

APPENDIX P. PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULE RESEARCH INVOLVING PLANTS

Appendix P specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant or synthetic nucleic acid molecule-containing plants, plant-associated microorganisms, and small animals. All provisions of the NIH Guidelines apply to plant research activities with the following modifications:

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

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

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

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

Appendix P-I. General Plant Biosafety Levels

Appendix P-I-A. The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant or synthetic nucleic acid molecule-containing plant genome, including nuclear or organelle hereditary material or release of recombinant or synthetic nucleic acid molecule-derived organisms associated with plants.

Appendix P-I-B. The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility, e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem.

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

Appendix P-I-D. BL1-P through BL4-P are designed to provide differential levels of biosafety for plants in the absence or presence of other experimental organisms that contain recombinant or synthetic nucleic acid molecules. These biosafety levels, in conjunction with biological containment conditions described in Appendix P-III, Biological Containment Practices, provide flexible approaches to ensure the safe conduct of research.

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

Appendix P-II. Physical Containment Levels

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

Appendix P-II-A-1. Standard Practices (BL1-P)

Appendix P-II-A-1-a. Greenhouse Access (BL1-P)

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

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

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

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

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

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

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

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

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

Appendix P-II-A-1-e. Concurrent Experiments Conducted in the Greenhouse (BL1-P)

Appendix P-II-A-1-e-(1). Experiments involving other organisms that require a containment level lower than BL1-P may be conducted in the greenhouse concurrently with experiments that require BL1-P containment, provided that all work is conducted in accordance with BL1-P greenhouse practices.

Appendix P-II-A-2. Facilities (BL1-P)

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

Appendix P-II-A-2-a-(1). The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-A-2-a-(2). The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.

Appendix P-II-A-2-b. Greenhouse Design (BL1-P)

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

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

Appendix P-II-B. Biosafety Level 2 - Plants (BL2-P)

Appendix P-II-B-1. Standard Practices (BL2-P)

Appendix P-II-B-1-a. Greenhouse Access (BL2-P)

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

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

Appendix P-II-B-1-b. Records (BL2-P)

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

Appendix P-II-B-1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-B-1-b-(3). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety

Committee, NIH/OBA and other appropriate authorities immediately (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Documentation of any such accident shall be prepared and maintained.

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

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

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

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

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

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

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

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

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

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

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

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

Appendix P-II-B-1-g. Transfer of Materials (BL2-P)

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

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

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

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

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

Appendix P-II-B-2-a-(1). The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-B-2-a-(2). The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas and is considered part of the confinement area.

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

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

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

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

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

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

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

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

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

Appendix P-II-C. Biosafety Level 3 - Plants (BL3-P)

Appendix P-II-C-1. Standard Practices (BL3-P)

Appendix P-II-C-1-a. Greenhouse Access (BL3-P)

Appendix P-II-C-1-a-(1). Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility.

Appendix P-II-C-1-a-(2). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL3-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Appendix P-II-C-1-b. Records (BL3-P)

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

Appendix P-II-C-1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

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

Appendix P-II-C-1-c. Decontamination and Inactivation (BL3-P)

Appendix P-II-C-1-c-(1). All experimental materials shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal, except those that are to remain in a viable or intact state for experimental purposes; including water that comes in contact with experimental microorganisms or with material exposed to such microorganisms, and contaminated equipment and supplies.

Appendix P-II-C-1-d. Control of Undesired Species and Motile Macroorganisms (BL3-P)

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

Appendix P-II-C-1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

Appendix P-II-C-1-e. Concurrent Experiments Conducted in the Greenhouse (BL3-P)

Appendix P-II-C-1-e-(1). Experiments involving organisms that require a containment level lower than BL3-P may be conducted in the greenhouse concurrently with experiments that require BL3-P containment provided that all work is conducted in accordance with BL3-P greenhouse practices.

