Growth Facilities Protocol Handbook

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Web page: https://gf.eeb.utoronto.ca/

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Codes of Conduct

Staff General Responsibilities

The Growth Facilities are managed by Chief Horticulturalist Bill Cole and Horticulturalist Tom Gludovacz. They are responsible for providing growth spaces for research organisms, controlled for temperature, light and sometimes humidity. They monitor, maintain and administer the growth chambers (N=65), rooms (N=22) and glasshouses (N=23). Space occupancy averages over 90 percent. Most of the space is dedicated to research projects followed by materials grown for undergrad courses. Our facilities are primarily used by two departments: Ecology and Evolutionary Biology (EEB) and Cell and Systems Biology (CSB). Occasionally an outside Department may rent space if it is available.

Specific Duties: Staff

- Program environmental controls as per user requirements.
- Maintain a teaching plant collection and grow plant material used in undergraduate lectures and laboratory exercises.
- Maintain and schedule repairs to the glasshouses, growth chambers and growth rooms.
- Charge back for facilities and materials are at cost or in the case of Biological controls, free. Charges are tabulated by month and invoices every three months (see Appendix 1 for current rates).
- 100% of the charges go to preventative maintenance, emergency repairs (failed compressors, cooling valves).
- Receive requests from researchers for growth space and potting material.
- Assign space to users and troubleshoot conflicts.
- Implement an integrated pest management program and inform users of action taken.
- Order seed stocks and potting material for growth facilities, track financials and invoice labs for resources used.
- Construct and maintain online resources for the growth facilities.
- Provide researchers a wide range of knowledge and experience.
- Test new technologies (LED lights, Pest control, Horticultural methods) to increase energy efficiency, improve growth conditions and accommodate researchers needs.

User General Responsibilities

Users of the Growth Facilities are expected to follow the guidelines outlined within this document. Users must be aware that disregarding these policies can have negative impacts on both their own research projects as well as the research projects of others using the facility. Users are expected to behave in a professional manner as outlined in the University's Guideline on Civil Conduct (https://hrandequity.utoronto.ca/wp-content/uploads/sites/34/2016/09/Human-Resources-Guideline-on-Workplace-Harassment-and-Civil-Conduct-Civ....pdf). All scientists, students, staff and visitors to the Growth Facilities are expected in all their conduct to encourage behaviour, language and attitudes, which will create a favourable environment, free of discriminatory actions, stereotyping and harassment. All communication, whether it be in person or electronically, should be kept appropriate and respectful for a professional audience including people of many different backgrounds and identities.

Specific Duties: Users

- Submit requests for space in a greenhouse or growth chamber by email.
- Notify staff when finished with space and conversely do not move into space without permission. Fees for space are by the month and will only stop after notification and all areas clean. Unoccupied space held in reserve will be charged to their lab as if the space were occupied with research material. Sitting on a chamber for future use is discouraged as it limits the ability to assign space for short-term projects. If you are planning a new project that will require significant growth facility resources, please approach us as soon as possible so we can make every effort to provide suitable space.
- Users are not to alter growth chamber parameters. If a change is required, a request should be made to staff by email.
- Report problems with the facilities and its equipment as soon as possible.
- Users are responsible for ensuring daily care of experimental material. This includes watering, feeding and containment of invasive species or controlled pathogens. Users are responsible for making special arrangements for watering over weekends and holiday breaks.
- Users are responsible for purchasing and storing their own plant pots and trays. Staff has catalogs from suppliers available.
- Users must maintain cleanliness in growing spaces and potting areas. Do not obstruct access to valves, lights, drains or other equipment essential to the function of the growing

space. This includes removal of excessive dirt and plant debris on greenhouse/growth chamber floors, maintaining open and unobstructed floor drains, removal of dead plants left for prolonged periods in greenhouse/growth chambers, removal of dirty pots left behind in greenhouse/growth chambers.

- Faculty members who are primary users of the facility should share in the responsibility of training and supervising their students and staff members to assure that sound scientific practices as well as Growth Facilities policies are being followed.
- Report clogged sinks and floor drains to growth facilities staff. Do not use a sink if its drain is clogged.
- Uncontaminated (NO PLASTIC, or Pest species) plant and organic material must be disposed of in the compost 'green' bins, and garbage disposed of in garbage receptacles. For a list of Plant Pests Regulated in Canada see the following link: (http://www.inspection.gc.ca/plants/plant-pests-invasive-species/regulated-pests/eng/1363317115207/1363317187811).
- All untreated soil that is foreign (from any other country) and from regulated areas in Canada must be sterilized prior to disposal.
- Follow the 'Waste Information and Procedures for Bio Labs' as outlined by the University's Office of Environmental Health and Safety (https://ehs.utoronto.ca/wp-content/uploads/2019/04/Waste-Information-and-Procedures-for-Bio-Labs_v3.1-09-14-2020.pdf). All university biosafety policy can be found at https://ehs.utoronto.ca/our-services/biosafety/.

Responsible use of ESC B120 (Autoclave Room):

- The potting table and floor should be swept after completing work. Brooms and dustpans are provided in the room.
- No seed harvesting allowed in the room. Seed harvesting is permitted in B111.
- Do not leave material soaking in the sink overnight. Sinks are in high demand and need to be shared.
- No plastic, glass or soil can go down sink drains. Prior to washing in sinks, ensure all plant material and loose planting media has been removed from the pots and put in the appropriate waste container.
- Compost bins are for disposing of potting media and plant material only. Non-organic materials such as plastic, broken pots, tape, twist ties and aluminum foil should be disposed of in the garbage bin.

