Management of PNTs in the College of Biological Science's Containment Level 2 (CL2) Phytotron

Introduction

The use of genetic manipulation and other techniques to produce plants with novel traits (PNTs) is common in plant sciences research. PNTs are regulated by the Canadian Food Inspection Agency (CFIA), Health Canada and Environment Canada until such time that they are approved for release into the environment and proven safe for use in food, livestock feed, and other non-food (e.g. industrial) uses.

Containment of unapproved PNTs in the research environment is essential for compliance and biostewardship. Guidelines for biosafety, biosecurity and operational procedures are available from several sources[1,2,3]. The College of Biological Sciences (CBS) has therefore created a Containment Level 2 (CL2) growth facility specifically for PNT research and has implemented requirements for biological containment and control of unapproved PNTs and recombinant DNA-derived organisms associated with plants. The following resource people oversee the facility:

Phytotron Coordinator…… Michael Mucci ext. 53960
Phytotron Committee Chair……. Hafiz Maherali ext. 52767

How are PNTs defined?

According to the CFIA PNTs by definition are:

- plants containing traits not present in plants of the same species already existing as stable populations cultivated in Canada, or are expressed outside the normal statistical range of similar existing traits in the plant species[1].
- PNTs that are subject to an environmental safety assessment are those plants that are potentially not substantially equivalent to their counterpart plants with regards to potential changes in weediness/invasiveness, gene flow, plant pest properties, impacts on other organisms and impact on biodiversity[1].
- Consistent with the Canadian approach, the CFIA recognizes that it is the presence of a novel trait in a plant that potentially poses environmental risk, and hence is subject to regulatory oversight, as opposed to how the traits are specifically introduced, e.g.,

introduction of novel traits by traditional breeding, mutagenesis, recombinant DNA techniques, etc[1].

Thus, plants developed through mutagenesis, somaclonal variation, wide cross, protoplast fusion or other techniques, as well as the plants developed through recombinant DNA technology may be considered PNTs[1]. Furthermore, conventional breeding may result in a PNT if the selected trait falls well outside the agronomic, nutritional and compositional range for that species in Canada[1].

Please speak with your supervisor or the Phytotron Coordinator if you are unsure about the designation of your plants.

Containment Levels for PNT Research

Biosafety levels for physical containment and operational performance, as specified in Canada's *Laboratory Biosafety Guidelines*, 3rd edition, are applicable to research with PNTs. Criteria for containment of PNT research are:

Criteria	**Biosafety Level**
Not a noxious weed or cannot outcross with one	1
Noxious weed or can interbreed with weeds	2
Contains complete genome of non-exotic infectious agent	2

In the context of PNT research, the prescribed Biosafety Levels *for environmental protection* may be interpreted generally as:

Biosafety Level 1 containment is appropriate for transgenic plants unable to survive and spread (if accidentally released) in the environment.

Biosafety Level 2 containment is necessary for transgenic plants that may exhibit a new weedy characteristic or may be capable of interbreeding with weeds or related species in the environment; any accidental release of associated organisms would have minimal biological and ecological impacts.

Primary containment is achieved by good microbiological practices and biological containment techniques. Administrative protocols and documented standard operating procedures (SOPs) enhance these efforts. Secondary containment of PNT material is achieved by the

physical structure of the greenhouse and certain engineered control systems.

The CBS Phytotron at the University of Guelph enables approved research work at Biosafety Level 1 and Biosafety Level 2. Containment for PNTs is achieved as follows:

(1) Structural Containment

The structure of the CL2 Greenhouse with its internal compartments provides physical containment. It has been constructed to prescribed standards with features for biological containment and control. Regular inspections of the physical condition of the greenhouse are performed by the Phytotron Coordinator. All authorized greenhouse users are nevertheless required to be vigilant for structural damage due to age-related wear and tear, seasonal influences, extreme weather, vandalism or other causes. Observations must be reported to the Phytotron Coordinator. Items to look for include:

- doors that are open or don't securely latch;
- worn door sweeps;
- loose glazing;
- cracks, breaks and damage to glazing;
- damage to screens over openings (which exclude arthropods);
- detritus on screens (which must be devitalized);
- evidence of arthropod vectors in the greenhouse;
- cracks in the concrete floor and other impervious surfaces;
- cracks in cement blocks and mortar;
- damaged or missing seals between structural components;
- damaged or missing seals around pipes and conduit.

The Phytotron Coordinator shall liaise with Physical Resources to arrange and oversee all maintenance activity in the Phytotron.

(2) Engineered Controls

The Phytotron has automated climate control systems for heating, ventilating and air conditioning, and an environmental alarm system for temperature and humidity that is overseen by the Phytotron Coordinator. Your environmental requirements and concerns about actual conditions should be discussed with the Phytotron Coordinator.

