



**Working with Permitted Plant Pathogens, Insect
Vectors, Nematodes, Human Pathogens and
Recombinant Microorganisms in Plant Pathology
Selby Hall
APHIS Facility 324**

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December 2010

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I. 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 ten years the Department of Plant Pathology (Wooster) has applied for and received a number of PPQ permits. For each permit we must prepare a standard operating procedure (SOP) outlining how we will receive, handle, and store the pathogen to prevent accidental escape. To ensure that our facility and procedures are in compliance, we are routinely inspected by APHIS and Ohio Department of Agriculture personnel. As our need to work with pathogens that are restricted has increased, the number of SOPs prepared and inspections performed has also increased. The objective of this document is to provide both a departmental general operating procedure (GOP) and SOPs 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 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 Institutional Bio-safety Committee (IBC) resources.

II. FACILITIES

Selby Hall is listed by USDA, APHIS as Facility 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 (color 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). The building houses the Department of Plant Pathology, the USDA-ARS Corn and Soybean 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 time outside of working hours.

A. Selby Hall Laboratories and Rooms Used for Permitted Organisms

i. Floor Plans

Floor plans for ground, first and second levels of Selby Hall are shown in Appendix I, Figures 1A-C. Rooms where permitted organisms are received, cultured, manipulated or stored are indicated by grey shading. The individual principle investigator (PI) or personnel responsible for each room and the equipment available in each room is listed in the table in Appendix III.

ii. Signage

1. 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.
2. 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.

B. Selby Hall Phytotron

i. Growth chambers

1. Lockable growth chambers (GC) are located near the Selby Hall head house in rooms 035 and 035A (Appendix XIII, color plate 2).
2. Access to the growth chambers can be made from one exterior door and three interior doors (two in the head house and one connected to the ground level laboratories).
3. The phytotron has twenty-one growth chambers of various sizes.
4. Two of the growth chambers have been authorized by the OARDC Safety Committee for use with human pathogens of biohazardous risk group 2 (BSL-2). The growth

chambers are clearly marked with OARDC approved biohazard signage (Appendix XIII, color plates 3 and 4).

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

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

ii. Insect rearing growth chamber

1. Growth chambers used for insect rearing contain sealed light fixtures to prevent the accidental escape of insects.

2. Yellow sticky insect traps hang from the ceiling outside of the GC to trap any insects that may escape.

3. 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, an exterior garage door or 7 side door entrances (Appendix XIII, color plates 5, 6 and 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 Plant Pathology personnel carrying a departmental key.

D. Selby Hall Containment Greenhouse

i. Floor plan

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

2. The Containment Greenhouse (CG) contains two units: CG1 and CG2. Access to both units is through a lockable door (marked as X on the floor plan, Appendix II; Appendix XIII, color plate 8). Access to CG1 is through a locked screened door and contains greenhouse (G) rooms G101, G102, G103, G104, G105, G106, G107, G108 and G109. Each room has its own separate entrance (color plate 9). Access to CG2 is from CG1 via a locked door (color plate 10) and contains rooms G110, G111, G112, G113, G114, G115, G116, G117, G118, G119, G120, G121 and G122. Each room has its own separate entrance (color plate 11). An air lock entry separates each unit (CG1 and CG2; color plate 12). Unless otherwise noted, permitted organisms are limited to CG2. There

is an emergency exit (exterior door) in CG2, which cannot be opened from the outside to prevent unauthorized entry (Appendix XIII, color plate 13).

ii. Physical containment standards

1. 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).
2. The location is more than 500 yards from commercial production fields.
3. The location is more than 300 yards from OARDC research plots.
4. The location is more than 40 miles from a commercial international airport.
5. The north, east and south sides are open (no barrier) and the west side is the greenhouse corridor (Appendix XIII, color plate 14).

iii. Structural description

1. 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 XIII, color plate 15).
2. Ceilings consist of 0.5 in.-thick polycarbonate panels.
3. Corridor floors are poured concrete. Room floors have a center aisle of poured concrete. Area under the benches is covered with a 6 in. layer of gravel (Appendix XIII, color plate 16).
4. Exterior and corridor walls are translucent, 0.5 in.-thick polycarbonate panes or panels.
5. Heating is provided by fin-tube radiators located on the outer walls of individual rooms (Appendix XIII, color plate 17).
6. Ventilation is provided by an outside air intake through a north-facing ridge vent in the central corridor (Appendix XIII, color plate 18).
7. Incoming air passes through two layers of antiviral screen (0.009 in., 1:1 ratio, 0.0105 in. x 0.0502 in.; Appendix XIII, color plate 19). Air is drawn into the individual rooms by circulation/exhaust fans located on the outside walls of the rooms (Appendix XIII, color 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 XIII, color 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 antiviral screen for CG2 compartments. No screens are present for the exit plenums of CG1 (Appendix XIII, color plate 22). The CG does not operate under negative pressure.

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

9. Hydrants are available in each room to provide water for plants. Open drains to city sewer system are indicated as open circles (○; Appendix II). Closed circles (●) are blocked (inactive) drains (Appendix XIII, color plate 23). Unless described otherwise in specific permits, water draining from pots is filtered through the gravel layer and earthen soil beneath the benches (Appendix XIII, color plate 24).

10. Benches are constructed of thick metal mesh attached to 2 in. by 4 in. wood frames mounted on concrete blocks (Appendix XIII, color plate 25).

11. A record of maintenance and repairs within CG1 and CG2 is maintained by the greenhouse manager and can be provided upon request.

iv. Signage

1. "Authorized Personnel Only" is posted on the entry door to CG1 and CG2.
2. An illuminated exit sign is posted on the CG2 emergency exit.
3. The PI and the name and extension number of the contact person for the existing project are posted on the room doors.
4. Special instructions for the existing project are posted on the room doors.

v. Disposal of Contaminated CG2 Materials

1. Soil, discarded plant material and miscellaneous lab materials (gloves, stakes ect.) are contained within biohazardous 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.
2. Used pots and collection trays are soaked in a 27% Clorox solution for five minutes. Pots will be completely dried prior to use.

vi. Disinfestation of CG2 Rooms

1. Upon completion of a project or expiration of the pathogen permit, materials in each room are disposed of according to the methods described in II-D-v.
2. Individual rooms are disinfested by the greenhouse manager. Work orders must be submitted to the head house manager prior to disinfestation. A copy of a work order can be found in Appendix X.
3. Benches, interior and exterior doors and door handles are sanitized with a sodium hypochlorite (1:1) treatment. Rooms may be re-occupied after a minimum of four hours drying time.

III. GENERAL OPERATING PROCEDURES

i. 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 or recombinant microorganism can be found in section IV of this document.
3. The PPQ permit number will be included on the first page of the SOP of the permitted culture.
4. The Institutional Bio-safety Committee of The Ohio State University (OSU-IBC) protocols will be attached to the SOP.

ii. 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.

iii. 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.

iv. Autoclaving

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, color plates 26, 27 and 28). Autoclaves housed in rooms 135 and 222 are maintained by the departmental research assistant. The head house 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 by the OARDC facility services department and one time per year by a STERIS Corporation (5960 Heisley Road, Mentor, Ohio 44060;

440-354-2600) technician. ***Only these autoclaves should be used to destroy permitted cultures.*** Autoclaves that are not to be used for permitted cultures are clearly signed (Appendix XIII, color plate 29).

