

Central European Institute of Technology BRNO | CZECH REPUBLIC

General rules for work with plants in phytotrons, greenhouses and cultivation chambers

Plant Sciences Core Facility, CEITEC MU 2021

These general rules for work with plants in cultivation chambers have been drawn up to ensure that such an environment is created inside the Core Facility where researchers can work together safely and productively while maintaining a pest-free environment.

Thus, the general rules will be continuously adapted to suit changes to our facility as it evolves. We strongly encourage users to bring to our attention all ideas how we can more effectively meet the goals of the facility. Please contact us with your comments, questions, and requests.

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General information:

- In order to maintain a work environment that is clean and functional for all Core Facility users, these general rules must be followed accurately!
- Make sure you book your space in the growth chambers in advance.
- >GMO training is required before starting the work in Core Facility area.
- Each person is responsible for logging their GMO plants and cultures into the GMO register.
- For information about growth chambers with non-standard growth conditions (multispectral LEDs, high light, phenotyping, cold or heat stress) and the environmental simulation service, please contact the core facility staff.
- All requests, comments or suggestions from users should be sent via email to the Core Facility.
- ➤You are responsible for your plants! Check them regularly.

Plant work:

- ➤ Always start with healthy plant material!
- ➤ Plants must either be grown under sterile conditions before transfer to soil or the seeds must be sterilized before sowing.
- Freezing seeds before using is strongly recommended as it prevents pest contamination.
- Clearly **label each tray** with your name! In case of the label is missing, the Core Facility shall not be responsible for such plants.
- ➤ Use **lab coats** when working in greenhouses/phytotrons. It is not allowed to work in growth chambers without lab coats.
- Ensure that you leave the working place clean before you leave the facility.
- ➤ NEVER transfer plants from the greenhouse to the phytotrons!
- Plants transported from the phytotrons into the greenhouses have to be covered with lids where possible.
- Always put the inflorescence of flowering plants into paper bags if you intend to collect the seeds to avoid contaminating neighboring plant trays with these seeds. Dry your non-infected plants in the greenhouse corridor's shelves or in the specified greenhouse chamber in case there is not enough space on the shelves. Infected plants must be left to dry where they are.
- Discard all plants that are not needed and harvest seeds as soon as possible. Plants and used

pots with old soil are a wonderful breeding ground for pests and infectious agents!

- Cold treatment of plants in the cold chamber: pots with seeds should be always covered with lids in this chamber.
- No ornamental plants are allowed in the labs where you plan to work with experimental plants (DNA isolation etc.).
- Avoid contact with any ornamental plants if you plan to enter the facility.
- To reduce risk of cross-infestation, finish all work first in the phytotrons or cultivation chambers. Do not enter the cultivation chamber or phytotrons after visiting greenhouse. Once you enter the greenhouse, going back to the phytotrons or cultivation chamber is strictly prohibited.
- >Always enter pest-free chambers first.
- Always clean the transport trolley with ethanol after you are finished working with it.
- Clean all phytotron shelves, banks or greenhouses when leaving after finishing of your experiment this will be checked by the Core Facility staff responsible.

Soil preparation area:

- Keep this area clean. This area must be cleaned when leaving.
- The preparation area is a one-way room. Do not bring any plant material back from the growth chambers into this area. The only exception to this is transferring plants from in vitro culture onto soil.
- ➤ Do not bring any seeds into this area to avoid contamination. You can sow your seeds into the soil on tables in the corridor or in plant working rooms.
- Close the soil bag after taking out soil prevents pest contamination.
- ➤ Available substrates:
 - Klasmann TS3 commonly used for Arabidopsis
 - Garden substrate rich for nutrients
 - Vermiculite, perlite
 - Sand

Plant working area:

- ➤ Keep plant working rooms clean at all times.
- All plant and seed work is allowed in these areas.

Exceptions:

- 1) Plants from the greenhouse: do not bring in any plants from the greenhouse
- 2) Infested plants from growth chambers (working with them is allowed only inside the

infested chamber)!

Growth chambers (phytotrons, greenhouses, cultivation room, cold room):

- ➤ Keep the growth chambers clean! Cleaning equipment is always available.
- ➤ Only use cleaning equipment belonging to the chambers you are in.
- If you notice anything that is not working properly, please inform the Core Facility staff directly.