(3) Administrative Protocols

Authorization

As the Phytotron is a secure, controlled access facility, authorization to use facility space for PNT and non-PNT research must be obtained from the Phytotron Coordinator. Prospective PNT researchers must submit:

- a **University of Guelph Phytotron Space Request form**;
- an operational Biosafety Permit if Level 2 work is proposed (consult the Biosafety Officer in Environmental Health and Safety as per http://www.uoguelph.ca/ehs/policies/11-02.pdf);
- your facility-related project-specific **PNT Risk Management Assessment Worksheet** including standard operating procedures (SOPs), and Contingency Plan for Breach of Containment.

All locations where PNT and DNA materials will be constructed, grown, stored and destroyed must be identified in this documentation.

Experiments that require CL1 containment may be conducted concurrently with those that require CL2 containment provided that all work is conducted in separate compartments with CL2 SOPs. Greenhouse access may be granted and compartment space will be assigned subject to reasonable research-specific conditions (e.g. mandatory facility orientation, use of dedicated footwear or collection of run-off if appropriate, the destruction of all PNT biomass at the end of the experiment, etc.) and explicit record-keeping conditions. All prospective users must agree to abide by the operational performance standards outlined in this document and the in the *Reference Manual for the Containment Facility for Plants with Novel Traits*. Use of compartment space and containment procedures will be subject to regular and random compliance inspections.

Upon completing their greenhouse work, users must formally decommission their activities, devitalize compartment and equipment surfaces, devitalize or destroy all remaining PNT material and waste, sanitize and vacate assigned space(s) and surrender their access cards and compartment keys to the Phytotron Coordinator.

Facility privileges may be withdrawn for non-compliance at the discretion of the Phytotron Coordinator and the Chair of the Phytotron Committee.

Access and Orientation

Restricting access to the CL2 Greenhouse reduces traffic and the likelihood that transgenic pollen, seed and propagule will be moved about or inadvertently removed from the facility. Authorized users will be issued greenhouse facility access cards and keys for their assigned compartments. The swipe card system maintains an audit trail used by the Phytotron Coordinator for security purposes. All investigators, whether working with PNTs or not, will be provided an orientation about the access, containment and control procedures at the CL2 Greenhouse.

Signs and Labeling

The principal investigator must post a "Caution – Experiment in Progress" sign at the entrance to his/her compartment. The sign (provided by the PNT Phytotron Coordinator) must indicate the Biosafety Level, the plant species and novel trait, viable microorganisms used, precautionary requirements, and must include 24-hour emergency contact information. Any risk to human health must be identified separately with a Biohazard Warning. Signage and labeling within the compartment must distinguish PNT and non-PNT research plant materials.

Experimental Equipment

Only equipment essential for experimental work may be brought into the CL2 Phytotron. Such equipment should be resistant to water and the chemicals needed for sanitization at the end of the project. It may be appropriate, for example, to contain the entire PNT experiment in bench-top glass boxes or screen boxes.

Transport of PNT Material

PNT materials and viable microorganisms must be transported between the approved research laboratory (which may have, for example, a biological containment cabinet needed for biohazardous aerosol-producing work) and the CL2 facility in labeled non-breakable leak-proof containers with lids. Similarly, plant and other PNT-related materials to be autoclaved must be securely contained, labeled and transported directly to the designated secure storage area in the facility in accordance with established procedures. Transport containers must be sanitized after each use.

Hand Washing and Hygiene

Hands must be washed prior to leaving the greenhouse compartment. Street clothes must be inspected for evidence of transgenic pollen.

Housekeeping

Research personnel must keep their compartments clean and orderly. All detritus must be devitalized in accordance with the project SOP and any instructions from the Phytotron Coordinator.

Pest Control

Arthropods and nematodes are potential pollen vectors. Their use in experimental work must be specifically approved.

The presence of unwanted pests must be reported to the Phytotron Coordinator. Pest containment and control measures will be prescribed.

Inspection and Record Logs

Compartment-specific PNT Logs will be provided and their use is mandatory. Chronological inspection records must be retained for the initial and pre-operational inspections, regular (weekly) operational inspections, post-experiment inspections and the final post-decommissioning inspection with the Phytotron Coordinator. Any unsatisfactory conditions must be reported immediately to the principal investigator and to the Phytotron Coordinator.

In order to track the whereabouts of PNT materials, records must be maintained of all such plant material removed from the Phytotron. The PNT Log must be annotated with such details (e.g. date, what was taken, how the container was sanitized, where it was taken, by whom).

Inspection and Record Logs must be surrendered to the Phytotron Coordinator at the time of decommissioning for safe-keeping and future audit by institutional or regulatory authorities.

