TECHNICAL NOTE 96.4

HPC Sampling and Analysis Protocols

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Acronyms and Abbreviations

EC: Electrical conductivity
FID: Flame ionization detector
GC: Gas chromatograph
HPC: Higher Plant Chamber
HPLC: High Performance Liquid Chromatography
LA: Leaf area
LAI: Leaf area index
ppb: Part per billion
TCD: Thermal Conductivity Detector
UAB: University “Autonoma” of Barcelona
UoG: University of Guelph

1. Introduction

This document describes the analytical methods required for sampling full maturity crop growth of lettuce in the UAB HPC1. For short-term experiments that test HPC system functioning, no plant harvesting or extra analysis is required. Many of the techniques described here can be employed for other basic plant growth and compositional analysis in HPC controlled environment plant research.

2. Equipment

1. Balance for fresh weight measurement (± 0.01g)
2. Balance for dry weight measurement (± 0.001g)
3. Drying oven (60 C)
4. Tweezers
5. Weighing dishes (consumable)

3. Sampling

Plant tissue sampling methodology will depend on the experimental design and desired results. For basic harvest yield studies, the only sampling requirement is to separate the plants into edible and inedible biomass. From this point, fresh weight, leaf area, and subsequent dry weight can be determined. More detailed plant analysis may assess nutrient uptake and nutritional composition, while more in depth protocols may involve the extraction and analysis of biochemical and sub cellular components. Such detailed analytical procedures are beyond the scope of this document, and specific sampling techniques can be obtained from analytical laboratories contracted to perform the desired analytical procedures. In the case of short-term (1 week) studies, no sampling is required. For long-term tests (eg. 40 days), the analytical requirements are listed in this document.

Proper sampling is the key to reliable analytical results. A sample can represent the status of one plant or the status of the entire plant growth chamber. Often, for nutritional and biochemical determinations, the actual laboratory analysis requires less than one gram of tissue. However, a good sample should contain enough leaves (roots, edible biomass, etc) to represent the area sampled. In the case of the HPC, representative samples can be taken from each trough and combined for a single sample for detailed tissue analysis, however experimental sampling protocols should be determined through consultation with a statistician. Depending on the type of analysis, either fresh or dry plant material will be required.

4. Analysis – Plant Material

Illustration 1: Plant analysis flow chart for lettuce harvest from HPC1

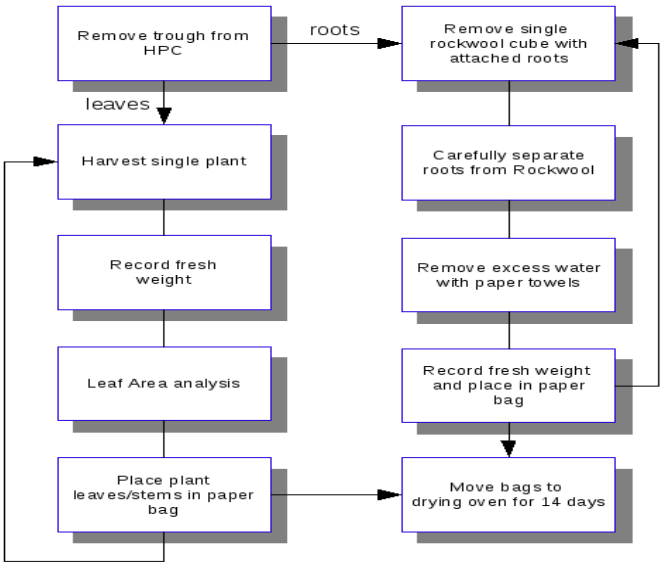


Illustration 1 outlines the basic sampling flow for the harvest and processing of batch lettuce grown in HPC1.

The analysis considered relevant for a full maturity test are outlined here below. The ones considered critical are the following:

- fresh weight
- dry weight
- percent carbon and percent nitrogen
- plant mineral analysis
- crop nutrient solution analysis

Since in the frame of the present call of order (COO6) only first tests for chamber validation were performed, it was not considered necessary to carry out the following analysis: leaf area, ash, fat and fiber analysis.

4.1 Fresh weight

Plant fresh weight is determined by removing the plant material from the growing medium and, if required, separating it into roots and leaves/stems, or roots, leaves/stems, and edible biomass. Each part is individually weighed to +/- 0.01g on a scale, the mass recorded, and the plant material placed into a labelled paper bag which will be then dried for dry matter determination.

Leaves/stem:

1. Remove one plant from the trough by cutting the stem at the junction where the stem enters the Rockwool block
2. Weigh the entire plant and attached stem to 0.01g and record
3. Proceed to leaf area measurement quickly to avoid leaf wilting
4. Return to step 1 until all the plants in the trough have been harvested

Roots:

Detailed procedure for manipulation with roots and rockwool is given in the procedure for Rockwool safe manipulation in the end of this document (Appendix 1).

4.2 Leaf area

Leaf Area (LA) is the total area of the leaf upper surface generally expressed on a per plant basis. Leaf Area Index (LAI) takes into consideration the ratio of total upper leaf surface divided by the surface area of growing area.

