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## **TN 101.3**

### **Test report for the characterization of lettuce batch cultures in HPC1**

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# MELISSA Pilot Plant

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Prepared by <i>Auteur</i>	Tikhomirova, N. , Munganga, C. and Peiro, E. <i>Euxim Peiro</i>	Date <i>Date</i>	29/07/11
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Checked by <i>Verifié par</i>	Fossen, A. and Stasiak, M. <i>[Signature]</i>	Date <i>Date</i>	18/08/11
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Approved by <i>Approuvé par</i>	Gòdia, F. <i>[Signature]</i>	Date <i>Date</i>	18/08/11
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Approved by customer <i>Approuvé par le client</i>	Lamaze, B. <i>[Signature]</i>	Date <i>Date</i>	26/06/2012
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## CHANGE LOG

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## 1. Scope

This document reports the results and conclusions from the lettuce batch cultures carried out with 20 days of duration in the HPC1 in order to validate the reproducibility of the culture conditions in the chamber. These cultures followed a previous culture campaign of 38 days duration (reported in RD1) performed in the frame of the previous contract COO6.

## 2. Reference and applicable documents

### 2.1 Applicable documents

- AD1 19071/05/NL/CP Memorandum of Understanding between MELiSSA Partners
- AD2 COO9: HPC1 characterization phase in the MELiSSA Pilot Plant
- AD3 TN 101.2: Protocol for batch culture experiments in the HPC1 of the MPP
- AD4 TN 96.3 "Test Protocols and procedures for lettuce cultivation."
- AD5 TN 96.4: "Sampling and analysis Protocols and Procedures for biomass, nutrient solution and gas phase."

### 2.2 Reference documents

- RD1 TN 96.12: Cultivation as-run procedures, test results and final test report
- RD2 Hanford et al (2006) Exploration life support baseline values and assumptions document. NASA Contract Report
- RD3 Massot, A. (2007) Engineering Photosynthetic Systems For Bioregenerative Life Support. PhD Thesis. Universitat Autònoma de Barcelona
- RD4 Waters et al (2002) Dynamic modeling of the higher plant chamber as a component of bioregenerative life support systems. PhD Thesis. University of Guelph, Canada



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## Acronyms/Definitions

MELiSSA	Micro Ecological Life Support System Alternative
MPP-CCL	MELiSSA Pilot Plant – Claude Chipaux Laboratory
HPC	Higher Plant Chamber
COO	Call-Off-Order
IDCG	Integrated Daily Carbon Gain
NCER	Net Carbon Exchange Rate
UPC	Universitat Politècnica de Catalunya
OP	Operating Procedure

## 3. Testing facility contacts

### 3.1 Prime testing facility

#### 3.1.1 Name of the testing facility

MELiSSA Pilot Plant – Claude Chipaux Laboratory

#### 3.1.2 Address of the testing facility

Departament d'Enginyeria Química  
Escola d'Enginyeria (EE)  
Universitat Autònoma de Barcelona  
Bellaterra Campus, Barcelona  
Spain

#### 3.1.3 Contact for technical information

Enrique Peiro  
MPP – CCL Technical Manager  
Phone Number: 0034935818172 and 0034 935868101  
Email: [Enrique.peiro@uab.cat](mailto:Enrique.peiro@uab.cat)

### 3.2 Subcontractors

#### 3.2.1 Name of subcontractor

Controlled Environment Systems Research Facility



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### 3.2.2 Address of the subcontractor

School of Environmental Sciences

University of Guelph  
Guelph, Ontario  
Canada

## 4. Customer contacts

### 4.1 Name of the Customer

European Space Agency

### 4.2 Address of the Customer

European Space Agency  
D/TEC-MCT  
P.O.Box 299, 2200 AG Noordwijk, The Netherlands