Breach of Containment

A breach of containment can occur due to weather related incidents, utility interruptions or failures, vandalism or human error (leaving

doors open, spills or improper handling, transportation or disposal of PNT material). All personnel associated with the CL2 Greenhouse research work must be familiar with the Contingency Plan for Breach of Containment. General instructions in this regard shall include:

- notifying the PNT Phytotron Coordinator;
- containing all flowering or seeding PNTs as necessary;
- taking steps to recover or prevent the spread of PNT material;
- correcting any deficient containment or procedures;
- determining if PNT material has escaped the Phytotron.

Any accidental release of unapproved PNT material to the natural environment must be reported forthwith to the Phytotron Coordinator and, via the Phytotron Coordinator, to University authorities, to the Canadian Food Inspection Agency and to Environment Canada.

Devitalization and Disposal

Steam heat (autoclave), dry heat, chemical inactivation and incineration are possible methods for the treatment or destruction of experimental and PNT materials. The processing of PNT biomass by autoclave is the preferred treatment method. All experimental materials (e.g. soil, gravel, stones, trays, pots, tools, irrigation water, work surfaces, etc.) and transgenic, PNT and non-PNT materials must be treated. All activities related to the treatment and destruction of such materials must be recorded in the PNT Log (i.e. date, description of material, biohazard bag ID, method used, and name of researcher).

PNT materials to be destroyed by autoclaving or incineration must be collected in orange biohazard bags. When full, these bags must be identified with coded labels provided and then taken to the secure area in the autoclave area for storage until processing can be arranged. Treatment will be arranged by the Phytotron Coordinator.

Experimental microorganisms must be rendered biologically inactive by an appropriate method before being destroyed outside of the CL2 Greenhouse.

(4) Standard Operating Procedures (SOPs)

The principal investigator shall document and issue standard operating procedures to his/her staff concerning the conduct and containment of experimental work, the growth and management of transgenic and PNT material, and the use of biological containment techniques. A

separate Contingency Plan for Breach of Containment must be included with the SOPs. A copy of this Contingency Plan shall be posted at all times outside the door to the greenhouse compartment. If appropriate, Material Safety Data Sheets (MSDSs) and any information about human biohazards must also be available outside the entrance to the compartment.

(5) Biological Containment Techniques for PNTs

Biological containment of the PNT material will ensure that no experimental plant material and associated plant pathogens are released. The focus of these efforts will be PNT reproductive biology based:

- cover or remove flower and seed heads to prevent pollen and seed dispersal;
- harvest plant material prior to sexual maturity or use male sterile lines;
- control the time of flowering so that pollen shed does not coincide with the receptive period of compatible plants;
- ensure that cross-fertile plants are not within the pollen dispersal range of experimental plants;
- localize transgenes in non-propagative plant parts.

(6) Biological Containment of Microorganisms

Effective dissemination of microorganisms beyond the confines of the Phytotron can be prevented by one or more of the following procedures:

- 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 these microorganisms, and confine these injections to internal plant parts or adherent plant surfaces;
- 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;
- 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;

- use viruses and other microorganisms (or their genomes) whose animal vectors are reasonably expected not to be present;
- use microorganisms that have obligate association with the plant; or
- 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 or organisms outside of the experimental facility.

(7) Biological Containment of Macroorganisms

Effective dissemination of arthropods and other small animals can be prevented by using one of more of the following procedures:

- use non-flying, flight-impaired, or sterile arthropods;
- use non-motile or sterile strains of small animals;
- conduct experiments at a time of year that precludes the survival of escaping organisms;
- use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or
- prevent the escape of organisms present in run-off water by chemical treatment or evaporation of the run-off water.

Summary

Adherence to the containment strategies, personal hygiene precautions, project management initiatives and record-keeping requirements will maintain the integrity of CL2 Greenhouse operations and will prevent the release of unapproved PNT or genetic material to the natural ecosystem. Exceptions or modifications to the foregoing SOPs must be approved by the CL2 Phytotron Coordinator.

Contact the PNT Phytotron Coordinator if you have any questions or concerns about biosafety or biosecurity, or about compliance with Phytotron PNT protocols or prescribed regulatory requirements.

Document updated November 1, 2007.