Once the plant has been harvested and fresh weight determined, the LA procedure must be performed quickly to avoid leaf wilting.

1. Zero the leaf area meter
2. Strip leaves from the lettuce stem one at a time and feed through the entrance of a leaf area meter (Li-Cor 3100, Li-Cor, Lincoln, NE, USA, Appendix 2, Illustration 2)
3. Record the total leaf area (cm^2) that is displayed
4. Place the leaves and stems in a labelled paper bag

5. Transfer to a drying oven for 14 days, or until the leaves/stems have dried completely

Detailed procedures on leaf area meter operation will be included in the instrument's user/operation manual.

Illustration 2: LI-COR LI-3100C Leaf Area Meter

4.3 Dry weight

After fresh weight and leaf area determination, the plant material is dried for a period of 2-5 days (until constant weight) in an oven set to 60-70°C. (the time required for complete drying depends on the tissue type and amount of water it contains, so this period may vary)Be sure that the material is completely dry before weighing. Dry weight is determined as follows:

1. Remove the plant material the paper bag and place on a tared weighing dish
2. Weigh to +/- 0.001g
3. Record the value

4.4 Harvest and sampling protocol

In the frames of the present call of order the harvest and sampling protocol is as follows:

a. 11-days crop test

1. Seven days after crop test start up, turn off irrigation pump
2. Open the exterior harvest air lock door
3. Open the inner harvest air lock door
4. Take photos of the plants in HPC1
5. Turn off the system of environmental control
6. From the side of module A remove 4 troughs and take photos of the plants
7. Remove one plant from the trough by cutting the stem at the junction where the stem enters the Rockwool block
8. Weigh the lettuce shoot (leaves and stem) to 0.01g and record.
9. Place shoots of a plant from the middle of the tray (central plant, plant #3 of each tray) into a glass vessel for liophylization.
10. Mix 3 central plants from each 3 trays in a one glass vassel. For 18 troughs there should be 6 glass vessels with lettuce shoots.
11. The last glass vessel will contain only 2 lettuce shoots harvested from the last 2 troughs
12. Place a rockwool cube with the root system into a plastic bag and put the bag into a container for further storage (for detailed procedure of rockwool safe manipulation during harvest, please refer to Appendix 1 of this document, paragraph c). Repeat for all 20 central plants.
13. Place the containers with rockwool cubes and lettuce roots into the cold cabinet of the department for a storage
14. Take 3 samples of nutrient solution from drain valve
15. Place the samples of nutrient solution into a fridge
16. Place glass containers with lettuce shoots into a freezer and leave them for 2 days at -80 ºC
17. Return all the troughs into the chamber at the same position, close the doors, enable system of environmental control and continue the test
18. Two days after harvest, take glass containers out of the freezer and start the process of lyophilization. The lyophilization process should last 2 days.
19. Weigh lyophilized samples to 0.01g and record their dry weight. Store the samples in a desiccator in a fridge for further elemental analysis
20. Eleven days after crop test start up, turn off irrigation pump
21. Repeat steps 2-8.
22. Place shoots of a plant #2 (counting from the wall where main nutrient collector is situated) of tray #1 into a glass vessel for liophylization.
23. Place shoots of a plant #4 (counting from the wall where main nutrient collector is situated) of tray #2 into the same glass vessel for liophylization.
24. Place shoots of a plant #2 (counting from the wall where main nutrient collector is situated) of tray #3 into the same glass vessel for liophylization.
25. In order to fill 7 glass vessels with lettuce shoots, repeat steps 21-23 for all the troughs.

26. The last glass vessel will contain only 2 lettuce shoots harvested from the last 2 troughs
27. Weigh the rest of the plants to 0.01g and record fresh shoot weight, place each lettuce shoot into a labelled paper bag
28. Place the paper bag with lettuce into a big plastic bag for further transportation
29. Repeat steps 12-19.
30. In the Veterinary School, place paper bags with lettuce shoots into a drying oven and leave to dry for 8 days at 70ºC.
31. Next day after harvest, deliver rockwool cubes with roots inside to the Veterinary School for separation and drying. Please refer to Appendix 1 of this document, paragraph d for detailed procedure of rockwool safe manipulation.
32. Place paper bags with the lettuce roots into the oven with lettuce shoots and leave them drying for 7 days.
33. Eight days after harvest, take the paper bags with lettuce leaves and shoots out of the oven, weigh them to 0.01g and record the dry weight.
34. Mill each dry root sample and store them in a desiccator in a fridge for further elemental analysis

b. 45-days crop test

1. Turn off CO_2 control mode
2. Take a sample of air for ethylene analysis
3. Turn off irrigation pump
4. Open the exterior harvest air lock door
5. Open the inner harvest air lock door
6. Take photos of the plants in HPC1
7. Turn off system of environmental control
8. From the side of module A remove 4 troughs and take photos of the plants
9. Remove one plant from the trough by cutting the stem at the junction where the stem enters the Rockwool block
10. Weigh the lettuce shoot (leaves and stem) to 0.01g and record.
11. Place the lettuce shoot into a labelled paper bag
12. Place the paper bag with lettuce into a big plastic bag for further transportation
13. Place a rockwool cube with the root system into a plastic bag and put the bag into a container for further storage (for detailed procedure of rockwool safe manipulation during harvest, please refer to Appendix 1 of this document, paragraph c).
14. Repeat steps 8-13 until all the plants are harvested