***Responsible use of ESC B111 (Plant Senescence Room)**:*

- The senescence room is for short-term storage of drying plants and harvesting of seed. Maximum occupancy period of **60 days**. Owners of material found to have been stored beyond 60 days will be notified their time is up. Material that has been abandoned is subject to removal. Researchers will be notified, material that is abandoned will be removed
- Plants should be stored in trays, and each tray should be labelled with the researcher's name, principal investigator and the date of entry. Reusable stakes and labels will be provided. Loose or unlabeled material will be subject to disposal.
- The worktable and floor should be swept after harvesting. Brooms and dustpans are provided in the room.
- Compost bins are for disposing of potting media and plant material only. Non-organic materials such as plastic, broken pots, tape, twist ties and aluminum foil should be disposed of in the garbage.

Access into building, chamber rooms and greenhouses

It is important that you always have your lab key on you as this is what gives you access into the greenhouse research wing and growth chambers rooms (ESC B112, B115, B119, B122). Specific keys are required for accessing chambers in RW, so advanced arrangements with Growth Facilities staff are required. A building key fob is necessary to enter the Earth Science or Ramsey Wright buildings on weekends and after hours (Monday - Friday, 6 am - 6 pm). Without these keys, the doors will be locked, and you will not be able to get into the building.

Emergency and other Contacts

- **9-1-1 is only to be used in emergency situations!** An emergency is any situation that requires immediate assistance from the police, the fire department or an ambulance.
- **City Police** Non-emergency phone line 416-808-2222 ask for "Communications".
- **Campus Police** 416-978-2222 (Emergency) -2323 (Non-emergency)
- **Engineers** 416-978-3000
- **Caretaking** 416-978-6252

Office of Environmental Health and Safety.
https://ehs.utoronto.ca/aboutus/contact-us/

- 416.978.4467

Growth Facility Contacts

- Chief Horticulturalist - Bill Cole (bill.cole@utoronto.ca) 416-729-7779
- Horticulturalist - Tom Gludovacz (Thomas.gludovacz@utoronto.ca) 647-327-9598
- Horticulturalists assistant – Alice DesRoches (alice.desroches@mail.utoronto.ca)

Growth Facilities Web Site

- https://gf.eeb.utoronto.ca/

Horticultural Methods

The Growth Facilities staff is available to train and advise on common horticultural or plant science protocols. These areas of knowledge may improve your ability to feel comfortable working with plants in the growth facilities.

General

To grow healthy plants, first think about where and how they grow naturally. This will guide your choices of what media to grow them in, pot size, watering schedule and environmental conditions. For example, if it is a perennial species from a temperate cline, it is not likely to do well under natural light late in the fall (shortening day lengths). To grow healthy plants is not difficult but it does require frequent monitoring and good planning. Check with peers who have grown this species (from your lab and published literature). The Growth Facilities staff can offer advice based on a wide range of experience with various plants.

Watering

There are several things to consider when watering. External conditions such as, outside temperature, sun and humidity factor into your decision of when and if to water. Other factors include appearance of plant, soil appearance, weight of the pot and feel of the soil. This is the hardest task to learn and the most important.

Rationale

Think of two pots of the same size, one with a plant in and one with just soil. Which one do you think will lose water faster?

The one with the plant in it will dry out quicker because the plant increases evaporation via transpiration.

Over watering is the most common mistake

The plant size and the soil volume affects rate of water use.

Root to soil ratio is the size of the root mass compared to the amount of soil in the pot.

Putting a small plant in a big pot does not help the plant. It is likely to result in soil that stays too wet or even goes anerobic. The roots can die from lack of oxygen or root fungi that attack the water logged roots. When the roots die the above ground leave may show signs of wilting. The

researcher may see wilt and decide it's because of lack of water when it was over watering that caused the problem.

Unlike short lived annuals, longer lived perennial plants that are kept in a small pot for a long time may become pot bound. This may stunt growth and require frequent watering.

General Considerations

- When a plant needs water, fill the pot **from the top until water comes out of the bottom**. Do not soak from the bottom as this can flood the lower levels and push the air up and out of the potting media.
- Overwatering or "Water logging" can lead to iron chlorosis, anaerobic conditions, root rot, and infestation with fungus gnats.
- Try not to disturb loose soil: turn down the water stream or use special nozzle attachments to make the stream less forceful.
- Most plants need water, when the top of the pot dries out. **Do not water plants that have wet soil, especially in small pots** (unless drying conditions are soon expected, i.e., sunny weather ahead).

Watering Process

1. Begin the process with a walk-through (or scan), checking for plants that are in a state of crisis and need to be immediately watered. A plant in a state of crisis may be identified by wilting or shriveled up leaves.
2. Whether you water with a hose or a watering can depends on how big the plants are and whether you are in a glasshouse, room or reach in chamber.
3. Return the watering can and or coil up hose without looping it too tightly and ready for next user.
4. Lastly, make sure the tap is shut off and the hose attachment is left open, which drains out any excess water.

How to determine if a plant needs water:

1. First, what type of plant is this: a succulent? An epiphyte? Is it leafy? Semi-aquatic? Plants come from all over the world, growing in different conditions with differing water needs.

2. Next, how root-bound is the plant? Is it a small plant in a big pot? Then there is a danger of overwatering it. Does it look like a plant that has outgrown its small pot? Then it will likely dry out most days, and under-watering is a bigger danger. Is it in a big pot? Large pots house large plants that often dry out, these are usually safe to water (unless they are dormant).
3. Lastly, what is the weather like outside? Is it a cool rainy day, cloudy all day, tomorrow? [→ less water]. How about a sunny hot day? [→ more water]. Err on the side of watering a plant if you think it might need water. On cold, sunny days in the winter the sun warms the greenhouse and the heat is on, drying out the plants - water more heavily these days too.

