UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE

CONTAINMENT GUIDELINES FOR PLANT PATHOGENIC NEMATODES

(Revised: Feb., 2010) (Nematodes)

CONTAINMENT GUIDELINES FOR NEMATODES

CONTENTS

CONTAINMENT GUIDELINES FOR NEMATODES

I. PURPOSE OF THIS DOCUMENT:

These guidelines are a reference to help you (a scientist of a state, federal, or commercial entity) design, build, maintain, and operate a containment facility for plant pathogenic nematodes that are non-indigenous or of limited distribution. Widely distributed indigenous nematodes may not require a containment facility. These guidelines are not intended for nematodes of medical and veterinary importance. If there is doubt as to the applicability of guidelines to specific organisms, contact Containment Facilities Staff (CF), or Permits and Risk Assessment (PRA) scientific personnel for appropriate guidance.

During inspections or reinspections of your facility, USDA, APHIS, PPQ personnel will review these guidelines and any risk mitigation instructions that may accompany your permit. When your facility meets containment standards and risk mitigation instructions it will be considered “adequate" from a containment standpoint; then permits may be issued to you.

The inspection and permitting procedures of the USDA, APHIS, PPQ are intended to prevent the release of nonindigenous plant pests to the environment of the United States. Accidental or purposeful release of these organisms is a violation of the PLANT PROTECTION ACT and is subject to civil and/or criminal penalties, loss of permits, and destruction of valuable cultures.

Components of this Document:

Your containment facility must meet the “Standards” listed in the grey, shaded boxes. To help you meet these standards, ask biotechnology industries, university Biosafety committees, and/or contractors for research hospitals and other research institutions to recommend specialized professional contractors. Professionals know state, local and federal laws that regulate construction, including the installation of emergency doors, incinerators, air intake and exhaust ducts, emergency lighting, plumbing, and many other features. PPQ’s permit specialists have little or no knowledge of these laws.

N. B. Notify PPQ of any structural changes prior to implementation, the development of blueprints, signing of construction contracts, start of construction, etc.

The “Suggestions” listed under each Standard are methods or equipment that are commonly used at this time to accomplish each containment standard. The design, construction and operation of your containment facility may vary, depending on the organisms you wish to contain, your research objectives, the functionality of your equipment and structural components, and your location. Again, we recommend professional advice on all design issues. [Some other useful containment references (non-USDA-APHIS) are: 1) "Containment Facilities and Safeguards for Exotic Plant Pathogens and Pests ". 1999. R. P. Kahn and S. B Mathur. (eds). APS Press, St. Paul, MN. 2) " A Practical Guide to Containment (Plant Biosafety in Research Greenhouses) ". Adair, D. and R. Irwin. 2008 (revised edit.). Information

Systems for Biotechnology, Virginia Tech, Blacksburg (http:// www.isb.vt.edu); 3) Containment Standards for Facilities Handling Plant Pests. 2009.(1st edition) Canadian Food Inspection Agency, Office of Biohazard Containment and Safety, (http://www.inspection.gc.ca/english/sci/bio/plaveg/placone.shtml)]).

Safety of facility personnel should not be compromised by containment requirements. Emergency exits should not be blocked by equipment; their operation should not be obstructed with tape or caulk.

We encourage Good Laboratory Practices (GLP) in all facilities. The standards and suggestions listed here only cover containment. Cross contamination of cultures within the containment may or may not signify poor containment.

USDA, APHIS, PPQ welcomes alternatives that are proven to meet or exceed the standards. To insure timely permitting, please review this document and research design alternatives. Once design options are narrowed, call or fax PPQ's Containment Facility staff at (301) 734-5592 or FX (301) 734-5392 and continue discussions as the facility is planned and built.

II. CONSTRUCTION STANDARDS FOR THE ENTIRE STRUCTURE

CONSTRUCTION STANDARD A. Locate the facility in areas with minimal human, agricultural and environmental risk. Identify the facility as dedicated and secure.

