K. M. DeAngelis 9/15/2006

Microcosms Protocol

Soil collection

Materials to bring to the field:

Plastic bags	Camera
Shovel	Work gloves
Cooloer	Latex gloves

Collect soil from top 10 cm in at least 5 different parts. Put into plastic bags and store in a cooler until ready to move on to the next step.

Preparing Soil

If the soil is very wet, allow it dry out for several hours or overnight.
Sieve the soil using a 2 mm soil sieve. Discard rocks and organic matter.
Mix the batch very, very well, and put it into labeled, dated plastic bags.
Store the soil at RT if less than a week, or at 4C if more than a week; If stored at 4C, allow one week to reach RT before re-homogenizing and planting.

Before filling the microcosms, (preferably, the day before), the gravimetric soil water content of the soil to be used should be measured in order to determine the mass of soil to be used. To do this:
Weigh a clean, dry aluminum tin (to 3 decimal places).
Add at least 15 g of the soil; weigh the tin + soil.
Place the tin in a drying oven set at 105C for at least an hour or to constant weight.
To weigh the dry soil, place the tin in a dessicator until room temperature, then weigh the tin + soil.

$\Theta_g \equiv \frac{m_{water}}{m_{drysoil}}$, or $\Theta_g = \frac{m_{wetsoil} - m_{drysoil}}{m_{drysoil} - m_{tin}}$ This is normally expressed as a percentage.

Starting Seeds:

Start the seeds about 4-5 days before the planting date.
Use the peristaltic pump to create a slow drip. Place 2 paper towels in a tray with holes or an old sieve inside a dish bucket (to catch the extra water). Wet the paper towels and put down the seeds. Put 2 more paper towels on top and wet these as well. Cover the whole thing with black plastic or tin foil to protect from light.
Fill a 2L flask with tap water and set the peristaltic pump to 2.0. Position the drip line so it waters the paper towels in the middle.
If the seeds germinate before the desired planting date, they may be stored in a loosely closed plastic bag wrapped in tin foil or black plastic in the refrigerator or cold room.

Preparing Microcosms

Empty the old microcosms and wash them (don't have to be spotless; we're filling them with soil). Place glass wool in the bottom hole. Put the solid plastic divider in the slot. If necessary, reinforce the double-sided tape holding the microcosm together, or use screws and wing nuts to fortify them before filling.

The microcosms are filled to about bulk density 1.2 g dry soil/cm^3 with one part sieved homogenized soil to one part silica sand. The sand can be obtained from the greenhouse.

Pour the soil carefully into the microcosm. Tap the sides of the microcosm to settle the soil. Try to pack the soil as evenly as possible, with no lines or areas of different density. If necessary, whack the microcosm on a table to encourage the soil to settle.

Planting

When the seedings have rootlets which are around 0.5 to 2 cm in length, they're ready to plant.

First, place the desired number of microcosms (usually 6) in a plastic tub. Gently add about 3-4 cm of water. Gently water the top of the microcosm a couple of times also.

When the water has soaked in, plant the seedlings. Make a hole in the soil with forceps. Very gently, place the seedling in the hole, and very gently replace the soil around it. All rootlets should be beneath the surface of the soil. Up to 0.5 cm of the shootlet may be beneath the soil (far better to have shootlet under soil than to have roots exposed). Very gently water the seedlings a bit. Do not direct the water onto the seedlings, as this may wash them out of the soil.

Allow the microcosms to sit for about 24 hours so that the wet soil can regain some structural integrity.

Wrap the microcosms in black plastic and secure with duct tape. Label them as to when the seeds were started and when planted, and bring them to the greenhouse. Always use the cart with the pneumatic tires so that the soil in the microcosms does not get compressed.

Day to Day Care

Water the plants with tap water every other day (can be every third day for young plants). Do not pour the water directly onto the plants. Do not pour too quickly. Do not add so much water that there is excessive draining from the bottom of the microcosm; I would like to avoid too much leaching of nutrients, since the plants will not be fertilized. Fill about half of the headspace for young plants, increasing as the plants get older.

All the plants in both chambers should be rotated randomly (both between and within chambers) on a weekly basis. Choose a day and do it.

A calendar and note with instructions should accompany all plants left in the care of greenhouse staff.