Tips for assessing plants:

- Look at plant - is it wilting? Is the soil color light or dark? Does the soil feel very dry and root bound? Is the pot light? →water IMMEDIATELY.
- Pick up pot to feel if it's heavy (wet) or light (dry)
- Touch the soil. If soil surface looks dry, you may stick your fingers at least an inch under soil surface to check for dampness (this method is not preferred).
    - If sufficiently wet/damp →DO NOT WATER,
    - Especially if the plant needs to fully dry out between waterings →DO NOT WATER.
- Think about plant size, root mass, foliage, and pot size. What is the soil to root ratio? Plants that are bigger with lots of leaves and/or a rootbound will use water faster.
- If you see a plant that does not look healthy, take a closer look for pests. Maybe remove the pot and look for signs of over watering (soil may smell of rot) , or pot bound (many roots circling the pot without any potting media.
- "When in doubt, let it dry out." If you are unsure about whether a plant needs to be watered, it is best not to water it or ask someone.

Light and Photoperiod

Plants need light to grow. The light should include the correct intensity and spectrum. Photosynthetically Active Radiation (PAR) is main portion of light used by plants and is between

400 and 700 nm. Photosynthetic Photon Flux Density (PPFD), the number of photons in the 400-700 nm range received by a surface for a specified amount of time, is normally measured in μmol $m^{-2}s^{-1}$.

With respect to growing *Arabidopsis*, a PPFD between 100 and 200 μmol m^{-2} s^{-1} is considered normal. A PPFD less than 100 can inadvertently encourage elongation and alter flowering time. Photoperiod is the relationship between the duration of the light period and dark period. *Arabidopsis* is a long day plant, with flowering stimulated with 16 hour or longer day lengths (See research from Perdue on 101 ways to grow Arabidopsis https://docs.lib.purdue.edu/pmag/).

Most plants will grow vegetatively in long photoperiods of 16 to 24 hours of light. Some "short-day plants" (e.g. *Coleus*) require a day length shorter than a critical maximum (e.g. 12 hour) to initiate flowering. Conversely, "long-day plants" (*Rudbeckia hirta*) will not flower unless the day length exceeds a critical minimum (e.g. 16 hours). Some plants are "day-neutral" and not affected by day length (e.g. tobacco). Refer to published literature for information on required photoperiod for your research.

Temperature and Air Circulation

Temperature and air circulation interact affecting plant transpiration evaporation and thus required watering frequency.

Air circulation in greenhouses and chambers is required for accurate measurement, control of temperature as well as reducing temperature gradients. Circulation of air also helps to replace CO_2-depleted air with CO_2-rich air (necessary for photosynthesis). For many plants, 25 ℃ is the optimal temperature for maximizing plant growth. Most researchers do not require maximized plant growth for their experiments, and therefore often use a 22 ℃ day temperature and 20 ℃ night temperature.

- Do not obstruct air flow in chambers or interfere with temperature probes. (i.e. don't pour water on the sensors)

With respect to *Arabidopsis* Perdue uses 21.5C day and 18C nights. Many of UofT researchers use 22/18C.

Air circulation in growth chambers allow accurate temperature control important for replicated phenotyping experiments but may increase the frequency of watering. Researchers that substitute water trays and sub irrigation risk root rot. Desiccation over a long weekend or

holiday break may be worse but over watering. Using a constant temperature day and night will increase water

Soil Mixes

The type of soil mix, volume and properties (pH, drainage, presence of mycorrhizal fungi) will determine the vigour the containers can support. The type of potting media will affect nutrient availability, water retention characteristics and how long a container can support healthy growth as the plants get bigger.

General:
When picking the media to grow your plants, consider how long you need to grow them. Drainage characteristics are important to avoid water logged soil. Does the experiment require standardized or quantify the Macro and Micronutrients?
Common media components include Composted peat, Vermiculite, Perlite, Sand, Silica, Topsoil, Compost, Bark chips, Turface, Styrofoam, Dried sphagnum. Components like sand and Turface increase drainage; Perlite, Vermiculite and Styrofoam can decrease media density, topsoil and compost can add nutrients and symbiotic fungi (see ProMix). Check published literature for materials and method s for growing your species.

- **Wear a mask when making the soil mixes to avoid inhaling dust.**

Seed Sterilization

Efficient and effective seed sterilization can be a limiting factor for successful plant science research. Seed collected from open fields or seed stored under improper conditions can be contaminated with unwanted microbes that can negatively affect germination rate. Surface sterilization of seed with sanitizing agents can improve germination rates seedling survival. Not all seed requires sterilization, however for the sake of experimental standardization, it is a recommend step prior to planting.
Several agents can be used for sterilization, including ethanol, bleach (sodium hypochlorite), hydrogen peroxide, chlorine gas and mercuric chloride. Bleach is the most common agent for cleaning seed and plant pots; however, it can leave residues if not washed thoroughly and the agent poses a safety hazard. It is also not the most effective sterilizer. At 3%, hydrogen peroxide is relatively safe to use. Decomposition of hydrogen peroxide liberates oxygen and heat. In dilute

concentrations the heat evolved is readily absorbed by the water present. Chlorine gas and mercuric chloride are highly dangerous to handle and is not recommended for use.
In a study (*Barampuram* et al., *2014*, DOI:10.1007/s11240-014-0472-x) comparing the efficacy of bleach, hydrogen peroxide and chlorine gas on sterilizing cotton seeds, a 7-hour treatment with hydrogen peroxide was found to be more effective than the other two agents in removing microbial contamination and improving germination rate.

The recommended protocol for seed sterilization with hydrogen peroxide is as follows:

1. Place seed into a falcon tube.
2. Fill falcon tube with 3% hydrogen peroxide.
3. Mount on rotatory shaker and agitate at 150 rpm for 7 hours.
4. Check tubes every 3-4 hours for leakage, as increased gas pressure can deform plastic caps.
5. After 7 hours of treatment, discard liquid and wash seed with distilled water.
6. Any seeds found to be floating are likely not viable.

