

Policy for the use of the Sears Plant Growth Facility Greenhouses and Growth Chambers

2019

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Training Requirements:

It is the responsibility of the assigned user to ensure that all personnel under their supervision are familiar with these policies. All principle investigators, staff and student workers are required to read this policy and pass a quiz with a score of at least 90% before swipe card access to the facility will be granted. Growth space will be periodically inspected by greenhouse staff and PI's (or designated contact) notified of violations. Failure to rectify violations by the given deadline will result in greenhouse staff stepping in to fix the problem at a recharge rate of \$50.00 per hour. Continual violations will result in loss of greenhouse/growth chamber space.

All persons using greenhouse or growth chamber space must be Worker Protection Standards trained. Contact Michelle Brooks to schedule a 30-minute appointment.

Use of Facilities and Sanitation

1. General Cleanliness - All users of the Sears PGF are expected to observe good housekeeping rules by returning equipment and unused supplies to proper storage areas and by cooperating in keeping the facilities clean and orderly. Sound sanitation practices are necessary to reduce, if not eliminate, disease and insect problems. Bench tops, floors and drains should be kept clean of plant/soil debris at all times. Brooms/dust pans and shop vacs are provided for daily use. Please contact Rich Wilman when you wish to use the power washer and he will place it in your greenhouse room. Reminder – any debris cleaned off of these areas must be autoclaved if you are growing transgenic plants in your space.
2. Tools/Containers - Use clean, sterile containers and tools. Avoid putting plant material, containers, or tools on the floor or other "contaminated" surface. The floor is not a desirable work area. All used pots and containers should be cleaned/sterilized immediately and returned to assigned storage areas.
3. Potting Media - No soil will be allowed, only use Promix provided. The use of premixed media is strongly encouraged and all media must be stored in sealed containers or tubs. Take care not to contaminate the sterilized media in tubs. Only use

clean scoops, dustpans, and brushes on bench surfaces. Promix is available on a recharge basis in the potting room. You will need to fill out the billing log sheet with DATE, PI and Promix USAGE AMOUNT when you use this material. Please indicate how many scoops or bales used. For questions about use of the Promix contact Michelle Brooks. If you plan to use a significant amount of media at once for a large project, please let Michelle or Rich know the approximate date and amount you'll be using. If you must use media components such as perlite, vermiculite or turface in your research, only order the amount you will use quickly. There is not room for storage of these media in the potting room and **storage of all media is strictly prohibited in greenhouses or growth chambers!**

4. Hoses - All watering hoses should be stored off the aisle walkways, and breakers/nozzles must be kept off the ground. **Do not drink from hoses!**
5. Pests - Report all insect and disease problems to the Michelle Brooks immediately so that their spread can be contained. All requests must be submitted in writing either by filling out provided form in the greenhouse hallway or by email to Michelle Brooks. After the request is submitted, the room will be scouted closely to determine necessary action. If pesticide application is needed, it will be done Tues. or Thurs. evenings after 4:30 pm by trained greenhouse personnel only. A treated greenhouse will be posted and locked until the Restricted Entry Interval is expired. No early entry by greenhouse users is permitted. It is strongly encouraged for other rooms to schedule a 2-4 week period in July that the greenhouse can be emptied, thoroughly cleaned and "cooked" out to help control pests. (See pesticide policy section for more detailed information.)
6. Storage - **Do not use greenhouse space for storage of pots or other supplies.** Storage space downstairs is limited so do NOT order large quantities of supplies! If you have not used it in a few years, throw it out! ALL items on shelves in the potting room must be labeled with the PI name! Storage cages must be kept neat and clean! Only items intended for use in the Sears Plant Growth Facility may be stored here. Do not use this facility to store supplies for your lab or field research!
7. Living material – No plant material, other than seed, should be brought into the Sears growth chambers, greenhouses or headhouse/potting room without prior approval by the Oversight Committee. Plants should not be moved from the growth chambers to greenhouses or greenhouses to growth chambers. Plants should not be moved from one greenhouse to another. Bringing plants from outside and moving plants between spaces only spreads pest problems and is prohibited. Seed material brought in from the field strictly for processing/storage should be taken directly to the drying room or the seed processing room and dealt with promptly. Make sure it is properly bagged to contain any possible insects during transport.
8. Hazardous Materials - The greenhouse modules and growth chambers in the Sears PGF are considered laboratories. Environmental Health and Safety conducts inspections biannually. No food or drink is allowed inside the greenhouse or growth chamber units. **Do not drink from hoses! ALL substances must be stored in a**

closed container and clearly labeled. This includes, DI wash bottles, fertilizers, etc. Culligan tanks must be secured to the wall.