15. Place the containers with rockwool cubes and lettuce roots into the cold cabinet of the department for a storage
16. Take off the lead of the nutrient tank and take 3 samples of nutrient solution from the top of the tank and 3 samples – from drain valve
17. Place the samples of nutrient solution into a fridge
18. Make analysis of ethylene in the air sample, taken from HPC1
19. In the Veterinary School, place paper bags with lettuce shoots into a drying oven and leave them for 10 minutes at temperature of 105ºC for enzymes inactivation. After place the bags into a drying oven and leave them to dry for 8 days at 70ºC.
20. Next day after harvest, deliver rockwool cubes with roots inside to the Veterinary School for separation and drying. Please refer to Appendix 1 of this document, paragraph d for detailed procedure of rockwool safe manipulation.
21. Place paper bags with the lettuce roots into the oven with lettuce shoots and leave them drying for 7 days.
22. Eight days after harvest, take the paper bags with lettuce leaves and shoots out of the oven, weigh them to 0.01g and record the dry weight.
23. Mix 5 lettuce dry shoots of 1 tray in order to have an average dry shoot sample per tray.
24. Mix 5 lettuce dry roots of 1 tray in order to have an average dry root sample per tray.
25. Repeat steps 23-24 for all 20 trays
26. Mill each average dry sample and store them in a desiccator in a fridge for further elemental analysis

4.5 Plant tissue analysis

There are a great variety of plant tissue analysis procedures and methods. Procedures selected will depend on the data required by the experimental design and equipment available to perform the analysis. As these specialized analysis are generally beyond the scope of the local UAB laboratory, the work should be contracted to laboratories better able to perform work of this type. Sample size is critical and must be determined prior to harvest. To determine the amount of plant tissue required for each analytical procedure, refer to the methods referenced below or contact the laboratory that will be performing the test(s). The following are analytical procedures (with example references) to be performed on lettuce root and leaf tissue at the end of the 40 days crop test:

Total Carbon and Total Nitrogen: The combustion gas analyzer method for total nitrogen and total carbon is often employed for this measurement. This analytical method quantitatively determines the total amount of

nitrogen and carbon in plant and other materials using a dynamic flash combustion system coupled with gas chromatographic (GC) separation and detection by thermal conductivity (TCD). The analytical method is based on the complete and instantaneous oxidation of the sample by "flash combustion" which converts all organic and inorganic substances into combustion gases (N_2, NO_x, CO_2, and H_20). The method has a detection limit of 0.01% and is generally reproducible within 5% (relative). This method is used for C, H, N determination in the lettuce after 7-days and 40-days crop tests.

Official Methods of Analysis of AOAC International, 16th Edition (1997), AOAC International, Arlington, VA.

Sheldrick, B.H. 1986. Test of the Leco CHN-600 Determinator for soil carbon and nitrogen analysis. Can. J. Soil Sci. 66(3):543-545

Sweeney, Rose A. 1989. Generic combustion method for determination of crude protein in feeds: Collaborative Study. J. Assoc. Off. Anal. Chem. 72: 770-774.

McGeehan, S.L. and D.V. Naylor. 1988. Automated instrumental analysis of carbon and nitrogen in plant and soil samples. Commun. Soil Sci. Plant Anal. 19:493

Proximate Analysis: Proximate analysis involves the determination of dry matter, ash, nitrogen and fat.

Official Methods of Analysis of AOAC International, 16th Edition (1997), AOAC International, Arlington, VA.

NO_3-N: Nitrate can be determined by extracting plant tissue samples using a 1 N KCl solution. Nitrate is determined by reduction of nitrate (NO_3-N) to nitrite using a cadmium column followed by spectrophotometric measurement.

Keeney, D.R. and D.W. Nelson. 1982. Nitrogen - inorganic forms. p. 643-687. In: A.L. Page, et al. (ed.). Methods of Soil Analysis: Part 2. Agronomy Monogr. 9. 2nd ed. ASA and SSSA, Madison, WI.

Plant Minerals (Excluding N and Cl): Plant minerals (B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn) can be determined by ICP analysis of a nitric acid digest. This method is used for determination of Na, S, Ca, Mg, K, Mn, B, Zn, Fe, P in lettuce after 7-days and 40-days crop tests and in the

nutrient solutions of HPC1. The determination of Cu and Mo is done by ICP-MS ICP analysis of a nitric acid digest.

Isaac, R.A. and W.C. Johnson. 1975. Collaborative study of wet and dry ashing techniques for the elemental analysis of plant tissue by atomic absorption spectrophotometry. J. Assoc. Off. Anal. Chem. 58: 436-440.