SUGGESTIONS:

1. Locate the facility in areas relatively free of agricultural zones, high-risk microclimates (e.g.. known flood zones) or other high-risk areas.
2. If possible design the containment facility as a separate, dedicated building. If this is not possible, design and build to prevent pest escape.
3. Install a 15 foot- wide buffer strip of a material that doesn't support plant growth outward from the foundation to surround the containment building(s) ; (no soil or host plants should be in this area)
4. Install barriers to prevent unlawful entry into the facility.
5. Post a sign with the following information:
   - Containment director/ containment officer name and contact numbers.
   - A sign stating: "ACCESS IS BY AUTHORIZED PERSONNEL ONLY".
   - Emergency telephone numbers.

CONSTRUCTION STANDARD B. Design the floor plan to prevent escape of the enclosed organism(s).

SUGGESTIONS:

1. Install one primary entry/exit.
2. The entry/exit should have a vestibule with self closing doors with at least 6 ft. separating them.
3. There should be a foot bath inside the vestibule constructed to decontaminate feet upon exiting the facility. Booties and disposal container and a rack for dedicated footwear would be useful.
4. Design the facility with laboratories and rearing rooms connected to a main laboratory.
5. Build restrooms outside of containment rooms. However, if restrooms must be built inside a containment room, use the same construction standards used throughout the facility and place the restroom next to low risk areas.
6. Build offices outside of containment areas.
7. Install self-closing doors throughout the containment structure(s).
8. Install exterior doors that lock.
9. Install a central closet for cleaning supplies.

CONSTRUCTION STANDARD C. Construct walls, ceilings and floors that are impenetrable to the enclosed organisms, and withstand repeated cleaning and decontamination

SUGGESTIONS:

1. Construct the walls and ceilings with building materials that resist moisture and withstand repeated decontaminations with bleach or other caustic solutions.
2. Walls and ceilings should be painted a light color for detection of dirt or debris which may harbor organisms.
3. Flooring cannot have any porous elements;
   Monolithic (in one-piece) floors, e.g. poured concrete, sheet linoleum, chemically resistant paint, etc. are desirable. Wood floors are not acceptable. Floor should be able to withstand repeated cleanings
4. Install floor drains to collect liquid wastes for sterilization.
5. Seal junctions, holes or penetrations of walls, ceilings, and floors with plaster, caulk, or equivalent materials.

CONSTRUCTION STANDARD D. If windows are necessary, install Windows impenetrable to the enclosed organisms.

SUGGESTIONS:

1. Install breakage resistant glazing (Plexiglas, Lexan, etc).
2. Install windows that do not open.
3. Seal joints between the windowsills, frames, etc. and walls with appropriate materials.
4. Store extra transparent panels nearby for emergency use.

CONSTRUCTION STANDARD E. Install Doors that contribute to the security of the facility.

SUGGESTIONS:

1. Install exterior doors that lock.
2. Install thresholds and gaskets that seal the exterior and interior doors with their frames. The space between seal and doorframe should not exceed 6.3mm (0.250 inch).
3. Emergency doors
   - Insure emergency doors are not commonly used as an entrance (may use " panic bars " on the interior side)

CONSTRUCTION STANDARD F. Design and install an HVAC System (Heating, Ventilation and Air Conditioning) that prevents escape of the contained organisms.

SUGGESTION:

While an HVAC system is not required for use with many nematode species, if for example, the nematode (such as *Bursaphelenchus* spp.) is vectored by an aerial organism an HVAC system may be required. Contact PPQ if necessary.

CONSTRUCTION STANDARD G. Design and install an Electrical System that maintains containment features under normal and emergency situations and is impenetrable to the contained organisms.

SUGGESTIONS:

1. Install an alarm to indicate power failure. (optional)
2. Install an alternative power source (generator, battery bank, etc.) for use when normal power is lost or interrupted. (optional)
3. Install weatherproof electrical boxes, receptacles, light fixtures, switches, etc.
4. Seal electrical boxes, lighting, switches, wiring, conduit, etc, with appropriate materials (caulk, foam, etc,) that are impenetrable to the contained organisms and withstand repeated decontaminations with bleach or other caustic solutions.