9. Potting Room use – Use the potting room for preparing media and filling pots. Seed should be sown in the greenhouse or growth chamber to avoid contaminating the unused promix with stray seed. **Clean up all of your things immediately when you are done!** The potting tables must be totally clear and clean. **Do not put spilled media from the floor back into the unused media container. It may contain unwanted debris, diseases or insects!** Any media that has been on the floor or work table must be discarded. Media remaining from pot filling that has been in **mixing table** can be retained and saved. Do not put media that has been moistened back in the storage bin. Only wet up what you know you will use! All carts should be vacuumed and cleaned.

The sink may be used for washing pots, however, you may not store dirty pots in the potting room for any period of time...**you must wash them immediately.** *Brush any loose dirt out of the pots into the trash can to avoid clogging the drain.* If you notice the water draining slowly, the soil trap may be full...contact Rich Wilman for cleaning.

Cleaned pots may be stacked on the sink for a short time for drying. As soon as they are dry, they should be moved to the appropriate storage area.

10. Disposal of unwanted material – Greenhouse rooms are equipped with two trash cans on dollies. ONE of these (marked General Trash) is for collection of daily general trash (non-transgenic plant trimmings, disposable gloves, paper trash, the occasional dead, non-transgenic plant) and will be emptied twice weekly by greenhouse staff. Currently, MU is not keeping plant material waste separate from general trash so there is no need to separate trash at this point. **Do NOT put transgenic material in this can!**

**When a non-transgenic experiment is terminated, it is the responsibility of the user to take this unwanted material directly to the dumpsters at the northeast corner of the building. It is recommended that you use a cart to move the plants directly to the dumpster. The trash cans are large and quite heavy when full, so I would not recommend filling it and then trying to dump it.

For transgenic material – it is the responsibility of the user to properly bag the transgenic material that is unwanted. Greenhouse personnel will then collect the bagged material, autoclave and dispose of it.

Greenhouses:

- Gather unwanted transgenic material in autoclave bags. Do not overstuff the bags! Overstuffed bags that are too heavy to handle will be returned to the user for splitting.
- Tie the bag shut or secure with a twist tie
- Place the tied bag in the trash can marked “Transgenic Only” in your greenhouse
- Additional autoclave bags are available in the designated area in the hallway.

Growth Chambers:

- Gather unwanted transgenic material in autoclave bags. Do not overstuff the bags! Overstuffed bags that are too heavy to handle will be returned to the user for splitting.
- Tie the bag shut or secure with a twist tie
- **Write your lab name or growth chamber number on the autoclave bag**
- Place the bag in the designated bin in the growth chamber room
- Additional autoclave bags are available in the designated area in the growth chamber room.

Seed Rooms:

- Gather unwanted transgenic material in autoclave bags. Do not overstuff the bags!
- Tie the bag shut or secure with a twist tie
- Place the bag in the designated bin in the seed sorting room.
- Additional bags are available in the designated location in the seed sorting room.

(Greenhouse personnel will NOT be handling unwanted material coming from the Plant Transformation Core Facility lab...that remains the responsibility of the lab personnel.)

Environmental Control

1. Greenhouse environmental control equipment (thermostats, vents, photoperiod shades, time clocks, environmental control system, etc.) shall be operated only by the Sears PGF Staff. These are not adjustable by users.
2. Changes in the environmental conditions in individual greenhouse spaces should be requested by email to Michelle Brooks. Malfunctions in the environmental control system in greenhouses can be reported to Michelle or Rich. Changes in the environmental conditions setup for growth chambers should be requested by email to Shawn Dey. Malfunctions in growth chambers should be reported to Shawn Dey.