### 4.3 Contact person for the Customer

Brigitte Lamaze-Lefebvre  
ESA Focal Point for the MPP-CCL

## 5. Test items

### 5.1 Short description of the test item

The first Higher Plant Chamber was installed into the MELiSSA Pilot Plant facility at Universitat Autònoma de Barcelona in the frame of the contract COO6. The main contributions expected by integration of this photosynthetic compartment into the MELiSSA loop were oxygen, water, vegetable food production and CO<sub>2</sub> consumption. Production characteristics of lettuce *Lactuca sativa* L. (cv. Grand Rapids), as a MELiSSA candidate crop, were investigated in that work in the first crop experiments in the MELiSSA Pilot Plant facility. The plants were grown in batch culture and totalled 100 plants with a growing area 5 m long and 1 m wide in a sealed controlled environment. It was shown that after 38 days of lettuce cultivation in the chamber a good productivity was obtained, but accumulation of oxygen in the chamber, which required purging of the chamber, and decrease in the food value of the plants were observed. Reducing the duration of the tests to 20 days allowed uninterrupted test without opening the system and also allowed estimation of the crop's carbon balance. Several replicates of the experiments were carried out and



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results of productivity, tissue composition, nutrient uptake and canopy photosynthesis of lettuce regardless of test duration are discussed.

## 6. Implemented test sequence

### 6.1 Objectives of the tests

Taking into account the results of the preliminary tests already performed in HPC1, and the corresponding protocols and procedures (i.e. including for sampling and analysis), a series of 2-3 replicate experiments of batch culture of lettuce in the HPC1 was performed, with the aim to optimize experimental variables, such as illumination, liquid medium feeding and circulation, air flow-rates and their distribution and assure culture homogeneity (See AD2).

### 6.2 Actual schedule of the tests

First 20 days Test: 04.01.10 – 01.02.10  
Second 20 days Test: 01.03.10 – 01.04.10  
Third 20 days Test: 12.05.10 – 11.06.10  
Fourth 20 days Test: 27.06.10 – 23.07.10

### 6.3 Implemented test sequence

For all the batch culture experiment replicates, the sequence was as follows:

- Phase 1 : Cleaning of the chamber, air handling unit and liquid loop and preparation of the culture campaign
- Phase 2 : Seedlings phase from planting to 8 days growth
- Phase 3 : Maturity phase inside the chamber, from day 8 till day 28 and harvesting

See AD3 for further details on each phase.

## 7. Implemented data collection plan

### 7.1 Implemented analysis/measurement plan (both on-line and off-line)

The following files were generated:

- First test: HPC1 28 days test – phase 1-01012010.csv / HPC1 28 days test – phase 2-01012010.csv HPC1 28 days test – phase 3-01012010.csv





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- Second test: HPC1 28 days test – phase 1-01032010.csv / HPC1 28 days test – phase 2-01032010.csv HPC1 28 days test – phase 3-01032010.csv
- Third test: HPC1 28 days test – phase 1-09052010.csv / HPC1 28 days test – phase 2-09052010.csv HPC1 28 days test – phase 3-09052010.csv
- Fourth test: HPC1 28 days test – phase 1-24062010.csv / HPC1 28 days test – phase 2-24062010.csv HPC1 28 days test – phase 3-24062010.csv

The dates of the files were the corresponding to the start of each phase of each test, according to the sequence described in Section 6.2 of this TN.

The files mentioned above were created as CSV text files by the HMI system and then translated into excel files for off-line analysis of process data.

- Calibration of instruments (see in Annex 2 the as-run procedures and, in particular, appendix 2 of the record corresponding to each test):
  - pH and EC electrodes were calibrated at the beginning of each test using standard buffer solutions
  - Gas analyser had been calibrated for the Functional tests (23.07.09)
  - PAR sensors were calibrated during the mapping (by UPC, 30.06.10)
  - For the rest of sensors, only the manufacturer’s certificates were available.
- Deviations and non conformances (see in Annex 2 the as-run procedures and, in particular, Appendix 3 of the record corresponding to each test): as explained in Section 9.3, the main deviation was that the validity period for instruments calibration had not been defined, so that most of the instruments only had the manufacturer’s calibration certificate.

## 7.2 Implemented sampling techniques :

For sampling purposes, the procedures described in AD5 were followed. Also when applicable, the procedures established by the external laboratories in charge of specific analysis were as well followed (elemental composition, minerals).