Havlin, J.L. and P.N. Soltanpour. 1989. A nitric acid and plant digest method for use with inductively coupled plasma spectrometry. Commun. Soil Sci. Plant Anal. 14:969-980.

Acid Detergent Fiber (ADF)-Neural Detergent Fiber (NDF): Fiber is determined gravimetrically following a liquid digestion process.

HORWITZ, W. (ed.) 2000b. AOAC official method 973.18. Fiber (Acid Detergent) and Lignin (H_2SO_4) in Animal Feed. In *Official Methods of Analysis of AOAC International*, section 4.6.03, AOAC International, Gaithersburg, MD.

Komarek, A.R. 1993. An improved filtering technique for the analysis of neutral detergent and acid detergent fiber utilizing the filter bag technique. Publication #101. Ankom Company®, Fairport, NY 14450.

5. Analysis – Nutrient Solution

In addition to pH and electrical conductivity which are continuously monitored, detailed nutrient solution analysis should be performed after the solution is mixed. In order to prevent nutrients accumulation or depletion in the solution, nutrient solution is to be changed every 5 days and 3 samples of nutrient solution should be taken before and after nutrient solution changeover. Detailed nutrient analysis of these samples should be performed. Typical methodology of nutrient solution analysis involves the use of ion exchange chromatography using a high pressure liquid chromatograph (HPLC). Anion and cation exchange columns are used separately for the analysis of calcium (Ca^{2+}), magnesium (Mg^{2+}), ammonia (NH_4^+-N), sodium (Na^+), sulfate (SO_4^{2-}), phosphate (PO_4^{3-}), nitrate (NO_3^--N), nitrite (NO_2^-), chlorine (Cl^-), and Potassium (K^+). There are numerous HPLC manufacturers, types of columns, and analytical methods employed for this type of analysis, thus specific methodologies are beyond the scope of this document.

Method of ion exchange chromatography is used for sulfate (SO_4^{2-}), phosphate (PO_4^{3-}), nitrate (NO_3^--N), nitrite (NO_2^-), chlorine (Cl^-) analysis in the nutrient solutions in the frames of the present call of order.

6. Analysis – Gas

Carbon dioxide and oxygen are continuously monitored by the HPC gas analyzer(s). Other gas phase components, while in minute quantities, can elicit detrimental effects to plant growth and development. The most important molecule to monitor on a regular basis is ethylene. Ethylene is a potent plant growth regulator with numerous biological effects including stem swelling, leaf epinasty, seedling hook opening, floral abortion, leaf abscision, and fruit ripening (Abeles, Morgan, and Saltveit, 1992). It is well known that most higher plants produce small amounts of ethylene during various stages of growth and development (Mattoo and Suttle, 1991), but in closed environments, there is the potential for ethylene to build up to levels which could be detrimental to plant growth and development. Atmospheric concentrations from as low as 4 ppb to 10 ppm can elicit responses in numerous plant species (Mattoo and Suttle, 1991). Ethylene has been shown to alter the rate of photosynthesis in a number of plant species (Gunderson and Taylor, 1988; Kays and Pallas, 1980; Woodrow and Grodzinski, 1989), and has been attributed to alterations in leaf angle (Woodrow and Grodzinski, 1989; Woodrow, Thompson, and Grodzinski, 1988; Woodrow et al, 1989). In soybean, ethylene has been shown to directly inhibit foliar gas exchange (Gunderson and Taylor, 1990) regardless of the epinastic movements of leaflets induced by the ethylene exposure. In controlled environments, ethylene has been shown to increase to potentially detrimental levels in soybean and other crops (Wheeler et al, 1996).

For ethylene monitoring in the HPC, an aliquot of air should be obtained through the air sampling port and analyzed at least weekly. Currently, the best method for low ppb measurement of ethylene involves the use of a gas chromatograph (GC) equipped with a flame ionization detector (FID) and gas analysis capillary column (example: 30 metre 0.53mm ID Supel-Q Plot, Supelco Inc). When properly configured, this type of system is capable of detecting ethylene levels as low as 10 ppb.

If ethylene is detected and removal is required, the simplest method involves passing a gas stream (ie. using the return line from the CO_2 analyzer) through potassium permanganate ($KMnO_4$) impregnated on a suitable porous material (for an example, see Hernandez et al, 2007).