Appendix P-II-C-1-f. Signs (BL3-P)

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

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

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

Appendix P-II-C-1-g. Transfer of Materials (BL3-P)

Appendix P-II-C-1-g-(1). Experimental materials that are brought into or removed from the greenhouse facility in a viable or intact state shall be transferred to a non-breakable sealed secondary container. At the time of transfer, if the same plant species, host, or vector are present within the effective dissemination distance of propagules of the experimental organism, the surface of the secondary container shall be decontaminated. Decontamination may be accomplished by passage through a chemical disinfectant or fumigation chamber or by an alternative procedure that has demonstrated effective inactivation of the experimental organism.

Appendix P-II-C-1-h. Greenhouse Practices Manual (BL3-P)

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

Appendix P-II-C-1-i. Protective Clothing (BL3-P)

Appendix P-II-C-1-i-(1). Disposable clothing (e.g., solid front or wrap-around gowns, scrub suits, or other appropriate clothing) shall be worn in the greenhouse if deemed necessary by the Greenhouse Director because of potential dissemination of the experimental microorganisms.

Appendix P-II-C-1-i-(2). Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal.

Appendix P-II-C-1-j. Other (BL3-P)

Appendix P-II-C-1-j-(1). Personnel are required to thoroughly wash their hands upon exiting the greenhouse.

Appendix P-II-C-1-j-(2). All procedures shall be performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and all experimental manipulations.

Appendix P-II-C-2. Facilities (BL3-P)

Appendix P-II-C-2-a. Definitions (BL3-P)

Appendix P-II-C-2-a-(1). The term "greenhouse" refers to a structure with walls, roof, and floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-C-2-a-(2). The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse.

Appendix P-II-C-2-b. Greenhouse Design (BL3-P)

Appendix P-II-C-2-b-(1). The greenhouse floor shall be composed of concrete or other impervious material with provision for collection and decontamination of liquid run-off.

Appendix P-II-C-2-b-(2). Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

Appendix P-II-C-2-b-(3). The greenhouse shall be a closed self-contained structure with a continuous covering that is separated from areas that are open to unrestricted traffic flow. The minimum requirement for greenhouse entry shall be passage through two sets of self-closing locking doors.

Appendix P-II-C-2-b-(4). The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

Appendix P-II-C-2-b-(5). Internal walls, ceilings, and floors shall be resistant to penetration by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix P-II-C-2-b-(6). Bench tops and other work surfaces should have seamless surfaces that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix P-II-C-2-b-(7). The greenhouse contains a foot, elbow, or automatically operated sink, which is located near the exit door for hand washing.

Appendix P-II-C-2-c. Autoclaves (BL3-P)

Appendix P-II-C-2-c-(1). An autoclave shall be available for decontaminating materials within the greenhouse facility. A double-door autoclave is recommended (not required) for the decontamination of materials passing out of the greenhouse facility.

Appendix P-II-C-2-d. Supply and Exhaust Air Ventilation Systems (BL3-P)

Appendix P-II-C-2-d-(1). An individual supply and exhaust air ventilation system shall be provided. The system maintains pressure differentials and directional airflow, as required, to assure inward (or zero) airflow from areas outside of the greenhouse.

Appendix P-II-C-2-d-(2). The exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air-HEPA filters and discharged to the outside. The filter chambers shall be designed to allow in situ decontamination before filters are removed and to facilitate certification testing after they are replaced. Air filters shall be 80-85% average efficiency by the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) Standard 52-68 test method using atmosphere dust. Air supply fans shall be equipped with a back-flow damper that closes when the air supply fan is off. Alternatively, a HEPA filter may be used on the air supply system instead of the filters and damper. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times.

Appendix P-II-C-2-e. Other (BL3-P)

Appendix P-II-C-2-e-(1). BL3-P greenhouse containment requirements may be satisfied using a growth chamber or growth room within a building provided that the location, access, airflow patterns, and provisions for decontamination of experimental materials and supplies meet the intent of the foregoing clauses.