3. The internal temperature of the A.K. Robins 240EF autoclave in the head house is checked at least one time per year with a biological indicator test such as Spordi* Test Kit (STERIS Corp.).

4. Cultures will be autoclaved for a minimum 20 minutes at 121 C (15 psi) 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.

v. 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, 125A or 134 (Appendix XIII, color plate 30). Class II BSC A/B3 laminar flow 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 C freezers located in rooms 029 and 225 (Appendix XIII, color plate 31) or in liquid nitrogen storage located in room 005 (Appendix XIII, color plate 32) 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.

vi. Personnel training

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

1. All personnel will be trained by the principle investigator or by the person named on the PPQ permit and/or OSU-IBC protocols.

2. All personnel will read the conditions attached to the PPQ permits and/or OSU-IBC protocols.

3. All personnel will read the SOP for the PPQ permit and/or OSU-IBC regulated organisms located in the principle investigators laboratory and sign a form (Appendix VII) indicating that they read and understand the bio-safety regulations 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 facilities.

vii. Containment director

1. The containment director 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 containment director will maintain and update the department SOP manual.

3. The containment director 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 containment director will maintain copies of all active PPQ permits and/or OSU-IBC protocols held by permittees in Selby Hall. ***Permittees will be responsible for providing copies of all current permits to the containment director.***

5. The containment director will maintain contact information for all permittees within Selby Hall.

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

viii. In the case of an Emergency

1. In the event of an unintentional release of permitted organisms the CG 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 CG, plants that remain will be placed in double sealed containers and locked in another part of Selby Hall. The containment director will be contacted immediately.

3. In the case of vandalism in the CG or storage rooms, the OARDC police and the containment director 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 containment director.

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

1. Standard operating procedure for plant pathogenic fungi

i. 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 125A or 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 125A are present inside and outside the safety cabinet and will be used to decontaminate both the cabinet and the laboratory. All contaminated material including gloves, tips, tubes, Petri plates and infected plant tissue will be placed in autoclavable containers and autoclaved before re-use or disposal. Cultures will be stored or grown in laboratory 134 and **not in laboratory 125A**. Molecular biology work will be performed in laboratory 134 or the assigned laboratory of the principle investigator.

ii. Fungal growth

Fungal cultures will be placed in closed, unbreakable plastic containers in laboratory 125A and transferred to a designated bench within the principle investigators laboratory or to an incubator in rooms 133 or 134 (Appendix XIII, color plate 34). 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.

iii. Fungal-inoculated plant growth

Plants, seedlings, and/or leaflets inoculated in laboratory 125A 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 or 134, 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 and/or sterilized under UV light in laboratory 125A prior to washing. Pots containing inoculated plants will be placed in plastic trays to avoid water run-off (Appendix XIII, color plates 35 and 36). Designated disposable laboratory coats, gloves, and pipette tips will be used and stored in the greenhouse compartment. All contaminated material will be stored in closed unbreakable containers and autoclaved before disposal. All plant material and soil from the greenhouse will be steam-sterilized twice before disposal.

iv. Culture storage and disposal

Cultures initially received will be opened in containment Class II BSC A/B3 laminar flow safety cabinets located in laboratories 125A or 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 C freezer located in room 225 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 OARDC dumpster located outside of the Selby Hall soil room.

v. Personnel training

The principle investigator 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 principle investigators 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 fungi. An annual review of Selby Hall standard operating procedures will be required by all personnel involved in handling biohazardous agents (see Appendix V).

vi. 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:

1. Liquid spills will be wiped with absorbent paper or cloth and the contaminated material will be placed in an autoclave bag, sterilized twice and disposed of according to the procedures described in section *III-iv* 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.
2. Solid material will be carefully removed from the surface using gloved hands, enfolded in absorbent paper or cloth placed in an autoclave bag, sterilized twice and disposed of according to the procedures described in section *III-iv* 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.

3. The spill and the completed clean-up will be reported to the principal investigator and containment director.

vii. 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 principle investigator and containment director will be contacted and The Ohio State University Wooster Campus Employee Accident Report completed (Appendix XI).

2. Standard operating procedure for plant pathogenic bacteria

i. 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 125A. 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 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 125A are present inside and outside the safety cabinet and will be used to decontaminate both the cabinet and the laboratory. All contaminated material including gloves, tips, tubes, Petri plates and infected plant tissue will be placed in autoclavable containers and autoclaved before re-use or disposal. Cultures will be stored or grown in laboratory 134 and ***not in laboratory 125A***. Molecular biology work will be performed in laboratory 134 or the assigned laboratory of the principle investigator.

ii. Bacterial growth

Bacterial culture plates will be placed in closed, unbreakable containers in laboratory 125A and transferred to a designated bench within the principle investigators laboratory or to laboratory 134. 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.

iii. Bacterial-inoculated plant growth

Plants, seedlings, and/or leaflets inoculated in laboratory 125A 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 and/or sterilized under UV light in laboratory 125A prior to washing. Pots containing inoculated plants will be placed in plastic trays to avoid water run-off. Designated disposable laboratory coats, gloves, and pipette tips will be used and stored in the greenhouse compartment. All contaminated material will be stored in closed unbreakable containers and autoclaved before disposal. All plant material and soil from the greenhouse will be steam-sterilized twice before disposal.

iv. Culture storage and disposal

Cultures initially received will be opened in containment Class II BSC A/B3 laminar flow safety cabinets located in laboratory 125A. 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 C freezer located in room 225 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 OARDC dumpster located outside of the Selby Hall soil room.

v. Personnel training

The principle investigator 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 principle investigators 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. An annual review of Selby Hall standard operating procedures will be required by all personnel involved in handling biohazardous agents (see Appendix V).

vi. 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:

1. Liquid spills will be wiped with absorbent paper or cloth and the contaminated material will be placed in an autoclave bag, sterilized twice and disposed of according to the procedures described in section *III-iv* 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.
2. Solid material will be carefully removed from the surface using gloved hands, enfolded in absorbent paper or cloth placed in an autoclave bag, sterilized twice and disposed of according to the procedures described in section *III-iv* 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.
3. The spill and the completed clean-up will be reported to the principal investigator and containment director.

vii. 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 principle investigator and containment director will be contacted and The

Ohio State University Wooster Campus Employee Accident Report completed (Appendix XI). Professional health care will be sought if necessary.