CONSTRUCTION STANDARD H. Design and install a Plumbing System to contain the organisms and remove liquid wastes.

SUGGESTIONS:

1. Seal sewer or drains (sink, floor, shower, etc.) with suitable filters (and perhaps prefilters) to prevent escape of infected host material, soil, and cysts or dormant stages of nematodes.
2. Sterilize effluents from sinks, floor drains, etc with steam or its equivalent before releasing them into the sewer system.
3. Install inline sink traps for laboratories and greenhouses.
4. In the alternative that large quantities of effluent cannot be handled, develop and submit an SOP that describes how effluent will be handled to reduce pest risk prior to disposal.

CONSTRUCTION STANDARD I. Install Vacuum Systems and Vacuum Aspiration Systems that prevent the escape of the contained organisms

SUGGESTIONS:

1. Use vacuum appliance only in facility.
2. Autoclave or sterilize vacuum filters and waste before disposal.

CONSTRUCTION STANDARD J. Install Communication System that allows communication between the interior and exterior of the facility and prevents organism escape.

1. Install a telephone(s) or intercom system.
2. Install a computer (LAN, modem, etc.) or Fax machine to allow for communication and data transfer to and from the facility.
3. Electronic devices (i.e., cell phones, etc.) may not be removed from containment area without suitable decontamination.

III. CONSTRUCTION STANDARDS FOR SPECIALIZED ROOMS.

CONSTRUCTION STANDARDS FOR SPECIALIZED ROOMS A. Build Greenhouses with security and containment features.

1. Inoculated plants may be confined in walk-in large growth chambers and growth rooms with HEPA filtered HVAC systems. This is preferable and more secure than greenhouses for containing organisms.
2. Construct the foundation of concrete, concrete block, brick, or similar material.
3. Extend the foundation below the soil line to insure a permanent and stable structure. Build the foundation at least 3 ft. above the soil line.
4. Construct greenhouse floors of materials that are impervious to the contained organisms and can withstand repeated disinfecting with caustic liquids.

5. Install a frame strong enough to support the translucent walls and ceilings.
    - Install translucent wall and ceiling materials strong enough to guarantee the security of the facility. Plexiglas, lucite, lexon, safety glass, and wire-reinforced glass are acceptable. Polyethylene, vinyl or plastic sheeting are NOT acceptable.
    - Seal the translucent panels to the frame with caulk or appropriate materials on the inside and outside surfaces.
    - Consider the installation of screens over the roof to protect it from hail.
6. Seal joints between the greenhouse and other containment rooms with caulk or other suitable material.
7. If containment greenhouse is detached from the primary containment facility, install a vestibule at each door. (See specialized room section on Vestibules.)
8. Install doors between the greenhouse and the rest of the facility that close completely, and seal to their frames. Use doors that are windowless or cover windows with blinds.
9. Ensure any HVAC system can be turned off to allow greenhouse fumigation.

CONSTRUCTION STANDARDS FOR SPECIALIZED ROOMS B. Vestibules

SUGGESTIONS:

1. Install a vestibule in each entrance and /or exit. (Have contractor check local construction codes on vestibules at emergency exits, as they may be prohibited.)
2. Shower rooms can count as a vestibule for the main entrance (see restroom construction).
3. Design vestibule to be at least 6 feet long from door threshold to door threshold.
4. Insure vestibule doors interlock, so that only one door can be opened at a time.
5. Insure thresholds and gaskets of doors seal to their frames.
6. Shoe racks useful if dedicated footwear is used.
7. Provide bin for discarded disposable footwear.
8. If no vestibule, then have a walkway with mats or shaker capability.

CONSTRUCTION STANDARDS FOR SPECIALIZED ROOMS C. Install Showers and Restrooms to prevent organism escape.

SUGGESTIONS:

1. Place showers/ restrooms near lowest risk rooms.
2. Ensure thresholds and gaskets of restroom doors seal to their frames.

