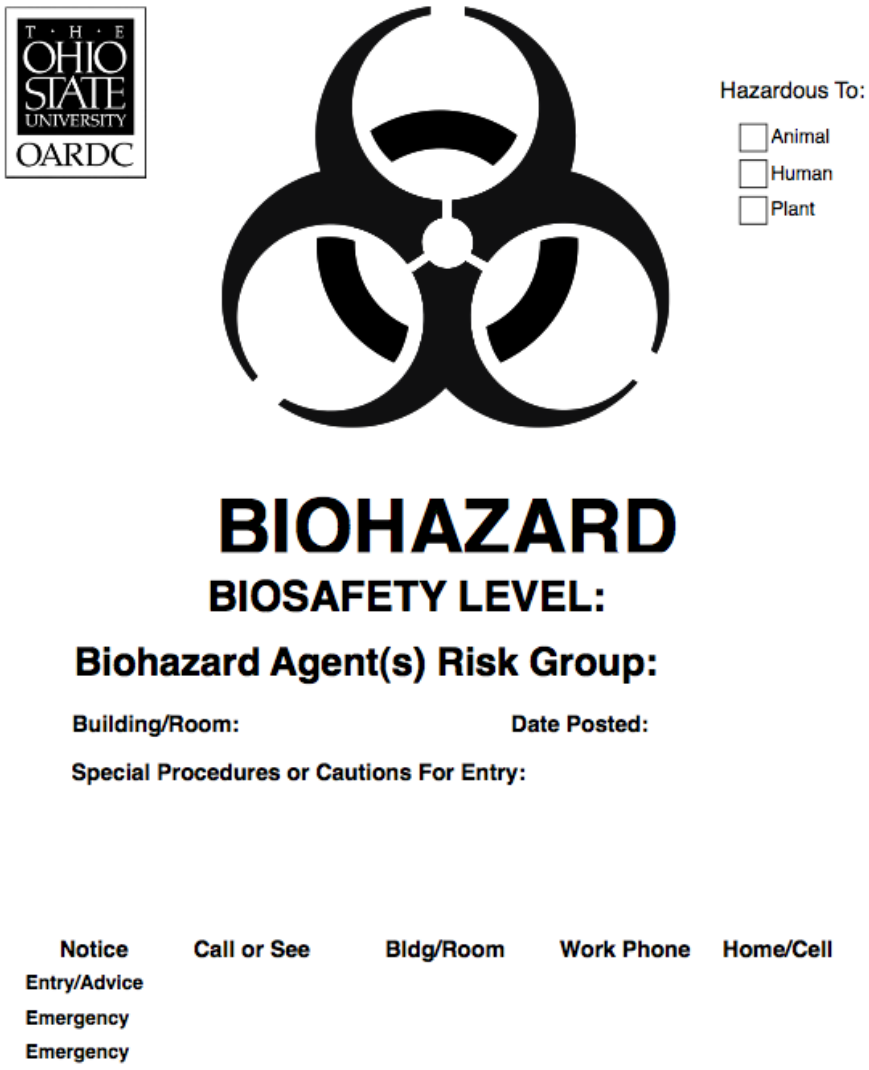
Employees have the right to apply for Workers' Compensation benefits. They have two years from the date of this accident to do so.
For more information regarding Workers' Compensation, call **614-292-3439**.

Submit this report to Integrated Absence Management and Vocational Services:
Fax: **614-688-8120** or Email: accidentreport@osu.edu

Office of Human Resources, EAR001, rev. 3/15 — Employee Accident Report, Page 1 of 4

APPENDIX XII

Biohazard sign for The Ohio State University/OARDC.
i. The sign will be printed on color paper (orange or red) and posted in all rooms within the department that use biohazardous materials.



APPENDIX XIII

Standard Operating Procedure for working in the biosafety cabinets.

Working in a Biosafety Cabinet (Class II / A2)

1. Slowly raise the sash until the bottom of the sash aligns with the sash indicator decal located on the left side of the work area.
2. Turn on lights and blower.
3. Check the air grilles for obstructions
4. Allow the cabinet to warm up for about 90 seconds.
5. Please wear appropriate personnel protective equipment (PPE) when working in this cabinet.
6. Wipe Down surface: To do this, raise the sash to its full open position with cabinet running. Wipe the surface area with a disinfectant (70% ethanol). Allow to dry.
7. While working keep all materials at least 4 inches (100 mm) inside the sash while preforming all contaminated work as far back into the hood as possible.
8. Segregate all clean and contaminated materials in the work area.
9. Use proper aseptic technique.
10. Avoid conducting work that disrupts the airflow pattern of the cabinet.
11. If there is a spill or splatter during use, all objects in the cabinet should be surface decontaminated before removal. Thoroughly disinfect the working area of the cabinet WHILE IT IS STILL IN OPERATION, to prevent the release of contaminants from the cabinet.
12. Once you are done working allow the cabinet to run for 2 or 3 minutes to purge contaminants from the work area.
13. Close the sash and turn off the light and blower.