Appendix P-II-C-2-e-(2). Vacuum lines shall be protected with high efficiency particulate air/HEPA or equivalent filters and liquid disinfectant traps.

Appendix P-II-D. Biosafety Level 4 - Plants (BL4-P)

Appendix P-II-D-1. Standard Practices (BL4-P)

Appendix P-II-D-1-a. Greenhouse Access (BL4-P)

Appendix P-II-D-1-a-(1). Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility or work in the greenhouse during experiments.

Appendix P-II-D-1-a-(2). Access shall be managed by the Greenhouse Director, Biological Safety Officer, or other individual responsible for physical security of the greenhouse facility; and access limited by means of secure, locked doors.

Appendix P-II-D-1-a-(3). Prior to entering, individuals shall be advised of the potential environmental hazards and instructed on appropriate safeguards for ensuring environmental safety. Individuals authorized to enter the greenhouse facility shall comply with the instructions and all other applicable entry/exit procedures.

Appendix P-II-D-1-a-(4). Personnel shall enter and exit the greenhouse facility only through the clothing change and shower rooms and shall shower each time they exit the greenhouse facility. Personnel shall use the airlocks to enter or exit the laboratory only in an emergency. In the event of an emergency, every reasonable effort should be made to prevent the possible transport of viable propagules from containment.

Appendix P-II-D-1-a-(5). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL4-P practices and procedures.

Appendix P-II-D-1-b. Records (BL4-P)

Appendix P-II-D-1-b-(1). A record shall be kept of all experimental materials brought into or removed from the greenhouse.

Appendix P-II-D-1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-D-1-b-(3). A record shall be kept of all personnel entering and exiting the greenhouse facility, including the date and time of each entry.

Appendix P-II-D-1-b-(4). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities immediately (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Documentation of any such accident shall be prepared and maintained.

Appendix P-II-D-1-c. Decontamination and Inactivation (BL4-P)

Appendix P-II-D-1-c-(1). All materials, except for those that are to remain in a viable or intact state for experimental purposes, shall be autoclaved prior to removal from the maximum containment greenhouse. Equipment or material that could be damaged by high temperatures or steam shall be decontaminated by alternative methods (e.g., gas or vapor sterilization) in an airlock or chamber designed for this purpose.

Appendix P-II-D-1-c-(2). Water that comes in contact with experimental microorganisms or with material exposed to such microorganisms (e.g., run-off from watering plants) shall be collected and decontaminated before disposal.

Appendix P-II-D-1-c-(3). Standard microbiological procedures shall be followed for decontamination of equipment and materials. Spray or liquid waste or rinse water from containers used to apply the experimental microorganisms shall be decontaminated before disposal.

Appendix P-II-D-1-d. Control of Undesired Species and Motile Macroorganisms (BL4-P)

Appendix P-II-D-1-d-(1). A chemical control program shall be implemented to eliminate undesired pests and pathogens in accordance with applicable state and Federal laws.

Appendix P-II-D-1-d-(2). Arthropods and other motile macroorganisms used in conjunction with experiments requiring BL4-P level physical containment shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

Appendix P-II-D-1-e. Concurrent Experiments Conducted in the Greenhouse (BL4-P)

Appendix P-II-D-1-e-(1). Experiments involving organisms that require a containment level lower than BL4-P may be conducted in the greenhouse concurrently with experiments that require BL4-P containment provided that all work is conducted in accordance with BL4-P greenhouse practices. When

the experimental microorganisms in use require a containment level lower than BL4-P, greenhouse practices reflect the level of containment required by the highest containment level microorganisms being tested.

Appendix P-II-D-1-f. Signs (BL4-P)

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

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

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

Appendix P-II-D-1-g. Transfer of Materials (BL4-P)

Appendix P-II-D-1-g-(1). Experimental materials that are brought into or removed from the greenhouse in a viable or intact state shall be transferred to a non-breakable, sealed, primary container then enclosed in a non-breakable, sealed secondary container. These containers shall be removed from the greenhouse facility through a chemical disinfectant, fumigation chamber, or an airlock designed for this purpose.