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

i. 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 BSC A/B3 laminar flow safety cabinet available in laboratory 139 in the Food Animal Research Program Building on the OARDC 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.

ii. Bacterial-inoculated plant growth

Plants, seedlings, and/or leaflets will be inoculated within the BLS-2 growth chambers located in room 035 of Selby Hall (Appendix XIII, color plates 37 and 38). 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. Designated disposable laboratory coats, gloves, and pipette tips used in room 035 will be stored in closed unbreakable containers below or within the growth chambers. All contaminated material will be stored in closed unbreakable containers and autoclaved before disposal. All plant material and soil from the greenhouse will be steam-sterilized twice before disposal. The plastic trays and covers and/or the closed containers will be surface decontaminated using 10% sodium hypochlorite. Personnel should 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 area.

iii. Signage

A hazard warning sign incorporating the universal biohazard symbol will be posted on all access doors in areas that BSL-2 bacterial pathogens are manipulated or stored. The hazard sign will identify the hazardous agent and list the names and telephone numbers of the principle investigators and any other responsible personnel. A copy of the hazard warning sign can be found in Appendix XII.

iv. Culture storage, transport and disposal

Cultures initially received will be opened in a Class II BSC A/B3 laminar flow safety cabinet available in laboratory 139 in the Food Animal Research Program Building on the OARDC campus. Disposable packaging will be destroyed by autoclaving (room 135 in Food Animal Research Program Building). Containers containing infectious agents must be placed in labeled, sealed, leak-proof containers for transport to the BSL-2 growth

chambers located in room 035 of Selby Hall. These containers will be carried within an additional unbreakable container on either a laboratory cart or state vehicle. All cultures will be stored in a locked -80 freezer in laboratory 139 in the Food Animal Research Program Building. After completion of experiments or when the permit expires, all cultures will be destroyed by autoclaving (121 C, 15 psi for 60 min.) or by the method indicated in the pertaining permit.

v. Personnel training

The principle investigator will ensure that the personnel involved in the handling of the biohazardous agents are adequately trained. Personnel may be required to complete human subjects training and be certified on the University Collaborative Institutional Training Initiative (CITI) program. 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 will be taught about the hazard of biological agents, how to handle them and how to dispose of them. In accordance to departmental policy, a SOP for each permitted BSL-2 human bacterial pathogen will be prepared and distributed to relevant personnel. A copy of the 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. A copy of the 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 principle investigators laboratory indicating that they have been informed of the hazards of working with biohazards and the proper procedures for working with BSL-2 human pathogenic bacteria. An annual review of Selby Hall standard operating procedures will be required by all personnel involved in handling biohazardous agents (see Appendix V).

vi. Spills

Spills or accidents involving biohazardous BSL-2 human bacterial pathogen materials must be immediately reported to the principle investigator or the laboratory supervisor if the principle investigator 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:

1. 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 section III-iv 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.
2. 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 III-iv 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.

3. 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).

4. Completion of clean-up will be reported to the principle investigator.

vii. Ingestion, inhalation or self-inoculation

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

4. Standard operating procedure for insect vectors

i. Insect rearing and live insect handling

Insects reared on plants are confined within insect-escape-proof rearing cages (Appendix XIII, color plate 39) in room 058A and growth chamber 2 in room 035. Insects are transported within these cages to room 033 or 058 for handling and manipulation. Insects are released, manipulated, captured and transferred to other cages within insect transfer cabinets (Appendix XIII, color plate 40) located in room 033. Room 033/058A is equipped with yellow sticky traps and insect zappers to trap any insects that escape from the transfer cabinet. Caged insects and plants are returned to room 058A or growth chamber 2 in room 035. Insects in the system are tracked using an insect container log form (Appendix VIII).

ii. Pathogen-infected Plant Growth

Insect-exposed plants that need to be transported to containment greenhouse 2 (CG2) will be inspected for insects inside the transfer cabinets, and subsequently placed into large plastic containers with sealable lids. The plants will be transported to room 112 of CG2, removed from the containers and sprayed with pyrethroid insecticide using manufacturers labeled rates, to kill any insects that may be remaining on the plants. After spraying, plants will be either transported to room 111 or 122 of CG2. Rooms 111 and 122 are sprayed weekly 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.

iii. 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 room 033/058A. Liquid nitrogen storage is located in room 005 within the MCIC facility. For freezing, vials containing the insects or plant material will be dropped into liquid nitrogen for 5 min. For fixation, insects will be paralyzed using CO₂ in room 033/058A then transported in closed tubes and/or vials within small disease transport chambers to the chemical fume hood in room 031 for transfer to fixative. Molecular studies or microscopy is performed on killed insects and plant material within the laboratory of the principle investigator or the MCIC (rm 003-006).

iv. Culture Storage and Disposal

All killed insects and plant material are stored long-term in either a -80 C freezers located in rooms 028B or in liquid nitrogen storage located in room 005 within the MCIC facility. Freezers and room 005 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 any unused insects or plant material will be frozen with a locked -20 C freezer located in room 035. Frozen insects and plant material will then be destroyed by autoclaving or by the methods indicated in specific permits. Autoclaved materials will be disposed of in the OARDC dumpster located outside of the Selby Hall soil room.

v. Personnel training

The principle investigator 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 principle investigators laboratory indicating that they have been informed of the hazards of working with biohazards and the proper procedures for working with insect vectors. An annual review of Selby Hall standard operating procedures will be required by all personnel involved in handling biohazardous agents (see Appendix V).

5. Standard operating procedure for plant pathogenic Oomycetes

i. Oomycete 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 125A. 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 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 125A are present inside and outside the safety cabinet and will be used to decontaminate both the cabinet and the laboratory. All contaminated material including gloves, tips, tubes, Petri plates and infected plant tissue will be placed in autoclavable containers and autoclaved before re-use or disposal. Cultures will be stored or grown in laboratory 134 and **not in laboratory 125A**. Molecular biology work will be performed in laboratory 134 or the assigned laboratory of the principle investigator.

ii. Oomycete Growth

For culturing, oomycete transformants will be placed in closed plastic containers and transferred to an incubator in room 133. This incubator will be labeled with appropriate biohazard signs and remain locked when not in use. Regularly, the plastic boxes will be surface decontaminated and sterilized under UV light in room 125A.

iii. Oomycete-inoculated Plant Growth

Plants, seedlings, and/or leaflets inoculated in laboratory 125A 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 rooms 133 or 134 or designated growth chambers. Disease symptoms will be examined directly through the transparent covers. The plastic trays and covers and/or the closed containers will be surface decontaminated using 5% sodium hypochlorite and/or sterilized under UV light in laboratory 125A prior to washing.

iv. Culture storage and disposal

Cultures initially received will be opened in containment Class II BSC A/B3 laminar flow safety cabinets located in laboratories 125A or 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 C freezer located in room 225 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 will be disposed of in the OARDC dumpster located outside of the Selby Hall head house.

v. Personnel training

The principle investigator 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 principle investigators 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. An annual review of Selby Hall standard operating procedures will be required by all personnel involved in handling biohazardous agents (see Appendix V).

vi. 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:

1. Liquid spills will be wiped with absorbent paper or cloth and the contaminated material will be placed in an autoclave bag, sterilized twice and disposed of according to the procedures described in section *III-iv* 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.
2. Solid material will be carefully removed from the surface using gloved hands, enfolded in absorbent paper or cloth placed in an autoclave bag, sterilized twice and disposed of according to the procedures described in section *III-iv* of this document. After the solid has been removed, the area in contact with the solid 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.
3. The spill and the completed clean-up will be reported to the principal investigator and containment director.

vii. 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 principle investigator and containment director will be contacted immediately. Professional health care will be sought if necessary.