Filling and Tilting

Materials to bring to greenhouse:

Clean, slotted dividers	Duct tape
Pliers	Scoops
Flathead screwdriver	Gloves
Razor blades	Experimental soil (no sand!)

Plants in microcosms are ready for the side-car to be filled with experimental soil after approximately 6-10 weeks from date of germination. This will depend on time of year, temperature and weather among other things.

Don't water the plants for 2 days before the filling and tilting, so that there is minimal settling of the already loaded soil.

Experimental soil, the soil that goes into the side-car, should not have any sand added. It can be collected separately, or if collected at the same time as main-car soil, it should have been stored at 4C then placed at RT for one week prior to planting.

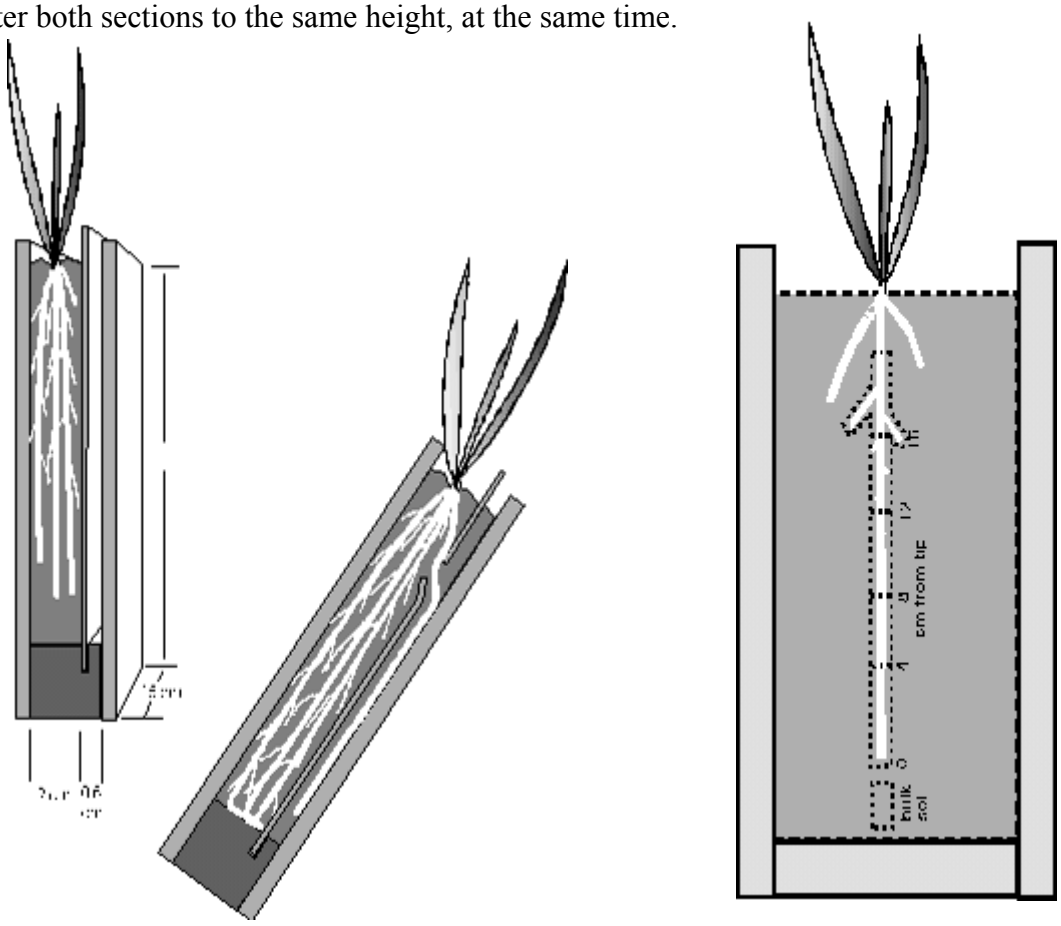
Bring the plants over from the greenhouse. Remove the solid plastic barrier (use **pliers** if necessary, try not to damage the microcosm or barrier, try not to compact the soil). Replace it with a **slotted barrier**. Press the slot down onto the roots/soil (so that it doesn't bulge out into the thin section). Attach a thick plexiglass plate to the microcosm with **screws and wingnuts**.