Appendix P-II-D-1-g-(2). Supplies and materials shall be brought into the greenhouse facility through a double-door autoclave, fumigation chamber, or airlock that is appropriately decontaminated between each use. After securing the outer doors, personnel within the greenhouse facility shall retrieve the materials by opening the interior door of the autoclave, fumigation chamber, or airlock. These doors shall be secured after the materials are brought into the greenhouse facility.

Appendix P-II-D-1-h. Greenhouse Practices Manual (BL4-P)

Appendix P-II-D-1-h-(1). A greenhouse practices manual shall be prepared or adopted. This manual shall include contingency plans to be implemented in the event of the unintentional release of experimental organisms.

Appendix P-II-D-1-i. Protective Clothing (BL4-P)

Appendix P-II-D-1-i-(1). Street clothing shall be removed in the outer clothing change room. Complete laboratory clothing (may be disposable) including undergarments, pants, and shirts, jump suits, shoes, and hats shall be provided and worn by all personnel entering the greenhouse facility.

Appendix P-II-D-1-i-(2). Personnel shall remove laboratory clothing when exiting the greenhouse facility and before entering the shower area. This clothing shall be stored in a locker or hamper in the inner change room.

Appendix P-II-D-1-i-(3). All laboratory clothing shall be autoclaved before laundering.

Appendix P-II-D-2. Facilities (BL4-P)

Appendix P-II-D-2-a. Greenhouse Design (BL4-P)

Appendix P-II-D-2-a-(1). The maximum containment greenhouse facility shall consist of a separate building or a clearly demarcated and isolated area within a building. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse facility.

Appendix P-II-D-2-a-(2). Outer and inner change rooms, separated by a shower, shall be provided for personnel entering and exiting the greenhouse facility.

Appendix P-II-D-2-a-(3). Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

Appendix P-II-D-2-a-(4). Access doors to the greenhouse shall be self-closing and locking.

Appendix P-II-D-2-a-(5). The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

Appendix P-II-D-2-a-(6). The walls, floors, and ceilings of the greenhouse shall be constructed to form a sealed internal shell that facilitates fumigation and is animal and arthropod-proof. These internal surfaces shall be resistant to penetration and degradation by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix P-II-D-2-a-(7). Bench tops and other work surfaces shall have seamless surfaces impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix P-II-D-2-a-(8). A double-door autoclave, fumigation chamber, or ventilated airlock shall be provided for passage of all materials, supplies, or equipment that are not brought into the greenhouse facility through the change room.

Appendix P-II-D-2-b. Autoclaves (BL4-P)

Appendix P-II-D-2-b-(1). A double-door autoclave shall be provided for the decontamination of materials removed from the greenhouse facility. The autoclave door, which opens to the area external to the greenhouse facility, shall be sealed to the outer wall and automatically controlled so that it can only be opened upon completion of the sterilization cycle.

Appendix P-II-D-2-c. Supply and Exhaust Air Ventilation Systems (BL4-P)

Appendix P-II-D-2-c-(1). An individual supply and exhaust air ventilation system shall be provided. The system shall maintain pressure differentials and directional airflow as required to assure inward (or zero) airflow from areas outside of the greenhouse. Differential pressure transducers shall be used to sense pressure levels. If a system malfunctions, the transducers shall sound an alarm. A backup source of power should be considered. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times. The integrity of the greenhouse shall have an air leak rate (decay rate) not to exceed 7 percent per minute (logarithm of pressure against time) over a 20-minute period at 2 inches of water gauge pressure. Nominally, this is 0.05 inches of water gauge pressure loss in 1 minute at 2 inches water gauge pressure.

Appendix P-II-D-2-c-(2). Exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air/HEPA filters and discharged to the outside and dispersed away from occupied buildings and air intakes. Filter chambers shall be designed to allow in situ decontamination before filters are removed and to facilitate certification testing after they are replaced. HEPA filters shall be provided to treat air supplied to the greenhouse facility. HEPA filters shall be certified annually.