Pest management:

- Inspect your plants regularly for any signs of pathogen infestation at a minimum of once per week. If you are doubtful about the health of a plant, please inform the Core Facility staff.
- ➤ In case of any infestation, **inform** the Core Facility staff **immediately**!
- Core Facility staff will also inspect your plants once a week for any signs of infestation.
- ▶ If possible keep your lab coat inside the growth chamber to prevent cross-contamination.
- Infested plants should stay in the growth chamber when you work with them, never move them outside. Trash infected plants whenever possible.

▶Black Flies (Fungus Gnat):

Do not overwater your plants, let the upper layer of the soil dry out regularly.

Hang up yellow sticky labels.

When you spot the first sign of infestation, inform Core Facility staff or use fresh nematodes (one scoop of nematodes for every three liters of water). Do not use nematodes as prevention.

Optional: use Gnat OFF for additional treatment - add 1ml per 1l for watering.

Other insects (thrips, aphids, red spiders):

In case of infestation, inform the Core Facility staff immediately! The handling of these infestation cases is the responsibility of the Core Facility.

Throw away all plants you do not need. Use autoclavable bags, close them and deactivate by autoclaving as soon as possible.

Core Facility will treat all plants in the contaminated chamber as soon as possible. When possible, after the first application infected plants will be transferred in covered boxes into a quarantine chamber.

Application of chemicals by the responsible person will be discussed with the user in

advance.

- All dirty and empty trays from the infected chamber should be immediately cleaned using disinfectant (Savo, Ethanol).
- >Do not enter a clean chamber after working in a contaminated chamber.
- ➤ Do not enter any infected chamber unless absolutely necessary. Infected chambers will be labeled and lab coats for work in this chamber will be placed inside.
- The user can choose the possibility to take care of pest management by herself/himself (all plants).

Waste management:

- Discard all **GMO** plant and soil waste into autoclavable bags in **blue bins** including pots and labels. Blue bins are placed in each phytotron or greenhouse section. Separate wooden sticks into a special bin.
- >Separate GMO substrate and other GMO contaminated wastes plates, gloves etc.
- ➤ Do not fill the waste bins more than half-full. Replace the bag when half full and insert a new bag. **DO NOT OVERFILL BAGS!!! THEY CAN GET VERY HEAVY!!!!**
- ➤ You can find new bags in shared shelves.
- ➤ Do not fill bags outside of a bin.
- Non-GMO waste (wild type plants with soil, pots, labels) should be put into black bags.

Reservation of cultivation space:

- ➤ Booking is obligatory for all shared growth chambers (phytotrons, greenhouses, Percivals, AlgaeTron).
- Always book your space in the growth chambers in advance (at least 2 working days before beginning the experiment).
- ➤ Minimum reservation time is 1 week (7 days).
- ➤ Users can book space in the growth chambers using the reservation system on the Core Facility webpage. The Core Facility can make reservation for users upon request.
- ➤ You can check for free space either in the reservation system or by sending an e-mail to the Core Facility.

➤ Information necessary for booking:

- researcher name/group
- number of shelves/banks,
- experiment starting date,

- expected length of the experiment,
- growth conditions (temperature, light intensity, humidity, day length)
- type of service to be provided (basic, advanced, full)

For large-scale experiments (more than two tables or shelves) please book the necessary space one month before the start or discuss availability with the Core Facility.

Any plants found without a valid booking will be discarded.

Categories of services provided by the Core Facility:

- **≻**Basic
- ➤ Advanced
- **≻**Full

Basic service:

- ➤ Setting up the cultivation conditions
- ➤ General cleaning of common areas
- ➤ Waste management
- > Pest control and treatment
- ➤ Attending to emergencies
- ➤ Maintenance of the CF: ordering consumables and services
- ➤ User training

Advanced service:

Basic service + preparing soil and pots + watering plants

Full service:

Advanced service + pinning of plants + bagging of plants + cutting plants when they are dry + cleaning shelf after experiment.

To maintain a work environment that is clean and functional for all Core Facility users, these general rules must be followed! Any violation of these Rules will attract appropriate consequences.

Thank you very much for your cooperation.