Planting Seed

When planting seeds, usually labs will provide detailed instruction as to soil type and specific requirements. Below is a recommended protocol to follow:

- Seeds will likely be sold with a bit of information about how to grow the seeds.

Tropical:

- If seeds are tropical, they are likely recalcitrant and should be planted immediately unless they are disturbance adapted species; specifically, large tropical seeds.
- In general **soak overnight** most recalcitrant seeds and large seeds to hydrate the seed before trying to germinate
- If seeds sink, they are likely viable. If they float, they are unlikely to germinate.

Temperate:

- Temperate species that are not disturbance adapted, frequently need cold stratification
- Any very small seeds should be surface sown onto pre-wetting media (the top layer of the germination mix may benefit with top dressed material pressed through a fine screen) and cannot be watered by the hose unless using a fine mist blocker.

Winter growing (Mediterranean Climate)

Species may need warm stratification, smoke treatment, scarification and/or a gibberellic Acid treatment. Gibberellic acid is a natural plant hormone that can be used to speed up the germination of seeds. It is mostly used on seed that is difficult to germinate or ones that takes a long time to germinate.

Difficult-to-germinate seeds:

Seeds from many species belonging to the following families (Arecaceae, Cycadaceae, Musaceae, Marantaceae) may require the "Plastic Bag Method" protocol for best results.

Using "**Plastic Bag Method**" seeds are blanketed in damp sphagnum moss and germinated in zipper-type, resealable plastic bags. Thoroughly saturate the sphagnum moss with water and wring it until no more can be expressed. Place the seeds and the sphagnum moss inside the plastic bags (along with a label) and keep the bags at 26-35°C. The bags may need to be flipped so that both sides receive an even amount of heat exposure.

Example plastic bag seed germination.

Labels:

- Below is information that may be required on ID labels
  - Genus species, variety
  - Family
  - Planting date, transplant date, start of flowering
  - PI, User, Treatment, Block, Id plant ID
  - Consider using coloured tags to easily distinguish important traits like treatment, block, population or species.
  - Use tags to last for the duration of the experiment. Waterproof sharpies on plastic tags are good for short term experiments, but the markings will fade, and the plastic becomes brittle over time. Pencil is visible for longer but impressions on a metal tag should be used for long experiments and harsh environmental conditions.
  - Add a tag to every pot (Relying on plant or tray position to track treatment is risky; what if as a result of a breakdown your plants must be moved?)

Container Size

Ideally the root mass should be enough to occupy most of the container. A small plant in a large container may suffer from soil compaction and low oxygen levels. Healthy roots create air spaces and can transport oxygen to the root zone. Some annuals do not tolerate transplanting disturbances, so container size is critical. Plant's that can tolerate transplanting do best when moved into successively bigger containers as they become pot bound.

Repotting

In general, the process of repotting is quite similar from species to species, but careful attention to the health of the roots, as well as how the roots are growing, are important indicators to note for choosing the kind of soil a plant may need, as well as the size of the pot the plant will be repotted to.

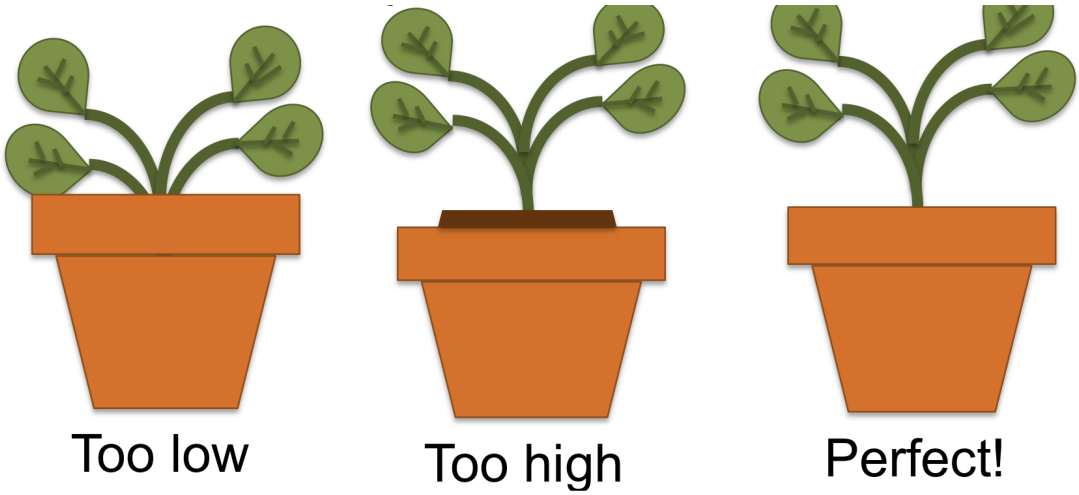
Repotting can take some time depending on the plant or the size of the plant you may be working with, so it is recommended to make sure that you have enough time to complete this task before getting started as it is not beneficial to the plant to have its roots exposed for long periods of time.

It will take some practice to become familiar with the kind of soil a particular plant may need as well as the size pot to use. Soil type and pot size are both extremely important aspects of the repotting process as choosing either of these two can lead to an unhealthy plant.

How to Repot

1. Carefully remove the plant from its current pot.
2. Gently free loose soil from around the root ball except for plants with brittle roots- cycads, Ericaceae, gymnosperms, cacti, and succulents.
3. Check the root system for rotting roots, dying roots, and to generally assess the root system. If the plant is rootbound, first break up the root ball manually, then with shears, cut into the root ball vertically.
4. It may be that depending on the condition of the roots, the plant may need to be repotted with a different soil mix. Always ask questions if unsure about an appropriate mix or size of the pot the plant will be going in.
5. Set plant into appropriately sized new pot. (Consider adding some coarse material like sand at the bottom of the container to facilitate drainage)

6. Add new soil around roots of plant. (Some media with high peat/sphagnum content should be wetted before use as water absorption may take a long time)
7. Make sure the soil is filled up to where it was before, if it is re-potted deeper you can risk crown rot.