Maintenance

1. Any problems with structure or non-dedicated-user equipment should be reported to Rich or Michelle.
2. Maintenance of plants and equipment dedicated to individual projects is the responsibility of the greenhouse user.
3. Please be aware that the greenhouse staff, as well as the Campus Facilities employees, must have access to certain areas in your greenhouse for maintenance purposes. The first area is the exhaust fans. There needs to be at least a 3 ft space between the south wall and your plants so that Campus Facilities can get to the fans to service them. The second area is the sumps and evaporative pads. There needs to be

a 3 ft space between the pads and your plants and there needs to be at least a 2 ft space between the sump and your plants so the greenhouse staff can get to them for weekly maintenance. Please do not leave anything on top of the sumps. Do not store anything on top of or behind the fin tube heating. This area should NOT be used for drying samples.

Alteration of Facilities

Approval must be obtained from the Sears PGF Oversight Committee before any changes, deletions, or additions are made to the permanent facilities.

Experimental Hazards

1. The use of hazardous materials, organisms, or systems (e.g., radioactive materials, dangerous non-pesticide chemicals, UV lamps, pollutants, etc.) in greenhouse experiments must be approved by the Sears PGF Oversight Committee. A minimum of two weeks notice is required prior to the use of any such materials or systems in the greenhouse.
2. Rooms in which hazardous materials/conditions are present must be kept locked. The rooms must be labeled with appropriate warning signs. Emergency contacts and procedures must also be posted.

Shoes and Clothing

Appropriate footwear is required for greenhouse staff, users, and visitors. No open-toed shoes should be worn in the greenhouse.

Nicotine

Smoking/vaping/chewing tobacco is absolutely prohibited in all areas of the Sears PGF. If you use tobacco, wash hands thoroughly before entering the facility to avoid bringing in viruses that may be contained in tobacco

Termination of Occupancy after Project Completion

It is the responsibility of the **greenhouse user** to properly dispose of the plant material in a timely manner after project completion. The module must be returned to its original state when a user terminates occupancy.

PESTICIDE POLICY

1. All pesticides are to be applied by trained greenhouse personnel only, not by greenhouse users. This includes soaps, oils, etc.
2. Everyone who works in the greenhouses or growth chambers is required to go through initial and annual Worker Protection Training with Michelle Brooks. Michelle will also go over the pesticide procedures with new hires during that initial training. If you hire new people, make sure they contact Michelle to set up a time to do this 30-minute training. For annual retraining, look for email announcement at the beginning of fall semester for large group trainings to attend.
3. It is up to you, the user, to keep an eye on your plants and submit a pesticide application request in writing to Michelle when you notice a problem. The best way to do that is to email Michelle with your request and the specific location you are requesting treatment for. Once Michelle receives a request, her crew scouts the room to confirm which specific pests are present and she will schedule the initial application. Michelle does her best to get the room treated as soon as possible but depending on when she receives your request and the severity of the problems in all the rooms she receives requests for, it may not be sprayed immediately. For example, if you turn in a request at 3:00 on Tuesday it will probably not get treated until Thursday. Pesticides are only sprayed on Tuesday and Thursday evenings after 4:30 pm so that you have time to get your work done during the day and the greenhouse will only be locked overnight. Warning signs will be posted on the door for the restricted entry interval during which time, the room will be locked. In the morning, they take down the signs and unlock the door. The green sign stating when and what was sprayed will be posted in the hallway for 30 days so you can go back and look to see what was done in your room. Beyond 30 days, you need to contact Michelle for that information.
4. After the initial application, greenhouse staff continue to scout the room and do follow-up treatments as needed. As required by most pesticide labels, spray intervals are at a minimum 7-10 days apart.
5. If you are taking data or something that you must have access to your greenhouse on certain Tuesday or Thursday evenings, you need to let Michelle know so she knows they can't spray those evenings. If for some reason there are plants that you do not want sprayed, you need to let Michelle know that too, however, keep in mind that if the entire room is infested, she cannot get control of the pest if there are infested plants that she can't spray.
6. Spray requests are good for 30 days. Usually in that time, with 1-3 sprays, greenhouse staff can get the pest population under control but will continue to scout and monitor that room for those 30 days. If another problem comes up a couple months later, you have to submit a new request.
7. Keep in mind that you should submit a request when you first notice a problem. If the plants are severely infested, it is much harder to control plus the chances are much

