

Standard Operating Procedures for the Insect Module

Last Updated: 30 July 2014

1. Introduction

- a. The Biotron insect module is a facility for the rearing of, and experimentation on, insects. Because many imported or modified insects may have the potential to be agricultural, economic or environmental pests, the facility is to be managed as a Plant Pest Containment Level 2 – Arthropod (PPC-2 Arthropod), a level suitable for work with arthropods that require containment or quarantine. Note that this level of protection is different from the Biosafety ‘CL2’ system, in that it has been developed specifically for work on arthropods. As a result, the use of disease vectors carrying a human pathogen may require additional containment, or be inappropriate for use in the facility.
- b. These SOPs and rules are specific to the insect module of the Biotron, and are in addition to any other rules covering overall use of the Biotron.
- c. All Occupational Health and Safety regulations under federal, provincial law or University regulations must be adhered to whilst using the Biotron. These laws and regulations take precedence over and are in addition to the SOPs outlined herein.

2. Training Requirements

- a. Access to laboratories in the Biotron and Biology require the following training courses:
 - i. WHMIS Comprehensive
 - ii. Laboratory safety and hazardous waste management
 - iii. Biosafety
- b. It is recognised that some courses are offered only intermittently. Access may be granted upon evidence that you have enrolled for this training.

3. Orientation to the Insect Module

- a. The New User Checklist covers items for orientation (WHMIS, Biosafety, Lab Safety, etc.). The Checklist must be completed and signed by the Project Supervisor and submitted to Biotron Administration before access will be given to individuals to the unit.

4. Responsible personnel

- a. The Principal Investigator of a project is responsible for his or her personnel, and for ensuring that all of these procedures are followed.
- b. Biotron staff maintain the integrity of the containment facility and the functioning of the insect module, however are not responsible for the maintenance of cultures or for cleaning up after users.
- c. Function and maintenance of the Biotron-owned equipment and chambers is the responsibility of the Biotron personnel.

5. Entering the Insect Module

- a. Before entering the outer door to the insect module, please leave personal possessions (i.e., outdoor coats, laptops, etc.) in the lockers outside the module.
- b. Please make very effort not to come directly in from the field or greenhouse into the insect module. If, however, it is unavoidable footwear must be changed prior to entering into the module, it is preferred that you change before entering the Biotron.
- c. Inspect yourself in the mirrors to check that there are no adhering arthropods.
- d. Give the outside of any containers and carts a quick vacuum to prevent too many things coming in. Pay particular attention to the undersides of any containers or carts.
- e. Note that the doors are interlocking, and that the lights go off when the doors are open. If the lights do not come on when the door is closed, flick the switch – they will still close when the inner door is opened.
- f. Protective laboratory clothing, properly fastened, must be worn by all personnel, including visitors, trainees and others entering or working in the laboratory; suitable footwear with closed toes and heels, covered by booties, must be worn in all lab areas.
- g. General Laboratory practices are in effect. (see General Rules below)

6. Exiting the Insect Module

- a. Before exiting the module, wash your hands and inspect yourself visually for any adhering arthropods.
- b. Walk through the door into the anteroom and remove your Biotron lab coat and booties (and or lab shoes) and hang on the appropriate hook.
- c. Inspect yourself in the mirror for adhering arthropods. Check the undersides of your shoes and under any flaps or collars.
- d. Vacuum the outsides of containers, carts etc. Please also vacuum briefly around your shoes and any cuffs etc.
- e. Inspect yourself in the mirror again.

- f. Check carefully around the door for any wayward creatures.
- g. You are now clear to leave the Insect module.

7. General rules

- a. Keep all doors closed when possible.
- b. No food, gum, drink, application of makeup, lip balm or insertion or removal of contact lenses while in the insect module.
- c. Restrain all long hair so it can't come into contact with insects.
- d. Loss of containment, accidents, escapes and near-misses must be reported to Biotron Administration via the Insect Escape Form, who will ensure that the SOPs have been followed and will maintain a written record of these events.
- e. Labelling of reagents and equipment
 - i. All reagents, materials, containers and instruments that are associated with a specific user or project must be clearly labelled with the user's name and (in the case of reagents) the date.
 - ii. Any special-use equipment must be clearly labelled as such.
 - iii. All labels for potentially hazardous reagents must conform to University of Western Ontario regulations.
- f. Please minimise visitors to the insect module. Social visits are unacceptable. Visitors assisting with specific procedures are allowed, but they must be supervised by a current user at all times, and should not be used as a loophole to avoid meeting other access requirements.
- g. Clean-up
 - i. Please note that there is only limited cleaning by custodial staff in the insect module. It is your responsibility to keep the module clean and tidy. Please avoid a tragedy of the commons.
 - ii. All bench space used must be cleared and cleaned immediately after use. If you must leave equipment set up, please label with a name, date and contact information.
 - iii. Media bottles (etc.) left to set or cure must be covered with cheesecloth and clearly labelled with (1) the media (2) the user, (3) the date and time of production and (4) the date and time of expected clean up. Media curing should take no longer than 24h. Ideally, do this on a cart in the food storage room.
 - iv. After use, all glassware must be cleaned and, when dry, the user is responsible for putting it away. DO NOT leave glassware on drying racks for more than 24 h.
 - v. After use, all benches and shelves used must be sprayed with bleach solution and wiped with a paper towel, followed by 70% ethanol and wiped with a paper towel.