IV. EQUIPMENT STANDARDS

EQUIPMENT STANDARD A. Use Benches, Tables and Other Furniture that are easy to inspect and clean.

SUGGESTIONS:

1. Install work surfaces and laboratory furniture (bench tops, etc.) impervious to water, and resistant to chemicals and heat.
2. Insure spaces between benches, wall cabinets, and equipment are easy to clean and inspect.
3. Dedicate cleaning equipment (mops, brooms, buckets, etc.) for use only in the containment area, and store it in the containment area.
4. Repair or replace dented, broken or cracked benches and tables if soil or dirt can become imbedded in the surfaces. Disposable blotter material maybe required as a liner between the work surface and the bench or table.

EQUIPMENT STANDARD B. Use equipment to sterilize contained organisms, soil, plant growing medium, plant material, and solid waste. Decontaminate solid waste and contaminated or infested articles before removing it from the facility.

SUGGESTIONS:

1. Install an autoclave. A double-door pass-through model is recommended. Conduct tests to evaluate effectiveness of autoclave on a regular basis.
2. Install a gas sterilizer for articles that would be damaged by steam. A double-door pass-through model is recommended.
3. If you are considering an incinerator within the facility, consult your contractor about state, local, and federal laws and ordinances.
4. Since large quantities of soil may be used in nematode research, rooms for sterile and contaminated should be kept separate, and should be equipped with a high pressure steam decontamination system to decontaminate the inside and outside of the rooms including walls, ceilings, and floors.
5. Clean potting soil and contaminated soil with other solid wastes must be subjected to a minimum temperature of 104°C (220°F) for three hours. [SEE USDA-APHIS TREATMENT MANUAL for soil--online]. The efficacy of the treatment must be verified by test organisms (such as *Geobacillus stearothermophilus* endospores teststrips/kits).

EQUIPMENT STANDARD C. Install a Biosafety cabinet to work with organisms.

SUGGESTIONS:

1. Install a biosafety cabinet, Class II, type A/B3 in which to open foreign source packages; it should be tested/certified annually by a professional technician.
2. Have access to a biosafety cabinet for initial opening of packages to ensure contents don't contain unpermitted materials and/or contaminants.

V. OPERATIONAL STANDARDS

OPERATIONAL STANDARD A. A Containment Director is responsible for the daily operation of the facility and its physical integrity.

SUGGESTIONS:

A Containment Officer is responsible for the organisms contained in the facility. He/she also maintains a copy of the Standard Operating Procedures (SOP) Manual for the facility. SOPs contain directions for normal use, maintenance, and disinfection of the facility and its equipment.

SOPs also describe how to:

- Respond to a typical emergency event (power outage, fire, glass breaks in containment area (flood, hurricane, earthquake, tornadoes, etc.). Maintain biosecurity for unauthorized removal of materials from containment area.
- Replace translucent panels in greenhouse.
- Monitor visitors.
- Clean the facility.

Copies of SOPs should be available to workers within the containment areas. Each revision must be dated.

Containment Officer:

- Implements the SOPs and conditions listed in permits for organisms held by the facility.
- Trains employees and/or authorized personnel in the SOPs.

- Updates copies of construction records (blueprints) for the facility.
- Maintains daily, weekly and monthly maintenance records of the facility.

The Containment Officer updates these lists:

- The names and phone numbers of those to call during emergencies as changes occur.
- The plant species in facility, as changes occur.
- Authorized personnel, as changes occur.
- Incoming and outgoing shipments of permitted organisms, including dead or destroyed incoming organisms, and submits list as per permit conditions to USDA, APHIS, PPQ, PRA (Permits & Risk Assessment) or by January 31 of each year (see address on last page.)

OPERATIONAL STANDARD B. Only Authorized Personnel have routine access to the facility.

Once your facility is adequate to receive plant pests, the behaviors of people who access your facility will have far more impact on the containment of the organism than any containment feature. Your selection of individuals to work in this facility is critical to maintenance of plant pest containment. In addition to picking good personnel, please consider the suggestions below.