6. Standard operating procedure for plant pathogenic viruses

i. 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 laboratories 035, 026, 029, 014, or CG (1 and 2) 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, unbreakable plastic containers. All disposable contaminated materials including gloves, pipette tips, tubes, plant tissue and soil will be placed in autoclavable containers and autoclaved prior to disposal. All plant material and soil from the greenhouse will be steam-sterilized before disposal. Before being released for other use, greenhouse rooms will be disinfested according to the procedure described in section *II-D-vi* of this document. 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 also be treated with sodium hypochlorite (5.25%) for 30 minutes prior to disposal in laboratory drains in rooms 014, 029, 031 or 035. Molecular biology work will be performed in the assigned laboratory of the principle investigator(s).

iii. 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 C or -80 C freezers in laboratories 014, 029 or 028B or in liquid nitrogen storage located in room 005 within the MCIC facility. Storage rooms 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 disposed of in the OARDC dumpster located outside of the head house.

iv. Personnel training

The principle investigator 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 principle investigators 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. An annual review of Selby Hall standard operating procedures will be required by all personnel involved in handling biohazardous agents (see Appendix V).

v. Arthropod infestations

Active surveillance will be in effect to prevent arthropod infestation within CG1 and CG2. Specific measures for controlling arthropods will depend on the species present.

1. A species-specific contact pesticide will be applied according to the manufacturers label to all plants in CG2.
2. Plants will be inspected regularly and the pesticide will be re-applied until the infestation is eliminated.
3. All arthropod infestations will be reported to the principle investigator and the greenhouse manager.

vi. Ingestion, inhalation or self-inoculation

Plant pathogenic viruses used in these experiments are not infectious to humans. In case of self-inoculation (e.g. with an infected needle) or accidental ingestion the principle investigator and containment director will be contacted immediately. Professional health care will be sought if necessary.

7. Standard operating procedure for plant pathogenic nematodes

i. Nematode rearing and handling

Nematodes reared on plants will be confined to a non-containment greenhouse (GH108). 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 sprayed weekly for insect pests by the greenhouse manager. 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.

ii. Nematode and egg harvesting

Pots with soil and infested roots will be placed in plastic autoclave bags and transported to laboratory 202 for nematode isolations. Shoots of infected/symptomatic plants will be removed prior to transport and discarded by placing tissue into plastic autoclave bags. Shoots will be sterilized in the autoclave located in the head house as described in Section III-iv.

Species of root-knot nematode (*Meloidogyne* spp.) will be isolated in laboratory 202 by removing sand bound to the roots and washing the roots with sterile water. Egg masses and release eggs will be dissolved with sodium hypochlorite (5.25%) and the eggs will be 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 GH108 for re-inoculation of healthy plants.

Species of cyst nematodes (*Heterodera* spp.) will be isolated in laboratory 202 by washing the soil and infested roots in autoclave tubs containing clean water. Cysts will be 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 GH108 for re-inoculation of healthy plants.

iii. Nematode and infected plant material disposal

All disposable materials that come in contact with nematodes, eggs, infested soil or plant material will be autoclaved in the head house autoclave according to the procedure described in Section III-iv. Glassware will be rinsed and washed using hot water in a cafeteria grade dishwasher located in rm 110. Sieves will be rinsed with water and placed into a drying oven located in rm 202 ($\geq 80^{\circ}\text{C}$) 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 Section III-iv. 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 disposed of in the OARDC dumpster located outside of the head house. All liquids used

in the isolation of nematodes will be autoclaved and disposed of in the public sewer (City of Wooster).

iv. Personnel training

The principle investigator 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 principle investigators 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. An annual review of Selby Hall standard operating procedures will be required by all personnel involved in handling biohazardous agents (see Appendix V).

v. 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 principle investigator and containment director will be contacted immediately. Professional health care will be sought if necessary.

V. 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 “Guidelines for Research Involving Recombinant DNA Molecules” (*NIH Guidelines*; http://oba.od.nih.gov/oba/rac/guidelines_02/NIH_Gdlines_2002prn.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.

i. 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.

ii. 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.

A. Biosafety Level I

1. Access to the laboratory will be limited or restricted at the discretion of the Principal Investigator when experiments are in progress.
2. Work surfaces will be decontaminated once a day and after any spill of viable material.
3. All contaminated liquid or solid wastes will be decontaminated before disposal.
4. Mechanical pipetting devices will be used; mouth pipetting is prohibited.
5. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area.
6. 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
7. Spray inoculations of plants and animals are not permitted (unless otherwise stated by the IBC accepted agreement).
8. In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, 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.
9. Contaminated materials will be placed in sealable, durable leak-proof containers before being removed from the laboratory.
10. Special containment equipment is not required for manipulations of agents assigned to BSL-I.

B. Biosafety Level II

1. All microbiological practices described for microorganisms in risk group BSL-I (Section V.ii.A.1-10) apply to microorganisms in risk group BSL-II.
2. 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 principle investigator or assigned supervisor about the potential hazards of working with BSL-II hazardous agents.
3. 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.
4. 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.
5. Gloves should be worn when handling experimental plants and when skin contact with the agent is unavoidable.
6. 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.
7. 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.

C. Biosafety Level I-Plants

The microbiological practices below will be used and *will supersede those in Sections V.ii.A and V.ii.B., 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

1. Access to the greenhouse will be limited or restricted, at the discretion of the principle investigator, when experiments are in progress.
2. 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.
3. A record shall be kept of experiments currently in progress in the greenhouse facility.
4. Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.
5. 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.

C. Biosafety Level II-Plants

1. All microbiological practices described for experiments in the greenhouse or growth chamber involving ***recombinant DNA-containing plants, plant-associated microorganisms, and small animals in Section V-ii- apply to those in BSLII-P.***
2. All work involving ***recombinant DNA-containing plants, plant-associated microorganisms, and small animals BSL-II*** will be conducted in the containment greenhouse facility.
3. A record will be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.
4. The Principal Investigator 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.
5. 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 IV.***).
6. Arthropods and other motile macroorganisms (e.g., flying arthropods or nematodes) will be housed in appropriate cages or within CG2.

iii. 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/>).

VI. APPLYING FOR PLANT HEALTH PERMITS

i. About The Animal and Plant Health Inspection Service (APHIS)

The Animal and Plant Health Inspection Service 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.

ii. About 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.

iii. When is a PPQ Permit Required?

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 organisms, soil, plants and plant products that would require a permit are listed below.

iv. Which PPQ permit is required?

1. The **PPQ 526** permit is used for importation, interstate movement, possession, and/or release into the environment of organisms 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, mycoplasmas, 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. The permits below are required for plants or plant products.