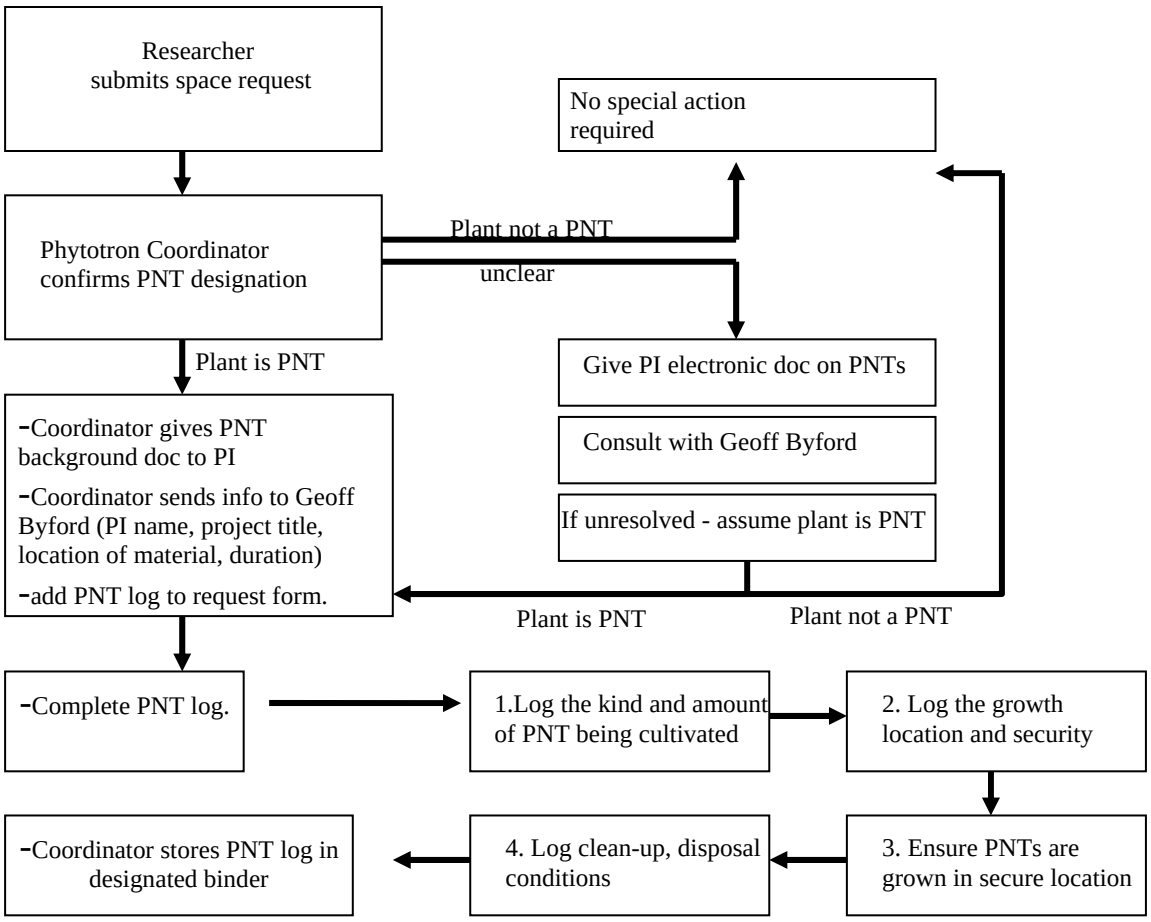
References

1. Regulatory Directive 2000-07: *Conducting Confined Research Field Trials of Plants with Novel Traits in Canada.* Canadian Food Inspection Agency (CFIA), 2004 (update), Ottawa. www.inspection.gc.ca/english/plaveg/bio/dir/dir0007e.shtml

2. *Laboratory Biosafety Guidelines*, 3rd Edition, 2004. Health Canada. http://www.phac-aspc.gc.ca/ols-bsl/lbg-ldmbl/

3. *NIH Guidelines for Research Involving Recombinant DNA Molecules.* National Institutes of Health, U.S.A., January 2001. http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html

PNT Flow Chart



Student PNT (Plants with Novel Traits) / IM (Infectious materials) Guide

1. Indicate PNT or IM on a Phytotron reservation form. If you are unsure about your plants, consult with your supervisor and the Phytotron coordinator.

2. Complete the PNT work sheet indicating the nature of the novel traits

3. Project number assigned by the Phytotron coordinator for space in the facility

4. Begin experiment, pick up or download the PNT/IM log and keep it in the designated area, usually in a folder outside the growth area. Use colored tags for PNT material, white tags for non-PNT material.

5. Use the PNT/IM log to record the amount of PNT/IM material in your area, the addition of new material, where and when any material is moved or destroyed.

6. As it becomes necessary to dispose of material, pick up orange autoclave bags and labels. Label all bags with the stickers provided by the Phytotron coordinator, note disposal Bag ID on your PNT/IM log, and bring them over to the PNT/IM disposal bin near the autoclave.

7. The Phytotron coordinator handles autoclaving duties for PNT/IM material.

8. Return your completed PNT/IM log to the Phytotron coordinator once your experiment is finished.