Sampling date and identification was performed in agreement with the MPP applicable procedures, and including always the date, the part of the crop (shoots/roots), the tray and the single crop when applicable. Also when applicable, according to the procedures of the external laboratories in charge of specific analysis.

For non available standard procedures, the traceability was assured through the technician logbook.



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- Place of sampling:
  - shoot and root samples were taken from the crops directly from the trays when harvested
  - hydroponic solution samples were taken in general from the bottom valve of the hydroponics tank, but some of them were as well taken from the top of this tank to check the homogeneity of composition inside the tank
  - gas samples for ethylene analysis were taken from the gas analysis loop (dedicated pipeline to the gas analyser)
- Conditions of sampling:
  - the procedure described in AD5 was followed
  - in some cases several samples were mixed for the analysis (seedlings, roots)
- Deviations and non conformances: N.A.

### 7.3 Implemented analyses/measurements

According to AD5, the following analyses were performed:

- Biomass analysis: fresh and dry weight (MPP), previous drying of lettuce samples in the Animal and Food Science Laboratory, Veterinary School, UAB.
- Nutrient solution analysis: anionic composition of hydroponic solution by HPLC (Chem. Eng. Department, by MPP technicians) and colorimetry (MPP)
- Gas composition analysis: O<sub>2</sub> and CO<sub>2</sub> (on-line, MPP)
- Elementary composition and mineral composition of crops and nutrient solution (Servei d'Anàlisi Química, UAB; Serveis Científicotècnics, UB)
- Ethylene, by GC (Chem. Eng. Department, by MPP technicians)
- Conditions of analysis/measurement
  - Biomass analysis were performed according to AD5
  - Anionic composition of the hydroponic solution was performed according to the Chem. Engineering department procedures (HPLC) or MPP quality control procedures (colorimetry)
  - Elementary composition and minerals were analysed according to the dedicated procedures in UAB and UB services, compatible with the specification defined in AD5.



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- Ethylene was analysed by GC (Agilent Technologies 6890N Network GC System, FID detector).

In particular, additional detailed description of the analysis performed by the MPP is provided below:

### Biomass analysis

All lettuce plants were harvested 20-days after crop seedling tests start-up. Fresh weight of lettuce was recorded immediately after harvest. Then the plants were dried in an oven at 70°C with preliminary fixation at 103°C during 10 minutes in order to stop the enzymatic process. Plant dry weight was recorded after weight stabilization (3-4 days of drying) and expressed as g dw.plant<sup>-1</sup>. Finally total plant productivity (g.dw.m<sup>-2</sup>.d<sup>-1</sup>) was calculated as the total biomass harvested divided by the production area and by the total days in the chamber.

### Nutrient solution analysis

Nutrient solution samples were analyzed using ion chromatography (ICS 2000 Dionex) to determine NO<sub>3</sub><sup>-</sup> and colorimetry (Xion 500 Dr Lange) to determine NH<sub>4</sub><sup>+</sup>. The results obtained were used for calculation of nitrogen uptake rates over the growth period. Nitrogen was chosen for nutrient mass balance estimation as main nutrient consumed by lettuce at the highest rate.

### Atmospheric composition analysis

HPC1 was equipped with a CO<sub>2</sub>/O<sub>2</sub> gas analyzer model 601P (California Analytical Instruments, Orange, CA, USA) which was used for online measurement of CO<sub>2</sub> and O<sub>2</sub> concentrations inside the chamber. CO<sub>2</sub> consumption was calculated using the amount of CO<sub>2</sub> injected by the CO<sub>2</sub> mass flow controller in order to maintain the chamber at a constant 1000 ppm CO<sub>2</sub> level.

- Deviations and non conformances:
  - Ethylene analysis was performed at the end of the second test, then it was cancelled as the obtained values were below the limit of detection of the equipment (1 ppm), when the adequate detection limit for the GC should be around 5 ppb for plant studies according to UoG experience.

## **7.4 Uncertainty budget evaluation**



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The uncertainty budget was not required for this particular test campaign.