greater than the pest can spread to neighboring rooms. On the other hand, if you see one thrips and turn in a request, if Michelle can't find the thrips when she scouts, the room will not be sprayed. Most of her pesticides are contact pesticides so it doesn't do any good to spray if the pest is not actually there. Michelle will scout the room for the next couple of weeks and if she finds thrips she will begin treatments. There are also lots of restrictions on how many times per year that Michelle can spray certain pesticides so she must be careful to make sure the pest problem is there before she can spray.

8. Those of you who work in both growth chambers and greenhouses should always go to the growth chambers before you go to the greenhouses and never move plants from the greenhouse to the growth chamber room. It's very easy to carry pests on plants or yourself from the greenhouses to the chambers. Michelle also recommends that you do not wear yellow clothing in greenhouses. Whiteflies are very attracted to yellow clothes and will land on you and be moved from one place to another.
9. Michelle's crew can only apply pesticides in the greenhouses and in the growth chamber room in the basement. Any growth chambers located in labs are the responsibility of the lab personnel.
10. Cleanliness in your greenhouse can help to keep pest populations under control. Any build-up of media or plant debris on benches or on the floor are places that harbor pests. Stacks of pots being stored in the greenhouse can harbor pests. There are brooms in the hallway and Michelle has a shop vac and power washer that can be checked out for use by emailing Michelle or Rich Wilman (wilmanri@missouri.edu). If there is any time that a growth chamber or greenhouse can be emptied out and thoroughly cleaned it will help.
11. Please also keep in mind that when Michelle's crew sprays a greenhouse or growth chamber, complete eradication is nearly impossible. There are just no pesticides that are relatively safe for people to handle that completely kill everything. Because most of them are contact sprays it's just very hard to hit every tiny pest so they do the best they can to kill as many as possible.

PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT DNA RESEARCH INVOLVING PLANTS

Adapted from the NIH Guidelines for Research Involving Recombinant DNA Molecules
(Amendment Effective January 24, 2002 Federal Register, November 19, 2001
(66 FR 57970)

<http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>

General Plant Biosafety Levels: The principle purpose of plant containment is to avoid the unintentional transmission of a recombinant DNA-containing plant genome, including

nuclear or organelle hereditary material of release or recombinant DNA-derived organisms associated with plants.

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 on the unintentional introduction and establishment of an organism in a new ecosystem.

Facilities (BSL1-P)

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.

The term “greenhouse facility” includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, which are considered part of the confinement area.

Physical Containment Levels: Biosafety Level 1 – Plants (BSL1-P)

Standard Practices (BSL1-P)

Greenhouse Access (BSL1-P)

Access to greenhouse shall be limited or restricted, at the discretion of the Bond LSC/Sears PGF Oversight Committee, when experiments are in progress.

Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BSL1-P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.

Records (BSL1-P)

A record shall be kept of experiments currently in progress in the greenhouse facility.

Decontamination and Inactivation (BSL1-P)

Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Control of Undesired Species and Motile Microorganism (BSL1-P)

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.

Arthropods and other motile microorganism shall be housed in appropriate cages. If microorganisms (e.g., flying arthropods and nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Concurrent Experiments Conducted in the Greenhouse (BSL1-P)

Experiments involving other organisms that require a containment level lower than BSL1-P may be conducted in the greenhouse concurrently with experiments that require BSL1-P containment, provided that all work is conducted in accordance with BSL1-P greenhouse practices.

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.

Effective dissemination of plants by pollen or seed can be prevented by one or more of the following procedures: (1) cover the reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity; (2) remove reproductive structures by employing male-sterile strains, or harvest the plant material prior to the reproductive stage; (3) 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 (4) ensure that cross-fertile plants are not growing within the known pollen dispersal range of the experimental plant.