The thin section gets about 150 g of dry soil. Filling the thin section is a little more difficult, but much more important, because this is the experimental soil. Try to make sure it goes in as evenly as possible.

Water the plants, water both the main section and thin section at the same time and to the same level (if you don't do this, there may be leaching from one section into the other). Wrap each microcosm in black plastic again and tape, and record the date. Wait several hours, and then transport the plants back to the greenhouse.

In the greenhouse, place the plants in the rack (at an angle) or otherwise tilt them. Make sure the thin section is facing down.

When you water these plants, put them upright, and let them stay upright until the water has soaked in. Water both sections to the same height, at the same time.

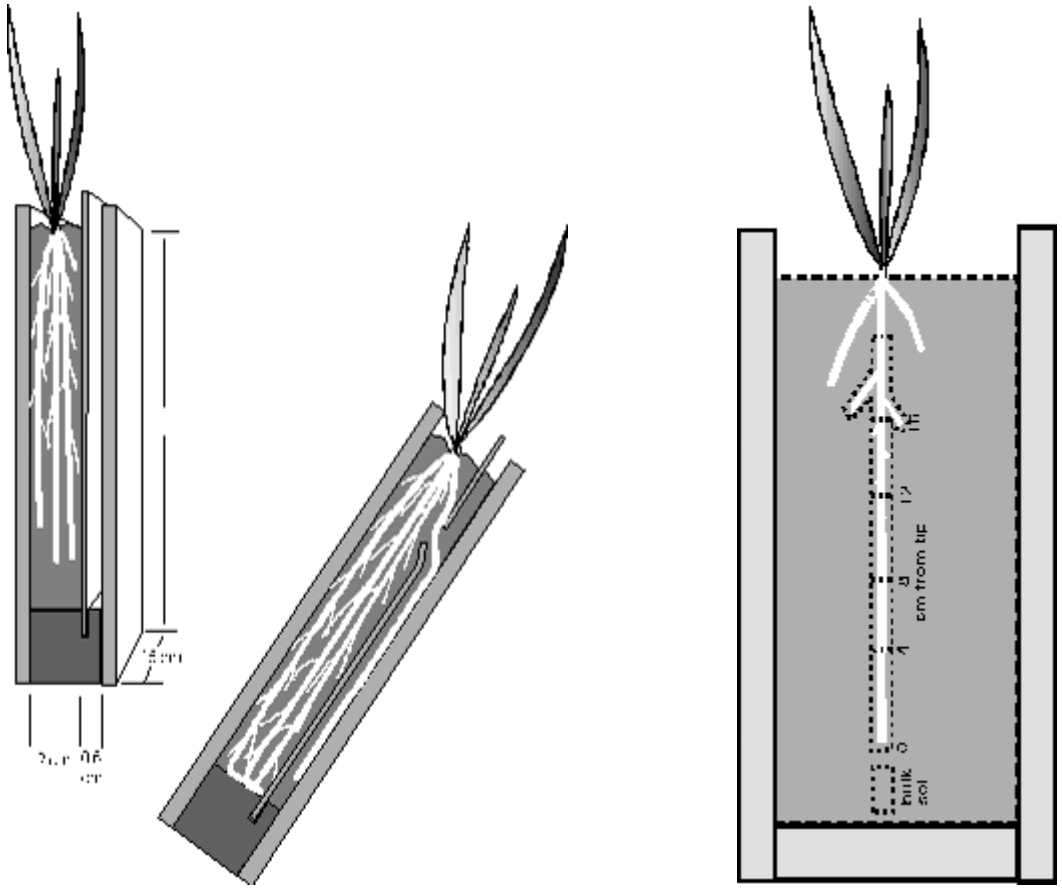


Schematic of the Microcosms. On the far left shows the set-up of the microcosms before the side-car is filled and the orientation of the plants in the greenhouse during the main growth phase. In the middle shows the set-up of the microcosms after the slotted divider is inserted and the side-car has been filled.

On the right is the view of the side-car from the front when the plexiglass cover is removed, illustrating the root zone sections to be harvested: **bulk soil** is at least 4mm away from any roots; **root tip** is 0-4mm from the root tip; **root hairs** are 4-8cm from the root tip, and **mature root** is 8-16 cm from root tip.

Dear GH Staff, for microcosms please:

tap water only
every 2nd day- see calendar



Thanks!

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