Within the frame of the present Call-off Order, ethylene analysis is performed with Agilent Technologies 6890N Network Gas Chromatograph System equipped with a flame ionization detector (FID) and gas analysis capillary column 19095P-QO4 HP-PLOT/Q length 30 metre ID 0,530 mm film 40 µm. The following method conditions are used:

Oven:
Initial temperature - 60ºC
Initial time - 5 minutes
Rate 1 - 20 (ºC/min)
Final temperature - 200 ºC
Final time 1 - 1 (min)

Front inlet (S/SL):
Mode – Split
Temperature - 250 ºC
Pressure – 9 psi
Split ratio – 5

Front detector (FID):
Temperature - 270 ºC
H_2 flow – 45 mL/min
Air flow – 400 mL/min
Mode – Constant makeup
Makeup (N_2) - off
Flame – on

7. References

Abeles, FB, PW Morgan and ME Saltveit; 1992; Ethylene in plant biology; Vol. 2, Academic Press, Inc. San Diego, CA, USA

Gunderson, CA, and GE Taylor Jr.; 1988; Kinetics of inhibition of foliar gas exchange by exogenous ethylene: an ultrasensitive response; New Phytol.; 110:517-524

Gunderson, CA, and GE Taylor Jr.; 1991; Ethylene directly inhibits foliar gas exchange in Glycine max.; Plant Physiol.; 95:337-339

Hernandez, A.B., Edralina P. Serrano and Ernesto J. del Rosario, Kinetics Studies of Ethylene Oxidation by Potassium Permanganate Adsorbed on

Rice Hull Ash, Lahar Ash or Coconut Coir Dust, Philipp Agric Scientist 90: 28-39 (2007)

Kays, S.J., and J.E. Pallas Jr. 1980. Inhibition of photosynthesis by ethylene. Nature. 285:51-52

Mattoo, A.K. and J.C. Suttle, 1991, The Plant Hormone Ethylene, CRC Press, Boca Raton, FL. USA. 337p.

Sisler EC and C Wood; 1988; Interaction of Ethylene and CO2; Physiol. Plant.; 73:440-444

Wheeler, RM, BV Peterson, JC Sager and WM Knott; 1996; Ethylene Production by Plants in a Closed Environment; Advances in Space Research; 18:193-196

Woodrow, L and B Grodzinski; 1989; An evaluation of the effects of ethylene on carbon assimilation in Licopersicon esculentum. Mill.; J. Exp. Bot.; 40:361-368

Woodrow, L, LJ Jiao, JM Tsujita and B Grodzinski; 1988; Whole plant and leaf steady state gas exchange during ethylene exposure in Xanthium strumarium L.; Plant Physiol.; 90:85-90

Woodrow, L, LJ Jiao, JM Tsujita and B Grodzinski; 1990; "Photoautotropic Systems: Ethylene and Carbon Dioxide Interactions from the Callus to the Canopy"; In: Polyamines and Ethylene:Biosynthesis, Physiology and Interaction, eds. H.E. Flores and R.N. Arteca. Current Topics in Plant Physiology, Vol 5, American Society of Plant Physiology Publishers, Washington D.C.

Woodrow, L, RG Thompson and B Grodzinski; 1988; Effects of ethylene on photosynthesis and partitioning in tomato, Lycopersicon esculentum Mill; J. Exp. Bot.; 39:667-684

8. Appendix 1: Procedure for the Grodan rockwool safe manipulation

a. Manipulation with rockwool - small cubes - Grodan AO 36/40 6/15W

1. Use Personal Protective Equipment:
particle mask,
gloves (3 pairs),
goggles,
lab coat.
As alternative to goggles and particle mask for better protection of face skin integral dust protection mask with face protective shield can be used. In order to prevent possible affect of gases that might be released from rockwool a mask with integral gas and dust filters can be used.
2. Place the two flats of rockwool cubes into a big plastic bag.
3. Fill this bag with deionized water until it covers all the cubes.
4. Having gloves on wash the hands.
5. Tie up the bag and let the cubes to dampen.
6. Wash the hands with the gloves on.
7. Place wet rockwool cubes into thermostable vessels.
8. Wash the hands with the gloves on.
9. Cover the thermostable vessels with aluminium foil and fix well with sticky paper tape.
10. Sterilise these rockwool cubes by autoclaving during 30 minutes at 120ºC.
11. Wash again the hands with the gloves on.
12. Take off the gloves preventing the contact of gloves “polluted” side with skin and put them into a garbage bag.
13. Put on another pair of gloves.
14. Take the thermostable vessels with sterile rockwool out of autoclave and leave them to cool down until room temperature.
15. Take off the gloves and put them into a garbage bag.
16. When the vessels with sterile rockwool are cooled down put on another pair of gloves.
17. Place rockwool cubes into 2 seed germination trays and follow procedure for seeds planting (TN 96.3).
18. In the end of planting manipulation take off the gloves preventing the contact of gloves “polluted” side with skin and put them into a garbage bag.

b. Manipulation with rockwool - large cubes – Grodan Delta 4G 42/40

1. Use Personal Protective Equipment:
   particle mask,
   gloves (1 pair),
   goggles,
   lab coat.
   As alternative to goggles and particle mask for better protection of face skin integral dust protection mask with face protective shield can be used. In order to prevent possible affect of gases that might be released from rockwool a mask with integral gas and dust filters can be used.
2. Fill this bag with deionized water until it will cover all the cubes.
3. Having gloves on wash the hands.
4. Tie up the bag and let the cubes to dampen.
5. Wash again the hands with the gloves on.
6. Take off the gloves preventing the contact of gloves “polluted” side with skin and put them into a garbage bag.