## 8. As run procedures

### *Annotated test procedures*

The annotated test procedures are shown in Annex 2, including the following pdf files, corresponding to the second, third and fourth tests:

MPP-REC-10-4101(0)-01  
MPP-REC-10-4102(0)-01  
MPP-REC-10-4103(0)-01  
MPP-REC-10-4101(0)-02  
MPP-REC-10-4102(0)-02  
MPP-REC-10-4103(0)-02  
MPP-REC-10-4101(0)-03  
MPP-REC-10-4102(0)-03

For the first test (04.01.10 – 01.02.10), the standard MPP protocols and records had not yet been created, so the operations were recorded in the HPC logbook.

For the fourth test (27.06.10 – 23.07.10), the record corresponding to the first phase was not filled out as this phase was not performed.

### *Deviations and non conformances*

The deviations and non conformances are shown in Appendix 3 of the annotated test procedures files previously mentioned.

## 9. Non conformances and deviations summary

### *9.1 Deviations from the test plan*

The following deviations were found in the tests:



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## 9.1.1 First 20 days Test: 04.01.10 – 01.02.10

N°	Deviation	Criticality	Corrective action	Due date	Status
1	11.01.10 Gas sampling without putting the analysis loop in OFF, creating perturbancies (CO2 level rised up to 1200 ppm)	Low	Issue the formal OP and REC to minimize the risk of wrong operations	01.03.10	Closed
2	21.01.10 Hydroponic loop stopped 1 hour for changing acid and base valves	Low	N.A. (not critical for the process)	N.A.	Closed
3	25.01.10 Temp./humidity loop stopped 10 min to change resistor in hot water	Low	N.A. (not critical for the process)	N.A.	Closed

## 9.1.2 Second 20 days Test: 01.03.10 – 01.04.10

First phase: 10 deviations to the test protocol

N°	Deviation	Criticality	Corrective action	Due date	Status
1	Unforeseen volume of ethanol	Low	Modify new version of the protocol	End COO9	Open
2	Not using mask against vapours	Medium	Reminder in safety meetings; reconsider in new version of the protocol	End COO9	Open
3	Disinfection of hydroponic loop not	Medium	Modify new version of the	End COO9	Open



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	performed as agreed with ESA		protocol		
4	Additional EC measurement (only pH measurement was foreseen)	Low	Reconsider in the new version of the protocol	End COO9	Open
5	Id. Dev. 3				
6	Id. Dev. 4				
7	Rinsing nutrient tank with distilled water	Low	Modify new version of the protocol	End COO9	Open
8	Id. Dev. 3				
9	Leak tests lasting 5 hours	Medium	Write an OP before staggered culture campaign	Oct 2010	Closed
10	Validity period for instruments calibration not defined	Medium	To establish an HPC1 maintenance plan before staggered culture campaign	Oct 2010	Open

## Second phase: 2 deviations

N°	Deviation	Criticality	Corrective action	Due date	Status
1	Some not critical tasks not performed when foreseen	Low	Increase flexibility in the new version of the protocol	End COO9	Open
2	No watering of seedlings as the cubes were still wet	Low	Reword the task to give more flexibility in the new version of the protocol	End COO9	Open



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Third phase: 0 deviations

## 9.1.3 Third 20 days Test: 12.05.10 – 11.06.10

First phase: 3 deviations

N°	Deviation	Criticality	Corrective action	Due date	Status
1	Lights not switched on for cleaning to avoid hot conditions (ventilation not started)	Low	Modify new version of the protocol	End COO9	Open
2	Leak test failure (Module C door not tight)	High	Close tightly the door and repeat the leak test	07.05.10, performed and checked	Closed
3	Not defined validity period for instruments calibration	Medium	To establish an HPC1 maintenance plan before staggered culture campaign	Oct 2010	Open

Second phase: 6 deviations

N°	Deviation	Criticality	Corrective action	Due date	Status
1	Sterilisation in advance of Stock A and B tanks, not recorded	Medium	N.A. (task performed; recording to be improved)	N.A.	Closed
2	Preparation in advance of Stock A and B tanks, not recorded	Medium	N.A. (task performed; recording to be improved)	N.A.	Open
3	Checking of seedlings germination performed	Low	Introduce more	End COO9	Open