8. Make sure the plant is centered and angled in a way that the base of the stem is straight to prevent the pot from tipping over and for it to grow upright, unless having the main stem being vertical is going to result in the plant likely tipping over. If there is a branch on one side that is bigger than the other, you may trim.
9. Add soil around roots of plant and firm soil ("pack the soil") around the plant.
10. MAKE SURE THERE IS A BASIN FOR WATERING.
11. When you repot, **thoroughly water** the plant.
12. Clean up counter with dustpan and then sweep up
13. THE BENCH SHOULD BE READY FOR WHOEVER NEEDS TO USE IT NEXT!

Propagation

Growing annuals or long-lived perennials in confined spaces may require propagation.
This may be from seeds, pruning back or starting clones from cuttings. Which method(s) are best depending on the life cycle (annual, perennials), mating system (self-compatible, outcrossed) and

research goals? If you are interested in a specific genotype starting again from seed will not work. For more information on getting successful cuttings see this related document (link here)

Plant Drying and Seed Collection

Plant senescence and seed collection is restricted to B111. Plants can be left in the room to passively dry. All trays must be labelled with the lab name and date of placement in the room. The maximum length of occupancy for a tray of plants in the room is six months. After six months, your lab will be asked to move them out of the room.

The worktable and floor should be swap after use. No garbage (plastic, aluminum foil, twist ties, tape) in the green bins.

Drying Ovens

Plants can be dried at an accelerated rate using the Ontemp ovens. Plant material should be placed in labelled paper bags. The oven should be turned on prior to putting material in.

1. To use the ovens, turn on the fan control and heater control knobs to on.
2. On the temperature control, press the yellow left button once. SP1 should be displayed.
3. Press the yellow ^ or v arrows to set the desired temperature. If you're not sure what temperature to use, 65°C is a typical temperature for drying material fast.
4. Press the yellow left button several times until PV is displayed.

After drying is complete, ensure the oven is turned off and debris is removed.

Integrated Pest Management

Maintaining pest free plants can be crucial to completing experiments. An integrated pest management approach will help prevent damage to research plants. Below is an expanded discussion of PREVENTATIVE MEASURES, BIOLOGICAL CONTROL, CHEMICAL CONTROL, PEST IDENTIFICATION & MONITORING

Preventative Measures

Preventative measures are the least expensive and most effective way to maintain pest free plants. This includes **Exclusion** of pests, **Growing healthy plants** which maybe more resistant to pests and **Monitoring** efforts to detect the first signs of pest infestation.

Exclusion

- Start plants in a clean growing space. Remove old plants, soil and debris. Clean surfaces with soap and water, dilute peracetic acid (*SaniDate 5.0*) or ammonium chloride formulation (*Kleengrow*). Make sure your growing space has been heat sterilized prior to use.
- Remove dead tissue from plants, tables and floor daily, as they can harbor pests and pathogens. Dispose of dead and dying plant material in provided green bins, and store green bins away from growing spaces.
- Transfer of pests from the outside to plants can be mitigated with personal hygiene (i.e. hand washing, showering), wearing lab coats and gloves when entering growing areas or accessing growth chambers. Hang lab coats outside chamber or room. Do not visit all spaces with the same coat. If you have plants that are infected with pests visit this space last.
- Reducing the frequency and length of time growth room and growth chamber doors are open will minimize exposure of plants to pests and temperature changes.
- If space is available, plants affected by pests can be quarantined by moving them away from unaffected plants (place inside a plastic bag or a container with a lid then move to an isolated area where the pests can be treated and or contained).

Growing Healthy Plants

- Sow plants in clean new media. Do not reuse soil. Soil-less mixes such as Pro-Mix or Sunshine Mix #1 are recommended.
- Both Sunshine mix #1 and PRO-MIX contains a **starter fertilizer** to help plants acclimate to a new rooting environment. We strongly recommended to initiate a **fertilizer program within 7-10 days after planting.** The fertilizer program you select should take into consideration water quality, crop type and the stage of plant growth.
- Water plants by watering directly onto the growing media (not soaking the tray from bottom). Avoid wetting foliage, as it can promote growth of fungal and bacterial diseases (e.g. powdery mildew).
- Incorporate a slow-release fertilizer such as Nutricote 14-14-14 into potting mix and (or) apply a dilute fertilizer (20-20-20, with micronutrients) as needed.
- For more tips see previous section on HORTICULTURAL METHODS

Monitoring

- Inspect plants daily or as frequency as possible. Yellow sticky traps can be placed next to plants to scout for common invertebrate pests such as thrips, aphids, fungus gnats, shore flies, leaf miners and whiteflies. Blue sticky traps are effective for western flower thrips (*F. Occidentalis*) as they are difficult to see on the yellow traps. Place traps at time of sowing and replace often.
- Indicator plants or sentinel plants, plants that are most likely to be infested with pests, can be grown in conjunction with research plants to be used for detection. Common bean (*Phaseolus vulgaris)* or Marigold *(Tagetes erecta L.)* are attractive species to thrips. It is much easier to spot damage on the bean leaves to thrip feeding than it is to see the thrips. If you are using Marigolds for thrip detection tap the flowers over a blue card which will show as yellow insects on the blue background.
- Traps should be inspected at least once a week. A hand lens or dissecting scope is helpful for pest identification.