- **PPQ 585** - Application for Permit to Import Timber or Timber Products (logs and lumber)
- **PPQ 587** - Application for Permit to Import Plants or Plant Products.
 - 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
- **PPQ 588** - Application for permit to import prohibited plants or plant products for experimental purpose
- **PPQ 621** - Application for protected plant permit to engage in the business of importing, exporting, or re-exporting protected plants (CITES)
- **PPQ 586** - Application for permit to transit plants and/or plant products through the U.S.

iv. How to apply for a PPQ permit.

1. All PPQ permits can be applied for on-line using the ePermits system (<http://www.aphis.usda.gov/permits/index.shtml>). 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 an eAuthentication account with Level 2 access.*** Detailed instructions on how to register for eAuthentication can be found at <https://eauth.sc.egov.usda.gov/eAuth/selfRegistration/selfRegLevel2Step1.jsp>.

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.

iv. Time line for submitting, processing and receiving a PPQ permit.

Applications for permits are processed in the order received. It can take from 6 weeks to more than six 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.

1. Registering for an eAuthentication account with Level 2 access will take 2-3 days.
2. Completing a permit application form will take 30-60 minutes.
3. APHIS-PPQ review of application for completeness and evaluation of pest risk will take 2-4 weeks.
4. Containment facility inspection and mitigation of risk will take 1-5 months. This time frame will be less if the facility has been recently inspected for use with a similar permitted organism.
5. 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.

6. Development of permit conditions and agreement of permit conditions by permittee will take 1-4 weeks.

7. Issue of final permit will take 1 week. An additional 1-2 weeks is required for delivery of red and white labels for importation. Red and white labels can be requested for a permit by email (redandwhitelabelrequest@aphis.usda.gov).

v. 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 are available at:

http://www.aphis.usda.gov/plant_health/permits/organism/containment_facility_inspectio ns.shtml.

vi. Importation and Shipping Requirements for Importing Regulated Organisms

1. 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.

2. 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.

2. 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.

3. Each shipment must contain a copy of the permit (for permits issued before 2007) corresponding to the original Red and White shipping label. For permits issued after 2007 a copy of the permit is not required but the following information must be included in the shipment:

- Name of Permit Holder
- Permit Number
- Label Number

4. 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 IX.

vii. Permit conditions and enforcement

1. Each permit comes with a clearly defined set of conditions.
2. Upon receipt of the permit all personnel who will be working with the permitted organism will review the permit conditions.
3. Failure to comply with the conditions of the permit may result in the immediate ***cancellation of one or all permits held by the permittee and the denial of future permits*** (Federal Plant Protection Act, 2000).
4. Failure to comply with the conditions of the permit may result in monetary fines up to \$10 000.00 or 5 years in jail or both.
5. Additional conditions may be placed on the permit for special requests by the permittee (ie. hand carrying of permitted organisms). These conditions will be read and followed carefully. ***Additional fines (up to \$250,000 per violation) may be issued for violations of these conditions. Criminal penalties may also apply.***

vi. General Contact Information

1. For questions pertaining to **organisms or soil permits** contact permit services:

Telephone: 301-734-0841
Toll Free: 866-524-5421
Email: Pest.Permits@aphis.usda.gov

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

Telephone 301-734-0841 or 877-770-5990
Fax 301-734-4300
Email: permits@aphis.usda.gov

3. Plant Inspection Stations:

Telephone: 301-734-7839
Fax: 301-734-5276

4. Select Agent Program:

Telephone: 301-734-8758

Fax: 301-734-3652

Email: Agricultural.Select.Agent.Program@aphis.usda.gov

VII. THE INSTITUTIONAL BIOSAFETY COMMITTEE

i. 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.

ii. 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://orrrp.osu.edu/ibc/about/>)

iii. How to determine if your research needs Institutional approval

Many variables of your project may require IBC approval. To determine which protocol review applications are required for your research you should complete the **Protocol Decision Logic Questionnaire** (<http://www.ehs.ohio-state.edu/index.asp?PAGE=biosafe.rpd1>).

iv. Applying for an IBC application

1. After May 1, 2010 all IBC applications must be applied for on-line using the e-IBC system (<http://orrrp.osu.edu/ibc/forms/>). The following IBC applications are available through e-IBC:

Exempt Recombinant DNA (rDNA) Research: This application will act as your registration for exempt rDNA research. Exempt protocols do not require submission of Annual Reviews.

Non-exempt Recombinant DNA Research: Each approved protocol will require an Annual Review to update the status of the research. All non-exempt protocols will

continue to require the submission of amendments to document changes in personnel, procedures, research location, agents / rDNA host vector systems, and/or changes in containment procedures.

2. To use e-IBC, you will register to receive an IBC profile/account. Detailed instructions on how to register can be found at (<https://rf.osu.edu/secure/eProfile/>). Using e-IBC you can submit protocols, view your existing IBC protocol records, check the progress of your protocols under review, and create new protocol submissions for committee review.

iv. 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. Submission deadline and corresponding meeting dates are listed at <http://orrrp.osu.edu/ibc/meetingdates/>.

iv. Contact Information

For assistance with IBC submissions you can contact:

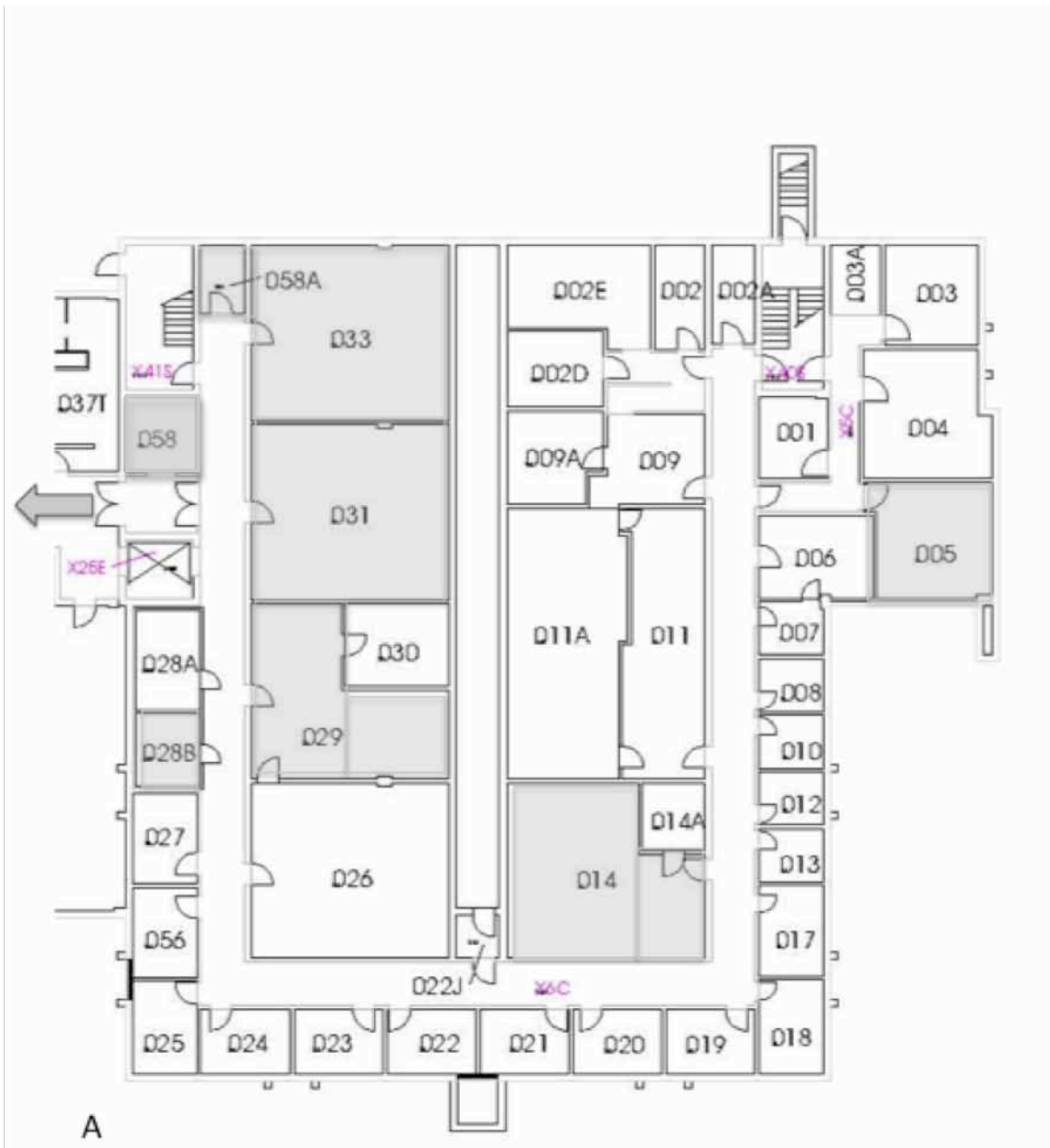
Jennifer Spohn
IBC Coordinator
614-247-1562
Fax: 614-688-0366
spohn.31@osu.edu

APENDECIES

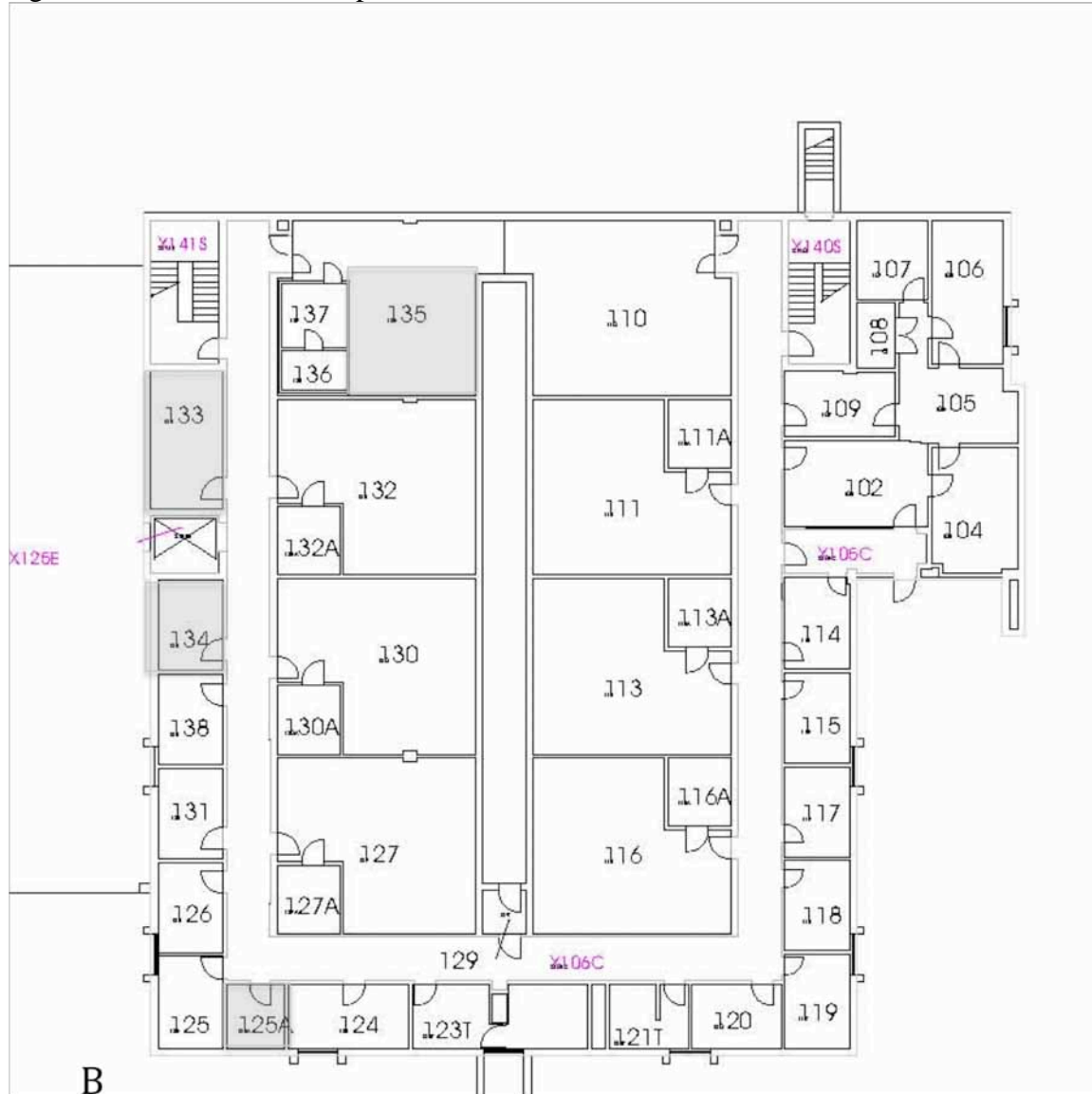
APPENDIX I

Selby Hall floor plans.

Floor plans for the ground (A). 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.



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



Floor plan of the second floor, showing various rooms and corridors. The plan is labeled 'C' in the bottom left corner. Rooms are numbered, and some are shaded gray. A central corridor is labeled '218'. A staircase is labeled 'X241S' and another 'X240S'. A room is labeled 'X206C'. The plan shows a complex layout with multiple rooms and corridors.