c. Manipulation with rockwool cubes during plants harvest

1. Use Personal Protective Equipment: particle mask, gloves, goggles, lab coat.
2. Take one of the 20 trays with plants from HPC1.
3. After cutting upper part of plant make sure that rockwool cube containing roots is wet. If not, dampen it with distilled water.
4. Take wet rockwool cube and place it into a plastic bag with zip, after zip up the bag and place it into a plastic box for storage and transportation to Veterinary School.
5. Repeat steps 2-4 for all the cubes in the 20 trays.
6. Having gloves on wash the hands.
6. After plastic box for storage and transportation is full, close it with appropriate lid.
7. Use more than 1 box if necessary.
8. Place plastic box/es for storage and transportation into cold room until in General Laboratory until it is transported to Veterinary School.
9. Wash again the hands with the gloves on.
10. Take off the gloves preventing the contact of gloves “polluted” side with skin and put them into a garbage bag.

d. Manipulation with rockwool cubes during samples preparation in Veterinary School

1. Use Personal Protective Equipment:
particle mask for manipulation with roots rinsing,
integral dust protection mask with face protective shield for manipulation with roots separation from rockwool,
longish gloves,
goggles,
lab coat and trousers,
protective cap,
safety shoes.
2. Turn on laminar flow cabinet and place plastic bags with rockwool cubes inside the cabinet under the hood.
3. Fill a plastic vessel with water and place it out of laminar flow cabinet next to a sink for roots rinsing.
4. Open plastic bag with rockwool cube and carefully separate roots from Rockwool using tweezers under the hood.
5. Rinse roots with water out of rockwool particles in the vessel out of laminar flow cabinet.
6. Dry out roots with paper towel and place them into a paper bag. Seal labeled paper bag and put it on a table out of laminar flow cabinet.
7. Empty vessel with rockwool contaminated water into a bigger vessel.
8. Rinse empty vessel with water using a sink in the same room where laminar flow cabinet is situated and fill it with clean water.
9. Repeat steps 3-8 for all rockwool cubes.
10. Place Rockwool wastes into a garbage plastic bag.
11. Clean thoroughly laminar flow cabinet with water and paper towels.
12. Throw contaminated paper towels into the garbage plastic bag.
13. Turn off laminar flow cabinet.
14. Clean vessels with water.
15. When a vessel containing rockwool contaminated water is full, place another empty vessel into a sink in the same room where laminar flow cabinet is situated and filtrate contaminated water into this vessel. After empty filtrate in the sink and throw sediment with the filter into the garbage plastic bag containing rockwool wastes.
16. Having gloves on wash the hands.
17. Take off protective Personal Protective Equipment in the same room where rockwool manipulation was performed.
18. Throw integral lab coat and all disposable equipment into the garbage plastic bag.
19. Put on another pair of gloves.
20. Clean non-disposable masks with paper towels wetted with ethanol and place the masks into separate clean plastic bags.

21. Throw contaminated towels into the garbage bag.
22. Having gloves on wash the hands.
23. Tie up the garbage bag.
24. Having gloves on wash the hands.
25. Place non-disposable personal equipment into separate clean plastic bag.
26. Place paper bags with roots into the oven.
27. Having gloves on wash the hands.
28. Take the garbage bag with you and throw it into appropriate garbage bin in Campus.

9. Appendix 2: LI-COR 3100C

LI-3100C
Area Meter

Fast, Precise, Easy Operation

LI-COR®
Biosciences

LI-3100C Area Meter

Rapid, Precise Area Measurement of Large or Small Leaves

- Adjustable Resolution: 0.1 or 1 mm²
- High accuracy and repeatability
- Individual or cumulative area
- Fast, continuous operation for large quantities of samples
- Large samples: 25 cm wide, 2.5 cm thick, 1mm² resolution
- Small samples: <1 cm² when using 0.1 mm² resolution
- Adjustable press roller to flatten curled leaves
- LED display
- Windows® software
- USB and Serial ports

Versatility

The LI-3100C Area Meter is designed for efficient and exacting measurement of both large and small leaves. Adjustable resolution settings provide versatility for diverse project requirements.