- Gently shaking plants can dislodge pests that are hidden from view. Use some blue card paper and tap pests out of indicator species. Use caution (bag them) when moving infested plants in and out of growing spaces.
- The Growth Facilities staff can assist in identifying pests and diagnosing problems.

Biological Methods

Biological methods should be used pre-emptively to reduce the frequency and severity of pests. Their long-term use is sustainable and safe for plants and humans.

- Use a potting media that includes Pro-Mix. It is inoculated with mycorrhizae, which will help grow stronger and healthier plants and can suppress some soil borne diseases.
- Application of natural predators is effective for suppressing pest populations. Apply products as soon as they are delivered, typically every other Wednesday.
- The Growth Facilities provides biocontrols upon request at no cost to researchers

Predatory Mites

- *Neoseiulus cucumeris* for control of thrips and mites.
- *Stratiolaelaps scimitus* for control of fungus gnats, shoreflies and thrips pupa.

Nematodes

- *Steinernema feltia* for control of fungus gnats and thrips.

n.b.: *Predator products should be applied to the surface of growing medium. Apply to plants most affected, the predators can move to neighboring plants. The nematode product S. feltia can also be applied to foliage. Avoid using in conjunction with pesticides, as it will interfere with their viability.*

More information from supplier: Natural Insect Control (pdf) or NIC, web pages at naturalinsectcontrol.com

Chemical Control

Chemical controls should be used to eradicate pests that have already infected or infested plants.

- Insecticidal soaps (potassium salts of fatty acids) or cinnamaldehyde (essential oil of cinnamon bark) are an effective first line treatment for plants infested with insects. They have low toxicity to humans and most plants; however, some plants that are stressed (i.e. by extreme temperature or water conditions) may show damage from these chemicals. Spot treatment on a couple plants can be done to test for toxicity prior to full application. The Growth Facilities staff recommends End All II, a mild insecticidal soap with a low concentration of pyrethrins.
- A 1% solution of oil (olive or vegetable), water and a drop of dish soap can be sprayed onto foliage to control for scales, aphids, mealybug, mites and whitefiles. Avoid application on water-stressed plants and during temperatures above 32 °C. Spot treatment on a couple plants can be done to test for toxicity prior to full application.
- For major infestations, consult the Growth Facilities staff for chemical control options.

Pest Identification & Monitoring

You are the eyes of the growth facilities. We rely on you to communicate any new pests or plant ailments that you come across. Scouting is learning how to look for & ID pests and communicate your findings to the team. You will learn quickly by observation. The following section summarizes the most common pests and possible control measures.

Common Pests **APHIDS**

Figure 1. Aphids with piercing mouth parts on an Eichhornia spp. leaf. Photo credit: André Santos-Severino.

Aphids are small sap-sucking insects and members of the superfamily Aphidoidea. A typical life cycle involves flightless females giving living birth to female nymphs without the involvement of males (fast asexual reproduction). Maturing rapidly, females breed profusely so that the number of these insects multiplies quickly. Winged females may develop later in the season, allowing the insects to colonise new plants. Aphids are among the most destructive insect pests on cultivated plants in temperate regions. In addition to weakening the plant by sucking sap, they act as vectors for plant viruses and disfigure ornamental plants with deposits of honeydew and the subsequent growth of sooty moulds. Insecticides do not always produce reliable results, given resistance to several classes of insecticide and the fact that aphids often feed on the undersides of leaves. On a garden scale, water jets and soap sprays are quite effective. Natural enemies include predatory ladybugs, hoverfly larvae, parasitic wasps, aphid midge larvae, crab spiders, lacewing larvae, and entomopathogenic fungi. (https://en.wikipedia.org/wiki/Aphid)

Aphids: Feeds on plant juices, results in wilting, yellowing and slowed plant growth.

- **Control: D**aily spraying with water during your regular watering schedule helps physically dislodge insects.. It's important to get the undersides of leaves.
- **Chemical control:** End All II (insecticidal soap with a low concentration of Pyrethrins).
- **Biological control:** Application of predatory mite (*Stratiolaelaps scimitus*) and Thrips Eliminator (*Neoseiulus cucumeris*).

FUNGUS GNATS

Figure 2. Fungus Gnat on an Eichhornia spp. leaf. Photo credit: André Santos-Severino.

Fungus gnats are small, dark, short-lived gnats, of the families Sciaridae, Diadocidiidae, Ditomyiidae, Keroplatidae, Bolitophilidae, and Mycetophilidae (order Diptera); they comprise six of the seven families placed in the superfamily Sciaroidea. The larvae of most species feed on fungi growing on soil, helping in the decomposition of organic matter.
The adults are 2–8 millimetres (0.08–0.3 in) long, and are occasionally pollinators of plants and carriers of mushroom spores. They also may carry diseases such as Pythium (which causes "damping-off" to kill seedlings) on their feet. They are typically harmless to healthy plants - and humans - but can inflict extensive damage to seedlings; their presence can indicate more serious problems. In houseplants, the presence of sciarids may indicate overwatering; they may be feeding on roots that have been immersed in water too long and are thus rotting, or the gnats may be attracted to fungus growing in saturated topsoil. Consequently, allowing the soil to dry may reduce their numbers.[8] The pests are sometimes also managed by placing a layer of sand. (https://en.wikipedia.org/wiki/Fungus_gnat)

Fungus Gnats: Larvae feed on roots and leaves close to soil surface.

- **Control:** Yellow sticky cards. Remove standing water.
- **Chemical control:** End All II (insecticidal soap with a low concentration of pyrethrins).
- **Biological control:** Application of predatory mite (*Stratiolaelaps scimitus*) from red cap bottle. Predatory nematodes (*Steinernema feltiae*) applied to soil.

THRIPS

Figure 3. Close up of Thrips and leaf damaged by Thrip feeding. Photo credit: André Santos-Severino.