8. Waste Management

- a. All chemical wastes must be dealt with as per Western regulations.
- b. All unused media waste, if non-hazardous, can be disposed of in the trash. Please be mindful of potential leaks (double- or triple-bag media), and also of the volume and weight of trash bags.
- c. All used media waste must be autoclaved prior to disposal in the trash. Follow the Western Autoclave SOPs and instructions provided by Biotron staff on operation of the autoclave.
- d. All insect waste must be disposed of as per permit regulations.
 - i. At the very least, please freeze all insect waste for at least 24 h before disposal in the trash.
 - ii. Autoclaving or preservation in Ethanol is required for waste of all insect species for which cold tolerance is unknown, and for all species imported to Canada under CFIA permits, as well as any species known to constitute an environmental, agricultural or economic risk.
 - iii. Insect waste includes both the insects themselves and any media or plant material with which they have come into contact.
 - iv. Please remove all waste from the module as soon as possible after treatment.

9. Diet room (120)

- a. The Diet room is a general-use room for users of the insect module to prepare media for insect cultures and perform experimental procedures associated with insects in culture in the facility that cannot be performed elsewhere. All rules and SOPs associated with the Biotron, and the insect module in general must be adhered to.
 - i. The cold room, balances, hotplates, glassware, autoclaves etc. are shared equipment. Please do not appropriate them for specific project use, nor remove them from the Diet Room. Please contact Biotron personnel with any issues. Please leave all equipment clean and tidy.
- b. Please label all non-common use equipment. Please request permission from the owner to use this equipment and observe the common courtesy of returning borrowed equipment clean and in working order.
- c. Storage for non-hazardous diet making components is available in a cupboard off the exit annexe. Please keep this space tidy, avoid spills and keep food components in airtight (and insect-proof) containers whenever possible. Please label all components with the lab name, and coordinate with other users if space is limiting.

10. *Drosophila* and the Biotron Insect Module

- a. Because this is a general insect facility, we ask that *Drosophila* users modify their habits to prevent escapes whenever possible. This is not only because we have an obligation to prevent all escapes from the Biotron, but also because flies can spread mites and microbes from cage to cage, and from room to room.
- b. Please keep the door to the fly room closed except when entering and leaving. Please turn the lights on immediately upon entering the room (*Drosophila* are usually positively phototactic).
- c. Please do everything you can to prevent unnecessary escapes. Please also do your best to kill/capture escapees.
- d. There are traps, fly paper and an electric trap in the fly room. Please check on their state before you start work, and replace/refill any traps that require it.
- e. Simple hygiene will prevent a build-up of flies (or transmission of mites):
 - i. Always wash benches and CO₂ pads down with ethanol before and after use.
 - ii. Never leave food lying around. Unwanted fly food, old cultures etc. should be stored in the freezer and autoclaved before disposal, and then taken out of the module as soon as possible thereafter.
 - iii. Please regularly sweep floors and wipe benches.

11. Escapes

- a. Escapes from rearing containers will happen from time-to-time. Our goal is to confine escapees to the insect module, and to quickly catch them.
- b. Please immediately catch/kill (as appropriate and possible) any insect you notice in the entry or exit anterooms. There are escapee containers provided for this purpose.
 - i. If you can identify the source of the arthropod, please place the container into the appropriate incubator and inform the owner. If not, place the container into the -20 freezer.
 - ii. Please fill in and submit (electronically or in hard copy) an Insect Escape form.
- c. If an insect you are working with escapes, please do everything you can to catch it immediately. There are insect nets available, as well as a shop vac. Please don't hesitate to ask for help from anybody else in the insect module, or to use the phone to call for help outside the biotron. Make use of the emergency contact numbers if necessary.
 - i. Please hang the 'warning, escapee' sign on the door to the exit anteroom
 - ii. **Be sure that if you call someone in from outside, you are careful to prevent the insect from getting into the anteroom.

- iii. Once the insect is caught, please deal with it appropriately (think before returning it to your experiment!), and fill in an escape form, copied to the user (if you know who it is) and their PI.
- d. If you cannot catch the arthropod
 - i. Place the 'Warning: Ongoing escape' signs on all entry and exit doors. This will inform other users to be particularly vigilant in their entry and exit procedures.
 - ii. Fill in an escape form, and email it, with copies to the user (if you know who that is) and their PI. Please use the subject line "Urgent: ongoing insect module escape". A warning will be forwarded to all current users.
 - iii. Continue to try to catch the arthropod, and be prepared to continue working with Biotron staff until it is caught.