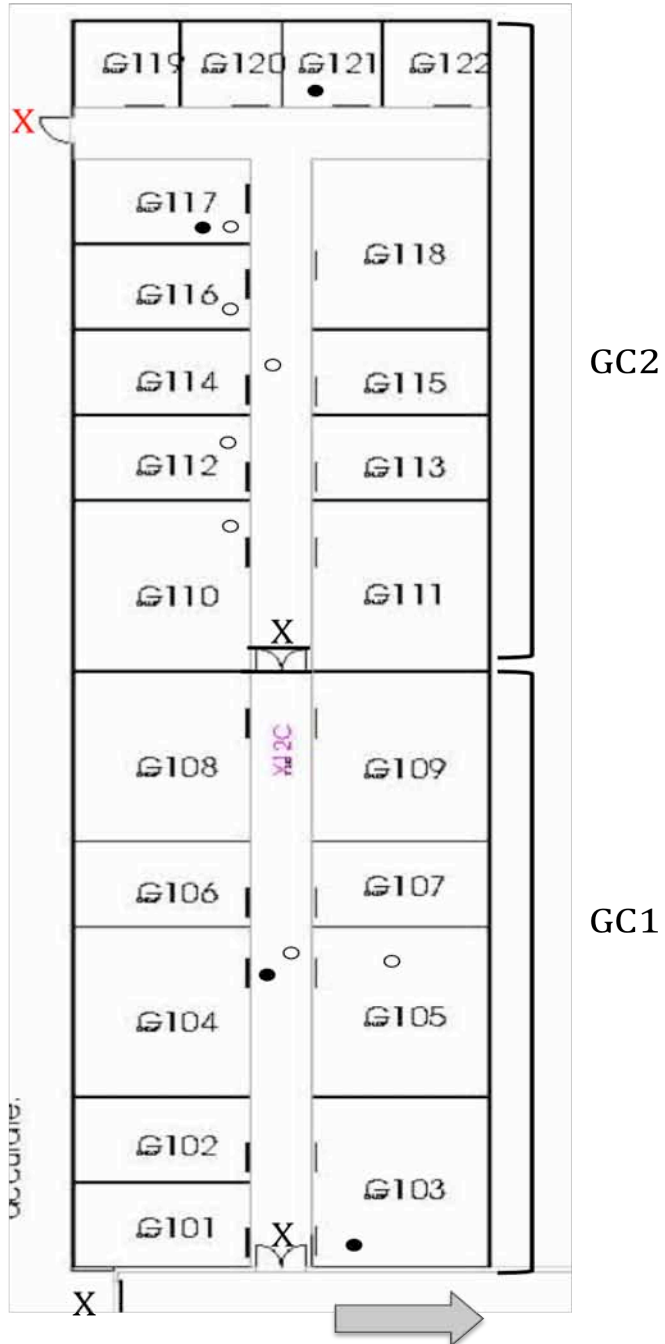
Plans for the phytotron, head house and greenhouse complex (D) of Selby Hall. Grey shading indicates rooms where permitted organisms are cultured, manipulated and/or stored. Containment greenhouse (CG) 1 and CG2 are indicated. Location of dumpster used to dispose of autoclaved permitted materials (not containing human pathogens) is indicated by a star.



APPENDIX II

The Containment Greenhouse (CG) Complex in Selby Hall.

i. The facility contains two units: CG1 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 drains to city sewer system are marked as ○ and blocked (inactive) drains are marked as ●. The grey arrow indicates the direction to the ground level of Selby Hall.



APPENDIX III

Listing of rooms in Selby Hall used for permitted organisms.

i. A current listing of the rooms in Selby Hall that are used for permitted organisms including the personnel responsible for the room, the activities performed in the room (using permitted organisms) and any equipment that may be used to work with permitted organisms. GR:ground level, 1: first level, 2: second level, and PYT:phytotron.

Level	Rm	Responsible Individual	Activities	Special Equipment
GR	054	Bill Bardall, Field Manager	- Sterilization - Soil preparation and storage	- Autoclave #1
GR	014	Lucy Stewart, ARS maize virologist	- Virus inoculations - Virus purification and characterization - Seed storage	- Inoculators - Locked storage cabinets
GR	005	Tea Meulia, Head of Molecular and Cellular Imaging Center	- Culture and virus storage	- Liquid N tanks
GR	026	Feng Qu, Assistant Professor	- Virus purification and characterization	- Fume hood
GR	028B	Peg Redinbaugh, ARS maize virologist	- Virus storage	Freezers (-80°C)
GR	029	Peg Redinbaugh, ARS maize virologist	- Transgenic pathogen transfer and analysis - Virus storage	- Class II BSC A/B3 #1. - Freezers (-80°C)
GR	031	Peg Redinbaugh, ARS maize virologist	- Purification and characterization	- Fume hood
GR	033/058	Peg Redinbaugh, ARS maize virologist	- Insect vector transfer	- Insect transfer hoods
PHY	035	Melanie Lewis Ivey, Research Associate Peg Redinbaugh, ARS maize virologist	- Plant-pathogen interactions - Insect destruction	- BSL-2P Growth chambers - Refrigerator (locked)
GR	058A	Peg Redinbaugh, ARS maize virologist	- Insect vector rearing	- Insect cages
1	125A	Melanie Lewis Ivey, Research Associate	- Experimental analysis of all pathogen groups (excluding human pathogens) - Culture transfer	- Door seals - UV sterilization lamps - Fume hood - Class II BSC A/B3 #2
1	127	Anne Dorrance, Associate Professor	- Fungal and oomycetes purification and characterization	
1	133	Anne Dorrance, Associate Professor	- Tissue and culture growth and storage	- incubation and storage shelves
1	134	Melanie Lewis Ivey, Research Associate	- Experimental analysis of all pathogen groups (excluding human pathogens) - Culture transfer, growth and storage	- Laminar flow hood (bench top) - Incubator - Refrigerator - Storage shelves
1	135	Laurel Leedy, Information Associate	- Sterilization	- Autoclaves #2
2	202	Chris Taylor, Assistant Professor	- Nematode purification and characterization	
2	219	Brian McSpadden-Gardener, Associate Professor	- Plant pathogen purification and characterization - Culture storage	- Laminar flow hood - Fume hood - Freezer (-80°C)
2	222	Laurel Leedy, Information Associate	- Sterilization	- Autoclaves
2	225	Laurel Leedy, Information Associate	- Culture storage	- Freezers (80°C) - Refrigerator
2	234	Sally Miller, Professor	- Plant pathogen purification and characterization	- Laminar flow hood - Fume hood

APPENDIX IV

Selby Hall Containment Director and Greenhouse/Phytotron Committee

i. Containment director

Lee Wilson, Research Associate, Department Safety Officer

Telephone: 330-202-35550 ext. 2865

Email: wilson.40@osu.edu

ii. Selby Hall Greenhouse and Phytotron Committee (May 2010)

B. Mc Spadden Gardener, Chair; 330-263-3565

A. Dorrance; 330-263-3560

M. Redinbaugh; 330-263-3965

W. Bardall; 330-263-3837

L. Madden; (*ex officio*) 330-263-3839

APPENDIX V

Annual review of standard operating procedures.

- i.* The principle investigator will ensure that personnel involved in the handling of the biohazardous agents are adequately trained as described in the pathogen specific standard operating procedures.
- ii.* 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 containment director will be responsible for organizing the annual review workshop, including but not limited to organizing the day, time, location and speaker/presenter (see *iii*) of the workshop.
- iii.* 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.

i. 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.

ii. 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).***

iii. 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.

iv. 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 (December 2010) USDA-APHIS-PPQ plant health safeguarding specialist is below.