*Thrips (order Thysanoptera) are minute (most are 1 mm long or less), slender insects with fringed wings and unique asymmetrical mouthparts. Different thrips species feed mostly on plants by puncturing and sucking up the contents, although a few are predators. Many thrips species are pests of commercially important crops. A few species serve as vectors for over 20 viruses that cause plant disease, especially the Tospoviruses. In the right conditions, such as in greenhouses, many species can exponentially increase in population size and form large swarms because of a lack of natural predators coupled with their ability to reproduce asexually (*https://en.wikipedia.org/wiki/Thrips *)*

Thrips: Feeds on plant juices, damages flowers and leaves (silvery streaks, black specks).

- **Control:** Yellow sticky cards.
- **Chemical control:** End All II (insecticidal soap with a low concentration of pyrethrins).
- **Biological control:** Application of predatory mite Thrips Eliminator (*Neoseiulus cucumeris*) from green cap bottle. Predatory nematodes (*Steinernema feltiae*) applied to soil.

MEALYBUGS

Figure 4. Mealybugs on coffee and cotton.

Mealybugs are insects in the family Pseudococcidae, unarmored scale insects found in moist, warm climates. Many species are considered pests as they feed on plant juices of greenhouse plants, house plants and subtropical trees and also act as a vector for several plant diseases. (https://en.wikipedia.org/wiki/Mealybug)

Mealybugs: Appear as cottony masses on all parts of the plant.

- **Control:** Physical disruption by spraying with water during your regular watering schedule helps. It's important to get the undersides of leaves, under the pot lip and under the pot.
- **Chemical control:** Intercept (Imidacloprid) drench
- **Biological control:** *Cryptolaemus montrouzieri* beetle larvae. The larvae are also white and cottony, but they are mealie predators. They can be distinguished from mealies by their larger, distinct, segmented bodies which are also usually motile, unlike non-moving mealies. (The adult stage of Cryptolaemus is a small brown and black colored beetle.)

Cryptolaemus montrouzieri beetle

larvae

WHITEFLY

Figure 5. Whitefly Trialeurodes adults & eggs.

*Trialeurodes vaporariorum, commonly known as the glasshouse whitefly or greenhouse whitefly, is an insect that inhabits the world's temperate regions. Like various other whiteflies, it is a primary insect pest of many fruit, vegetable and ornamental crops (Solanaceae i.e. tomatoes). It is frequently found in glasshouses (greenhouses), polytunnels, and other protected horticultural environments. Adults are 1–2 mm in length, with yellowish bodies and four wax-coated wings held near parallel to the leaf surface. (*https://en.wikipedia.org/wiki/Greenhouse_whitefly*)*

Whitefly: Whitefly usually fly up from plants when disturbed. Upon close inspection, these appear as tiny white moths, usually found on the undersides of leaves along with eggs and larvae. Alert management immediately whenever you find white fly.

- **Control:** Yellow sticky cards & weekly Cinnamon oil spray are effective controls. Plants need to be monitored daily and additional chemical may be called for.
- **Chemical control:** Only Cinnamon oil spray has any utility. They are resistant to current chemicals.
- **Biological control:** *Amblyseius swirskii*, beneficial predatory mite.

SPIDER MITE

Figure 6. Spider mites in webbing Spider mite adults. Photo credit: André Santos-Severino.

Spider mites are members of the Acari (mite) family Tetranychidae, which includes about 1,200 species.[1] They generally live on the undersides of leaves of plants, where they may spin protective silk webs, and they can cause damage by puncturing the plant cells to feed.[2] Spider mites are known to feed on several hundred species of plants.
*(*https://en.wikipedia.org/wiki/Spider_mite*)*

Spider mites: One of our most damaging and difficult pests to control. It can be easily detected by white webbing and leaf the damage that it causes on leaves. The leaf area will not look perfectly green and normal. The mites are removing chlorophyll (green pigment) from leaf cells with their stylet-like mouthparts, causing the leaf to look like it is chlorotic or a bronzing effect. Upon close inspection on the leaf underside, you will find numerous adult mites, their eggs and their young.

- **Control:** Alert management immediately whenever you find spider mites. Daily spraying with water during the regular watering schedule is an effective way of slowing them down. It's important to get the undersides of leaves. Plants need to be monitored daily and additional chemical may be called for.
- **Chemical control:** Cinnamon oil spray.
- **Biological control:** *Phytoseiulus persimilis* and other beneficial mites. This is one of the Biologicals included in our Bi-weekly control program

SLUGS

Figure 7. Slug damage on cabbage and a Slug eating Lettuce.

Slug, or land slug, is a common name for any apparently shell-less terrestrial gastropod mollusc. The word slug is also often used as part of the common name of any gastropod mollusc that has no shell. The shell-less condition has arisen many times independently during the evolutionary past, and thus the category "slug" is a polyphyletic one. (https://en.wikipedia.org/wiki/Slug)

Slugs: The best way to scout for slugs is to look for leaf holes or slime trails.

- **Control:** If possible**,** keep the plant on the dry side**.** Sluggo or iron phosphate, can be applied on top of the soil.
- **Chemical control:** Sluggo® (iron phosphate).
- **Biological control:** No current beneficials.

SCALE

Figure 8. Scale insects on stem and Scale insects on the underside of leaf.