Appendix P-II-D-2-d. Other (BL4-P)

Appendix P-II-D-2-d-(1). Sewer vents and other ventilation lines contain high efficiency particulate air/HEPA filters. HEPA filters shall be certified annually.

Appendix P-II-D-2-d-(2). A pass-through dunk tank, fumigation chamber, or an equivalent method of decontamination shall be provided to ensure decontamination of materials and equipment that cannot be decontaminated in the autoclave.

Appendix P-II-D-2-d-(3). Liquid effluent from sinks, floors, and autoclave chambers shall be decontaminated by heat or chemical treatment before being released from the maximum containment greenhouse facility. Liquid wastes from shower rooms and toilets may be decontaminated by heat or chemical treatment. Autoclave and chemical decontamination of liquid wastes shall be evaluated by appropriate standard procedures for autoclaved wastes. Decontamination shall be evaluated mechanically and biologically using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes are decontaminated with chemical disinfectants, the chemicals used must have demonstrated efficacy against the target or indicator microorganisms.

Appendix P-II-D-2-d-(4). If there is a central vacuum system, it shall not serve areas outside of the greenhouse facility. In-line high efficiency particulate air/HEPA filters shall be placed as near as practicable to each use point or vacuum service cock. Other liquid and gas services to the greenhouse facility shall be protected by devices that prevent back-flow. HEPA filters shall be certified annually.

Appendix P-III. Biological Containment Practices

Appropriate selection of the following biological containment practices may be used to meet the containment requirements for a given organism. The present list is not exhaustive; there may be other ways of preventing effective dissemination that could possibly lead to the establishment of the organism or its genetic material in the environment resulting in deleterious consequences to managed or natural ecosystems.

Appendix P-III-A. Biological Containment Practices (Plants)

Appendix P-III-A-1. Effective dissemination of plants by pollen or seed can be prevented by one or more of the following procedures: (i) cover the reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity; (ii) remove reproductive structures by employing male sterile strains, or harvest the plant material prior to the reproductive stage; (iii) ensure that experimental plants flower at a time of year when cross-fertile plants are not flowering within the normal pollen dispersal range of the experimental plant; or (iv) ensure that cross-fertile plants are not growing within the known pollen dispersal range of the experimental plant.

Appendix P-III-B. Biological Containment Practices (Microorganisms)

Appendix P-III-B-1. Effective dissemination of microorganisms beyond the confines of the greenhouse can be prevented by one or more of the following procedures: (i) confine all operations to injections of microorganisms or other biological procedures (including genetic manipulation) that limit replication or reproduction of viruses and microorganisms or sequences derived from microorganisms, and confine these injections to internal plant parts or adherent plant surfaces; (ii) ensure that organisms, which can serve as hosts or promote the transmission of the virus or microorganism, are not present within the farthest distance that the airborne virus or microorganism may be expected to be effectively disseminated; (iii) conduct experiments at a time of year when plants that can serve as hosts are either not growing or are not susceptible to productive infection; (iv) use viruses and other microorganisms or their genomes that have known arthropod or animal vectors, in the absence of such vectors; (v) use microorganisms that have an obligate association with the plant; or (vi) use microorganisms that are genetically disabled to minimize survival outside of the research facility and whose natural mode of transmission requires injury of the target organism, or assures that inadvertent release is unlikely to initiate productive infection of organisms outside of the experimental facility.

Appendix P-III-C. Biological Containment Practices (Macroorganisms)

Appendix P-III-C-1. Effective dissemination of arthropods and other small animals can be prevented by using one or more of the following procedures: (i) use non-flying, flight-impaired, or sterile arthropods; (ii) use non-motile or sterile strains of small animals; (iii) conduct experiments at a time of year that precludes the survival of escaping organisms; (iv) use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or (v) prevent the escape of organisms present in run-off water by chemical treatment or evaporation of run-off water.