SUGGESTIONS:

1. Ensure only authorized personnel have keys.
2. Train authorized personnel in the SOPs.
3. List the personnel authorized to enter the facility.
4. Require that visitors sign a logbook upon entry and exit. Authorized personnel must accompany visitors at all times to insure they do not bring in or carry out pests.
5. Insure emergency exit doors are not used routinely as an entrance.

OPERATIONAL STANDARD C. Wear, sterilize, and handle personal Apparel to minimize the risk of organism escape.

SUGGESTIONS:

1. Insure visitors and employees wear laboratory coat in the containment area and remove it prior to leaving the containment area. Foot covers, rubber aprons, and/or gloves may be appropriate.

2. Prohibit entry of overcoats, hats, backpacks, purses, etc. into the containment areas, as these articles may allow infested soil to adhere and organisms escape containment.

OPERATIONAL STANDARD D. Use Personal Cleanliness to contain organisms.

SUGGESTION:

1. Ensure authorized personnel and visitors clean and disinfect shoes before exiting.
2. Ensure laboratory coats are periodically washed in hot water.

OPERATIONAL STANDARD E. Clean and Disinfect the interior of the facility and its equipment regularly.

SUGGESTIONS:

1. Clean and disinfect the facility, its furniture, and its equipment regularly with bleach or a similar disinfectant.
2. Eliminate undesired pests , pathogens and weeds from the facility.
3. Autoclave or sterilize solid wastes (cultures, plant materials, soil, trash, etc.) prior to disposal.

OPERATIONAL STANDARD F. Open and Handle packages of permitted organisms to prevent organism release.

SUGGESTIONS:

1. Establish one enclosed area that is easy to disinfect, and use this area to open packages received from foreign sources if a biosafety cabinet is not available.
2. Place foreign source packages in the biosafety cabinet before opening.
3. Autoclave or incinerate packing materials immediately after the removal of specimens and cultures.
4. Establish procedures to eliminate hitchhiking pests (insects, entomopathogens, fungi, bacteria, etc.)

OPERATIONAL STANDARD G. Maintain nematode populations with as few contaminants as possible. Cross contamination indicates poor laboratory practice. However, it may not indicate containment problems.

SUGGESTIONS:

1. List all arthropod and/or plant materials used to maintain nematode populations.
2. Sterilize/destroy all packing materials from shipments and contaminants shortly after receipt.
3. 3. Autoclave or sterilize used media prior to disposal.
4. Destroy contaminated populations as soon as the contamination is detected.
5. Store tissue cultures in screw- top vials, sealable tissue culture containers or the equivalent.

OPERATIONAL STANDARD H. Follow all PPQ Regulatory Requirements for organisms received, reared in, or released from the containment facility.

SUGGESTIONS:

1. Meet all PPQ requirements or conditions as listed in permits for organisms kept in the facility. Permits from other Federal and State Agencies may also be required for certain plant pests. Receipt of USDA Plant Pest Permits does not relieve applicants from the responsibility of obtaining other permits. USDA Permits may be withheld or revoked if other Federal and State requirement are not satisfied.
2. Send SOP and blueprints to:

   Containment Facilities Program
   USDA, APHIS, PPQ, PPB, CF, Unit 133
   4700 River Road, Riverdale, MD 20737
   Phone (301) 734-5592
   FAX (301) 734-5392

3. Obtain permission from PPQ prior to shipping regulated organisms outside of the facility. Only ship regulated organisms to permit holders who supply a copy of their valid permit to receive these organisms. Follow all permit conditions that specify shipping requirements in their permit.

4. Maintain a log of all organisms that leave and enter the facility under permit.

Submit the above list to USDA APHIS PPQ (at address below) by January 31 of every year

USDA, APHIS, PPQ, PPB, Unit 133
4700 River Road, Riverdale, MD 20737
Phone (301) 851-2046
FAX (301) 734-4300

5. If the facility stops operating as a containment facility, either temporarily or permanently, immediately notify PPQ.