The scale insects are small insects of the order Hemiptera, suborder Sternorrhyncha. Most scale insects are parasites of plants, feeding on sap drawn directly from the plant's vascular system. Many scale species are serious crop pests. The waxy covering of many species of scale insects protects them effectively from contact insecticides, which are only effective against the first-instar nymph stage known as the crawler. However, scales often are controlled by use of horticultural oils, that suffocate them, systemic pesticides that poison the sap of the host plants, or by biological control agents such as tiny parasitoid wasps and Coccinellid beetles. Insecticidal soap may also be used against scales. (https://en.wikipedia.org/wiki/Scale_insect)

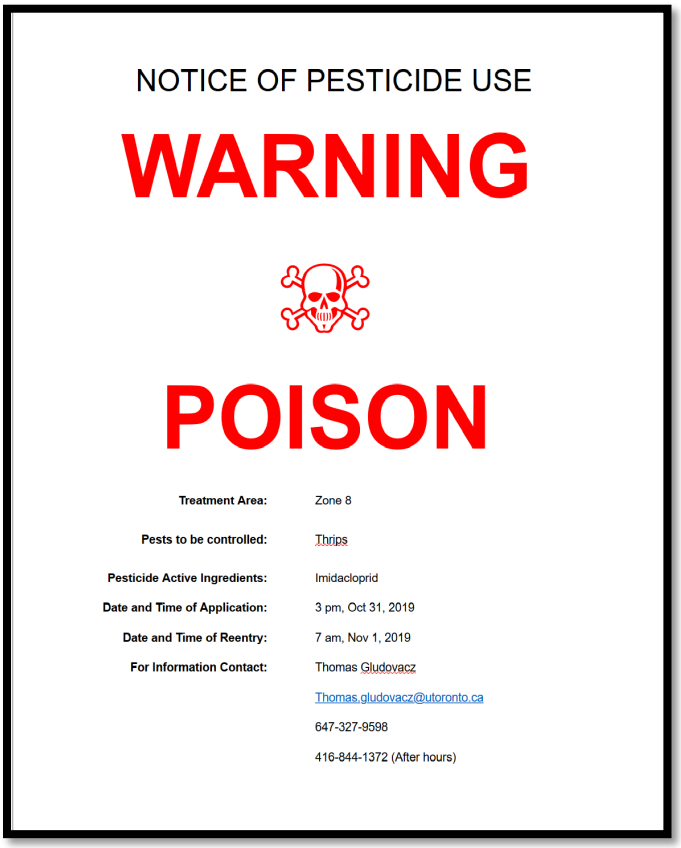
Scale: Appears as a brown, dome-shaped scale-looking outer shell. The insect feeds on the plant for under the protective scale.

- **Control:** Scale can be scraped off with soap and water or alcohol. Alert management verbally.
- **Chemical control:** Intercept (Imidacloprid) drench.
- **Biological control:** None known.

Pesticide Application Warning Signs

Will be posted on the room door, stating chemical used and the REI (Re-entry Interval), which is typically 12 hours.

Note: We cannot use pesticides in the basement walk-in rooms or reach in chambers as they are connected to the building air circulation system. Occasionally critical plants can be bagged and moved to a glasshouse to try and treated to collect seed or propagate novel genotypes.



NOTICE OF PESTICIDE USE

WARNING

POISON

Treatment Area:	Zone 8
Pests to be controlled:	Thrips
Pesticide Active Ingredients:	Imidacloprid
Date and Time of Application:	3 pm, Oct 31, 2019
Date and Time of Reentry:	7 am, Nov 1, 2019
For Information Contact:	Thomas Gludovacz
	Thomas.gludovacz@utoronto.ca
	647-327-9598
	416-844-1372 (After hours)

Pesticide Applicator License

Only licensed pesticide applicators can purchase, handle, mix, apply & store regulated pesticides. There are some products that student workers can apply with training and if wearing designated PPE (personal protection equipment).

n.b. The development of this document was inspired by and adapted from the University of Guelph Phytotron Policy by M. Mucci, L. Illman and H. Maherali, by University of Minnesota College of Biological Sciences Conservatory Protocol Handbook by L. Philander and by The Dunlap Institute for Astronomy and Astrophysics at the University of Toronto Values Statement and Code of Conduct. An expanded Horticultural method, Integrated pest management and Common pests was added and edited by Thomas Gludovacz and Bill Cole, U of Toronto Growth Facilities (2019) and by Bill Cole (Oct 2022).

Appendix 1. Current facility charge back rates per month

Growth Chambers + Rooms		EXAMPLE
Model	**Fall 2022**	Number
Biochamber Room ER120	120	44,45
Biochamber Room SR-240	180	43
Conviron A1000	50	60
Conviron ATC13	50	58
Conviron CMP650	120	20
Conviron E15	60	70
Conviron E7	50	90
Conviron Growth Rooms 1-3	115	
Conviron Growth Rooms 4-9	225	
Conviron MTR30	70	50
Conviron PGR 36	180	99
Conviron PGR15	60	23
Enconair AC40	90	92
Enconair AC60	115	1
Enconair AC80	115	13
Enconair GC20	90	6
Percival Sci F30B	45	25
Thermotron (-40C freezer)	120	59
Glasshouses		
Glasshouse-West Zones 2 ,3, 4	170	
Glasshouse-West Zone 1*	190	
Glasshouse West Zone 5	210	
Glasshouse West Zone 6	330	
Glasshouse-East Zones 7-14	125	
Glasshouse-East Zone 15	190	
Glasshouse-ESC 5007	120	
RW Glasshouse	115	RW 702
* LED light surcharge Dec+		15

Soil and Nutrient Supplies Price List

	Supplier	**Fall 2022**
Sunshine #1 (79 L)	SunGro	$20
Promix BX (107 L)	Plant Products	$40
Turface / Profile (50 lb)	Plant Products	$31
Perlite (10kg)	Plant Products	$36
Vermiculite (8.2kg)		
Sand	New Canadian Lumber	$15
Custom Mix White Bin	Bespoke mix of soils to researcher specification.	$80 (minimum)
NPK 20-20-20 (15 kg)	Plant Products	$85
Nutricote 14-13-13 (20 kg)	Plant Products	$120