12. Moving arthropods in and out of the Insect Module

- a. All insects must be moved in containers that are as escape-proof as possible, and tightly closed.
- b. If insect have been imported under a permit, the package must not be opened in the diet room, but preferably in a walk-in chamber. The packaging material must be disposed of as though it were infested (or cleaned thoroughly).
- c. If the insect has been imported under a permit, please
 - i. Submit an 'imported insect' form plus a copy of the permit to Biotron Administration.
 - ii. Add the imported arthropod information to the inventory on the wall of the diet room.
 - iii. Please update this information when you have finished with the animals.
- d. Please check all containers and cultures (inside and out) for unwanted individuals or hitchhikers before removing them from the insect module. You will then check them again as part of the normal exit procedure.
- e. If insect is a "plant pest" imported under a permit the package must be opened in a sleeved cage in the chamber to house the insect. The packaging must be disposed of as though it were infested.

13. Diseases and parasites

- a. We wish to avoid the introduction of parasites, mites etc. Please try to avoid the introduction of such organisms.
 - i. For *Drosophila*, please adhere to standard anti-mite practices (anti-mite paper on shelves, benzyl-benzoate-treated stoppers, etc.
 - ii. Please monitor all stocks (*Drosophila* and otherwise) regularly for mite infestation.

- iii. If a culture with food mites must be used in the insect module for unavoidable reasons, or an infestation is found in a stock that cannot be discarded, the following protocols must be followed:
 - 1. Anti-mite paper must line all shelves in the incubator.
 - 2. Tight-fitting stoppers treated with mite repellent (benzyl benzoate) must be applied to all containers.
 - 3. The incubator, including the external surfaces and the floor around it, must be wiped with 70% EtOH followed by 10% bleach every three days. Please keep a record of this procedure.
 - 4. All handling of cultures on lab benches must be performed on anti-mite paper and the surface wiped with ethanol and chlorox (as above) immediately after use. Clean (in ethanol) any tools used (paintbrushes, forceps etc) before storage.
 - 5. A separate lab coat and gloves must be worn when dealing with the contents of an infested incubator and these must be changed before contacting non-infested incubators.
- iv. Please be careful to avoid transmission of fungal or viral infections. Careful hygiene (as above) plus disposal of infected material (in ethanol or autoclaving) is essential. Deposit fungus-infected corpses into plastic bags or containers that have been misted with water to reduce aerosols of fungal spores.
- v. The most common source of infestations and disease is plants or soil brought in from the greenhouse. Please be very careful with such material, and move it in cages or sealed containers wherever possible.

14. Use of Insecticides

- a. For the most part, we aim to avoid the use of insecticides in the insect module. If you plan to use insecticides as part of your research, please discuss this with the other users and the Biotron staff. Be prepared to develop detailed, specific SOPs that will prevent damage to other projects. Please allow at least 3 months' lead time to give adequate time for this process.
- b. Use of insecticides for control of infestations will only be conducted under exceptional circumstances, and will be subject to additional discussion among users and those responsible for the Biotron.

15. Labelling of Samples, Cultures and Materials

- a. All chambers must be clearly labelled on the outside with
 - i. The User's name
 - ii. The name of the species

- iii. Temperature, humidity and light conditions, including the times of any changes (ie: '12:12 L:D' must also include 'lights on 9am' or similar)
- iv. UWO and after hours contact details of at least three people who can come to help deal with problems. These people must have some idea of what to do about escapes, as well as be able to manage the cultures in the case of an emergency (e.g. loss of temperature control). Please discuss this with them, and leave instructions on the door of your chamber.
- b. Each container and culture must be labelled with suitable information: Name, date, species etc, to allow them to be identified in the case of an emergency shutdown that sees the removal of cultures from your chamber.
- c. All materials, reagents etc. in storage and elsewhere must be labelled with (at the least) a name and contents. It helps to collate small items (spatulas, paintbrushes etc) onto a plastic tray to keep them together. In this case, the tray can be labelled. Reagent and chemical labels must also conform to WHMIS and Western regulations.

16. Changes to Project

- a. If your project changes (e.g. you start using different organisms, or move to a different room), please completed a new Project Application and Insect Module Exit form and submit to Biotron Administration. Do not move any current specimens until authorization has been given by Biotron Admin.

17. Project Completion

- a. Please remove all of your material, reagents, etc.
- b. Please clean out the incubator or chamber, and wipe it down with bleach and then ethanol solution.
- c. Complete an Insect Module Exit form and submit to Biotron Administration. Once specimens are removed and form is submitted the Biotron will then be able to heat-treat the chamber prior to it being made available for another user.

18. These SOPs are subject to change. All users are bound to follow new SOPs when they are instituted.