Thank you!

Lee
Selby Hall Safety Officer
Room 229, x2865, wilson.40@osu.edu

APPENDIX XIV

1. Selby Hall

2. Phytotron (room 035)

3. Phytotron signage

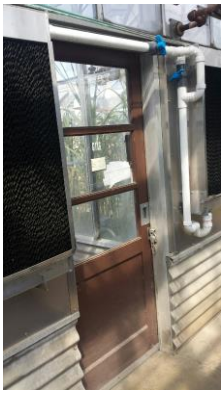
4. BSL-2 growth chamber (room 035)

5. Greenhouse access doors from head house

6. Greenhouse exterior garage door

7. Greenhouse side doors and head house access doors

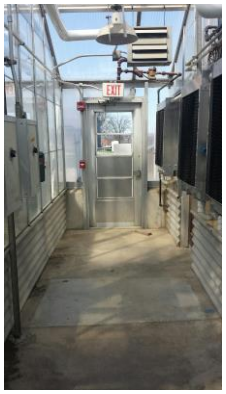
8. Containment greenhouse (G1 and CG2) access door



9. Containment greenhouse (G1) individual room door

10. Containment greenhouse (CG2) main access door (remains locked)

11. Airlock space between G1 and CG2



12. CG2 emergency exit door (interior view)

13. CG2 emergency exit door (exterior view)

14. CG2 exterior view

15. Greenhouse concrete exterior walls and polycarbonate panels

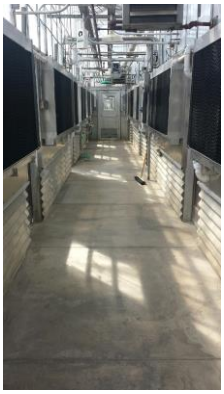
16. Greenhouse room concrete floors

17. Fin-tube radiators

18. North-facing air intake (top of greenhouse)

19. Anti-insect screens on North-facing air intake

20. Circulation/exhaust fan



21. Fiber water percolation pads

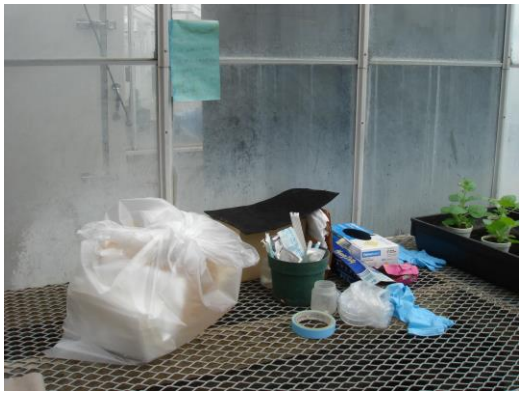
22. Screened exit plenums (CG2 houses only)

23. Open exit plenums (G1 only)

24. Inactive (left) and active (right) drains

25. Water drain in greenhouse room concrete floor.

26. Greenhouse bench



27. Laboratory supplies stored within CG2

28. Inoculated plants in CG2 contained within trays to avoid water run-off

29. Room 058A showing bug zapper.

30. Insect-escape-proof rearing cages

31. Sealable containers to transport insect-infested plants

32. Entry door to 036 insect transfer room (within vestibule)

33. Bug zapper in 036 vestibule leading to insect transfer room

34. Bug zapper and insect transfer hood (room 036A)

35. Culture growth and storage room with ClassII/A2 biocontainment hood (room 134, remains locked)

36. -80°C freezer in culture growth and storage room (room 134, remains locked)

37. Certified autoclaves (room 135)

38. Certified autoclave (front, room 222)

39. Signage indicating non-certified autoclave (not for APHIS permitted materials)

40. Certified autoclave (head house)

41. Class II BSC A/B3 laminar flow hood (rooms 029)

42. Liquid nitrogen storage (MCIC, Room 005)

43. Walk-in incubator (room 133, remains locked)

APPENDIX XV

i. Abbreviations of terms frequently used in this document.

APHIS:	Animal and Plant Health Inspection Service
BSL:	Biosecurity Level or Biohazardous Risk Group
BSL-P:	Biosecurity Level or Biohazardous Risk Group-Plant Containment
CG:	Containment Greenhouse
GC:	Growth Chamber
GOP:	General Operating Procedure
IBC:	Institutional Bio-safety Committee
MCIC:	Molecular and Cellular Imaging Center
PI:	PI
PPQ:	Plant Protection and Quarantine
SOP:	Standard Operating Procedure