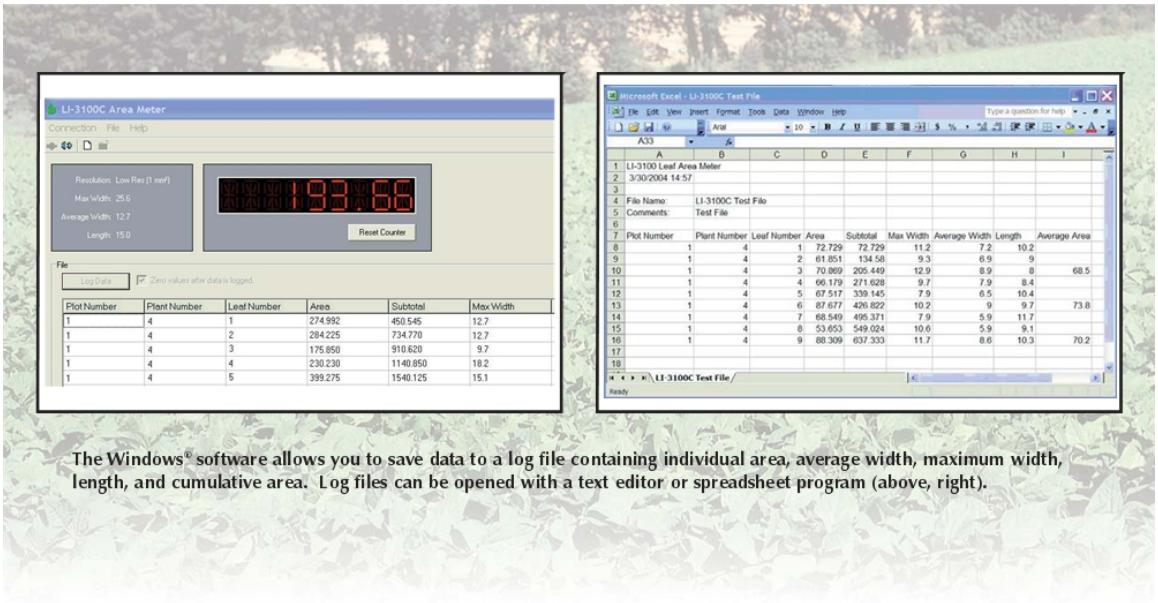
A wide variety of leaves can be measured, ranging from larger samples such as corn, tobacco, and cotton to smaller samples such as wheat, rice or alfalfa. Small leaves or leaf discs are measured with the same precision as larger leaves. The LI-3100C can also handle conifer needles, perforated leaves and leaves with irregular margins. This is especially important in determining leaf damage and insect feeding trails.

Operation

Samples are placed between the guides on the lower transparent belt and allowed to pass through the LI-3100C. As the sample travels under the fluorescent light source, the projected image is reflected by a system of three mirrors to a scanning camera. This unique optical design results in high accuracy and dependability.

An adjustable press roller flattens curled leaves and feeds them properly between the transparent belts. This provides for accurate measurement of small grasses, legumes, aquatic plants and similar types of leaves.

As samples pass under the light source, the accumulating area in mm² is shown on the LED display or on a computer screen when using the Windows® software. Calibration adjustments are easily accomplished using a standard area calibration disk (included) and turning the calibration screw located near the display.



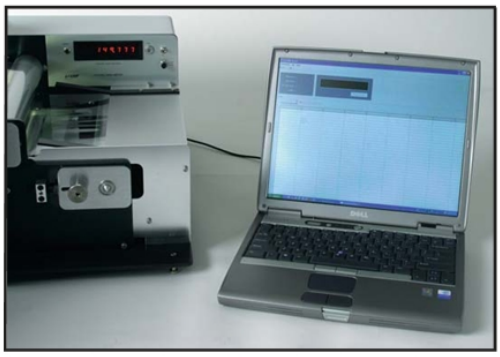
The Windows® software allows you to save data to a log file containing individual area, average width, maximum width, length, and cumulative area. Log files can be opened with a text editor or spreadsheet program (above, right).

Data Analysis

The LI-3100C Windows® Interface software allows users to monitor data on a computer and store readings in a log file. The log file includes individual area, maximum width, average width, length, and cumulative area. Remarks can be entered for each logged value. Most text editor or spreadsheet programs can open LI-3100C data files.

The Windows® Interface software also features:

- Display of individual area, leaf length, average width, and maximum width
- Indicator of resolution setting on the LI-3100C
- Area counter reset, independent of the LI-3100C LED display
- Support for both USB and serial connections



The LI-3100C can be connected to a computer via a serial or USB connection. Simple Windows® software allows you to log data to a file.

Simplified Maintenance

Cleaning the LI-3100C is simplified by convenient access to all belt surfaces and mirrors. The transparent belts are rugged and durable. The fluorescent lamp and belts are easy to replace when needed.

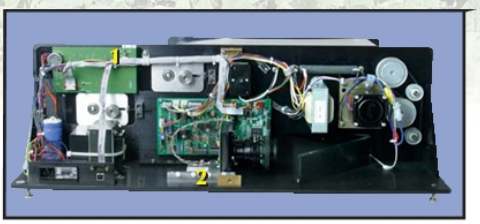
Specifications:

Resolution:	1 mm² or 0.1 mm² (adjustable)
Scanning Area:	1 mm² Resolution: 1 mm x 1 mm
	0.1 mm² Resolution: 0.300 mm W x 0.333 mm L
Display Capacity:	1 mm² Resolution: 999,999.99 cm²
	0.1 mm² Resolution: 99,999.999 cm²
Display:	Full 8-digit LED
Width:	25.4 cm max; 1.5 to 3.0 mm minimum
Thickness:	Up to 2 cm, user-expandable to 2.5 cm
Length:	Unlimited
Conveyor Belt Speed:	8.0 cm/s at 60 Hz; 6.7 cm/s at 50 Hz
Light Source:	15 W fluorescent tube
Transparent Belts:	Rugged clear vinyl
Power Requirements:	108-126/216-252 VAC, 48 to 66 Hz, 100 W max
Operating Temperature:	+15 to + 55 C
Storage Temperature:	-20 to + 65 C
Size:	25.0 H x 60.0 W x 73.0 L cm (9.8" x 23.6" x 28.7")
Weight:	43 Kg (95 lb)