David L. Lentz, Jr.
Plant Health Safeguarding Specialist
USDA-APHIS-PPQ
79 Glessner Avenue, Suite B
Mansfield, OH 44903-2413
419-525-3500 office
419-525-3502 fax
614-546-7059 cell
david.l.lentz@aphis.usda.gov

APPENDIX VII

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.

i. The insect container log form will be used to monitor the status of permitted insect vectors.

Sample of a yearly Insect/Container Log Sheet

Permit item	Insect carried from last inventory	Date of receipt	Date container examined and treated	No. Live items in Shipment	Parasite or other notes	Date dead removed and treated	Date dead removed and treated	Date dead removed and treated	No. live insects remaining year end

APPENDIX IX

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 X

Work order request form for the Department of Plant Pathology (Selby Hall).

GREENHOUSE, PHYTOTRON AND FIELD WORK REQUEST

Department of Plant Pathology

Name:

Principal Investigator:

Date of request:

Date needed:

Request:

Date completed:

Personnel involved:

Comments:

APPENDIX XI



Employee Accident report form for The Ohio State University (Wooster Campus).

Report # The Ohio State University Wooster Campus Employee Accident Report											
EMPLOYEE INFORMATION (Print in Ink)											
Name:	SSN:	Employee ID#:									
Home Address:	City:	Zip Code:									
Sex: M F	Date of Birth:	Age:	Home Phone #:								
Job Title:	Department:	Shop:									
Full Time:	Part Time:	Work Phone #:	Work Address:								
Supervisor's Name (printed):			Supervisor's Phone #:								
Supervisor's Address (Room & Building):											
ACCIDENT INFORMATION											
Accident Date:	Time:	am pm	Time Shift Began: am pm								
Location of Accident (Room # & Building):			Room Use (Lab, Shop, etc.):								
What was being done before the accident occurred?											
What happened?											
Was this part of normal job duty? Yes No Body part(s) affected or injured:											
Type of injury or illness: What object or substance directly harmed the employee?											
Witnesses (Name & Phone #):											
Report prepared by (if different from the injured employee):			Phone #:								
If you have been exposed to human blood or body fluids, refer to Medical Center Blood and Body Fluid Exposure protocol call Employee Health 614-293-8146 for instructions (see medical treatment section on reverse side) Hospital Medical Record # of source person:											
<i>I understand that it is my right to apply for Workers' Compensation benefits and that I have two years from the date of this accident to do so. For more information regarding workers compensation, University and James Hospitals employees, call 614-293-3571; Employees in other departments call 614-292-3439. I also authorize release of medical information regarding this accident to OSU BWC claim administrators.</i>											
EMPLOYEE SIGNATURE:			DATE:								
SEND EMPLOYEE FOR TREATMENT TO THE CENTER FOR OCCUPATIONAL MEDICINE WITHIN 72 HOURS AFTER ACCIDENT IS REPORTED Regional campus employees should be sent to local health care provider. (Do Not Leave form with Medical Provider)											
SUPERVISOR / CHARGE PERSON											
This accident was reported to me on: Date: Time: Cost Center / Department #:											
Is further investigation required? Yes No Supervisor / Charge Person Signature:											
HEALTH CARE PROVIDER											
Treated by Center for Occupational Med Yes No If No, treated by?											
Diagnosis / Assessment:											
Body part(s) affected:											
Is this a re-aggravation of previous injury? Yes No Date of initial injury: Lost Time or Restricted Duties? Yes No											
Medical Provider Printed Name:			Medical Provider Signature:								
OSHA300 Recordable Code(s):			1	2	3	4	5	6	7	8	Medical Record #:
Copies sent to:		Employee	OARDC Safety Office or EHS	OSU WC	OSU Employee Health	OSU EH&S:	Supervisor / Dept:				
		Fax Numbers	263-3767	88-8120	Fax: 83-8018	Fax: 82-6404					
ATTENTION: This form contains information relating to employee health and must be used in a manner that protects the confidentiality of employees to the extent possible while the information is being used for occupational safety and health purposes.											
Revised for Wooster Campus 09/04											

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 bioharazardous materials.

		<p>Hazardous To:</p> <p><input type="checkbox"/> Animal</p> <p><input type="checkbox"/> Human</p> <p><input type="checkbox"/> Plant</p>
<p>BIOHAZARD</p> <p>BIOSAFETY LEVEL:</p> <p>Biohazard Agent(s) Risk Group:</p> <p>Building/Room: _____ Date Posted: _____</p> <p>Special Procedures or Cautions For Entry: _____</p>		
Notice Entry/Advice Emergency Emergency	Call or See	Bldg/Room Work Phone Home/Cell

APPENDIX XIII

Color plates.



1. Selby Hall



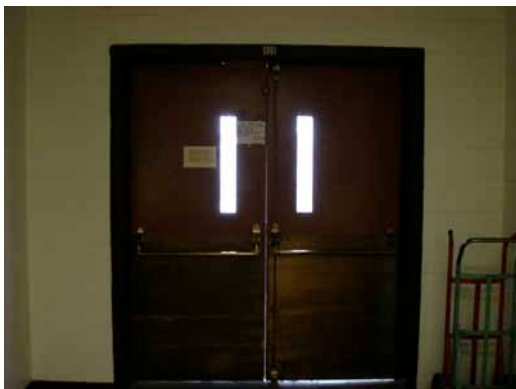
2. Growth chambers (Room 035)



3. BLS-2 growth chambers (Room 035)



4. Signage on BLS-2 growth chamber



5. Greenhouse access doors from head house



6. Greenhouse exterior garage door



7. Greenhouse side doors and head house access doors



8. Containment greenhouse (CG1 and 2) access door



9. CG1 individual room doors



10. CG2 access door (remains locked)



11. CG2 individual room doors



12. Airlock space between CG1 and CG2



13. CG2 emergency exit door



14. CG2 exterior view



15. Greenhouse concrete exterior walls and polycarbonate panels



16. Room floors showing concrete aisle and gravel beneath benches



17. Fin-tube radiators



18. North-facing air intake



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



20. Circulation/exhaust fan



21. Fiber water percolation pads



22. CG2 screened exit plenums



23. CG1 open exit plenums



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



25. Water drainage through earthen soil and gravel floor



26. Greenhouse benches



27. Autoclaves (room 135)



28. Autoclaves (room 222)



29. Head house autoclave



30. Signage indicating autoclave not for APHIS permitted cultures



**31. BSC A/B3 laminar flow hood
(rooms 029 and 125A)**



**32. -80 C freezers (room 225, room
remains locked)**



**33. Liquid nitrogen storage
(MCIC, room 005)**



**34. Room 125 with UV sterilizing
capabilities**



**35. Incubator (room 133,
remains locked)**



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



37. Laboratory equipment stored within CG2



38. Insect-escape-proof rearing cages



39. Insect transfer cabinet (room 033)



40. Sealable containers to transport insect infested plants

APPENDIX XIV

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:	Principle Investigator
PPQ:	Plant Protection and Quarantine
SOP:	Standard Operating Procedure