Accuracy — Sample Area

Resolution	10 cm²	3 cm²	1 cm²	0.3 cm²
1 mm²	± 2.0%	± 3.0%	± 6.0%	± 10.0%
0.1 mm²	± 1.0%	± 1.5%	± 3.0%	± 5.0%

Combined accuracy and precision to 99% confidence with correct calibration on verifiable shapes. Better accuracy can be achieved by calibrating the LI-3100C and/or placing the leaf on the middle section of the belt. Use the 0.1 mm² resolution for conifers, roots and other similar objects and expect the accuracy to be about 5% less than normal leaves.



Area-sensing resolution is shifted between 1 and 0.1 mm² with a simple procedure. For 1 mm² resolution, the display is switched for two decimal places (1), and the camera lens is moved to the appropriate pre-marked location (2). For 0.1 mm² resolution, the display is switched for three decimal places, and the camera lens is moved to a second pre-marked location. The lens focus is set by LI-COR.

Ordering Information

LI-3100C	Includes both 0.1 and 1 mm² resolution, one each 3100TBL and 3100 TBU transparent belts, two 3100LAMP fluorescent lamps, 3100-500 Windows interface software, RS-232 serial cable, USB cable, dust cover and instruction manual
3100TBL	Lower Transparent Belt
3100TBU	Upper Transparent Belt
3100LAMP	Fluorescent Lamp

LI-COR®

Biosciences

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980-07694

10. Comments

TN 96.4
Sampling and Analysis Protocols

Comments

General comments

The protocols included in this TN were discussed during the Progress meeting on HPC1 and TRR functional tests with SCHNEIDER **(**MPP-MOM-09-4105(0)-EP-20090716, page 8, second paragraph). The TN was updated accordingly after this discussion, mainly regarding:

- Ion analysis in the hydroponics solution
- Leaf area analysis
- Ethylene analysis

Detailed comments

Page/paragraph	Comment
3/Section 3	*"In the case of short-term (1 week) studies, no sampling is required. For long-term tests (eg. 40 days), the analytical requirements are listed in this document."* We are missing a clear sampling protocol which I cannot find in any of the document provided. A protocol is added to the paragraph 4 (Analysis)
4/Illustration 1	*"Illustration 1 outlines the basic sampling flow for the harvest and processing of batch lettuce grown in HPC1."* Please clarify if all analysis are performed on each single plant. This remark is linked to the general one on sampling protocol /strategy. Clarified in the analytical protocol (Section 4.4).
9/Section 4.5	Where do we find then the exact procedures used for our tests? My understanding is that we should somehow follow always the same analytical methods to authorize a comparison of results. Please clarify.

	Exact procedures used for the tests are provided below in the doc., and additional clarification in the text (pages 9-12): *"Total Carbon and Total Nitrogen: This method is used for C, H, N determination in the lettuce after 7-days and 40-days crop tests."* *"Plant Minerals (Excluding N and Cl): This method is used for determination of Na, S, Ca, Mg, K, Mn, B, Zn, Fe, P in lettuce after 7-days and 40-days crop tests and in the nutrient solutions of HPC1. The determination of Cu and Mo is done by ICP-MS ICP analysis of a nitric acid digest."* *"Nutrient solution: Method of ion exchange chromatography is used for sulfate (SO_4^{2-}), phosphate (PO_4^{3-}), nitrate (NO_3^--N), nitrite (NO_2^-), chlorine (Cl^-) analysis in the nutrient solutions in the frames of the present call of order."* Included description of method conditions for ethylene analysis (page 12).
9/ Section 4.5	*"Total Carbon and Total Nitrogen:"* Sorry I do not remember if other analysis were to be performed on other elements? Can you refresh my memory? Thank you Yes, we agreed to perform analysis also on H, P,S, Ca, Mg, K, Na, Fe, Cu, Mn, B, Zn, Mo, Cl.
11/Section 5	*"In addition to pH and electrical conductivity which are continuously monitored, detailed nutrient solution analysis should be performed after the solution is mixed, and then every 5 days until the end of the experiment. "* I do not understand this sentence as the nutrient solution is renewed every 5 days *("In order to prevent nutrients accumulation or depletion in the solution, nutrient solution is to be changed every 5 days.")* Please clarify. Removed the phrase: " *and then every 5 days until the end of the experiment"* Added the following: *"and 3 samples of nutrient solution should be taken before and after nutrient solution changeover. Detailed nutrient analysis of these samples should be performed."*
14/Section 9	*"Data file: 3100C.pdf"* Is it possible to include the file in the document? Document included.