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	at a different time than initially scheduled		flexibility in the new version of the protocol		
4	Id. Dev. 3				
5	Regulation of pH with acid or base was not needed as pH was in good levels, but it was defined as non compliant	Low	N.A.	N.A.	Closed
6	Leak in the nutrient solution collector	Medium	Collector to be repaired	21.05.10, performed and checked	Closed

Third phase: 3 deviations

Nº	Deviation	Criticality	Corrective action	Due date	Status
1	Ethylene analysis not performed (not available column and not adequate limit of detection)	Medium	Search for alternative method/equipment	TBD	Open
2	Several tasks considered not critical performed at different times than initially scheduled	Low	Increase flexibility in the new version of the protocol	9.1.4 End COO9	Open
3	Id. Dev. 1				

## 9.1.5 Fourth 20 days Test: 27.06.10 – 23.07.10

Second phase: 7 deviations

Nº	Deviation	Criticality	Corrective action	Due date	Status
1	Test phase 1 not performed	Low	N.A. (test oriented to		Closed





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			communication)		
2	Not checking seedlings germination when foreseen but task performed	Low	Let more flexibility in the new version of the protocol	End COO9	Open
3	pH not checked and watering performed later than initially scheduled	Medium	N.A. (not critical)		Closed
4	pH of seedling solution not adjusted	Medium	Reconsider in the new version of the protocol	End of COO9	Open
5	Irrigation system was not stopped before taking the samples	Low	N.A. (not critical)		Closed
6	Samples not taken from the top	Medium	Define better the sampling procedure in new version of the protocol or corresponding OP	End of COO9	Open
7	Not defined validity period for instruments calibration	Medium	To establish an HPC1 maintenance plan before staggered culture campaign	Oct 2010	Open

### Third phase: 10 deviations

Nº	Deviation	Criticality	Corrective action	Due date	Status
1	Ethylene analysis not performed (adequate equipment not available)	Medium	Search for alternative method/equipment	TBD	Open
2	Id. Dev. 1				
3	Id. Dev. 1				
4	Id. Dev. 1				
5	Environmental	High	Remote alarm	End COO9	Open



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	conditions in the chamber failed (probable power cut during week-end)		system or presential supervision during week-end		
6	Some not critical tasks not performed when foreseen	Low	N.A. (not critical)		Closed
7	Placing only one scale for weighing	Low	To make the new edition of the protocol more flexible	End COO9	Open
8	Id. Dev. 6				
9	CO <sub>2</sub> control not disabled for ethylene determination	Low	N.A. (no impact on the experiment)		Closed
10	Weighing for dry weight determination not performed sequentially but in the end of drying	Low	N.A. (not critical)		Closed

## 9.2 Resolved non conformances and deviations

There were not non conformances created.

The following deviations were solved at the date of edition of the report:

- Gas sampling without putting the analysis loop in OFF (Section 9.1.1, deviation 1)
- Hydroponic loop stopped for 1 hour (Section 9.1.1, deviation 2)
- Temp./humidity loop stopped for 10 min (Section 9.1.1, deviation 3)
- Leak test failure (Section 9.1.3, First phase, dev. 2)
- Preparation of tanks in advance, not recorded (Section 9.1.3, Second phase, deviation 2)
- Regulation of pH not needed, defined as non compliant (Section 9.1.3, Second phase, deviation 5)
- Leak in nutrient solution collector (Section 9.1.3, Second phase, dev. 6)
- Test phase 1 not performed (Section 9.1.4, Second phase, deviation 1)
- Delayed check of pH and watering (Section 9.1.4, Second phase, deviation 3)
- Sampling without stopping irrigation loop (Section 9.1.4, Second phase, deviation 5)
- Some not critical tasks not performed when foreseen (Section 9.1.4, Third phase, deviation 6 and 8)



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- CO2 control not disabled for ethylene determination (Section 9.1.4, Third phase, deviation 9)
- Dry weight determination not performed sequentially (Section 9.1.4, Third phase, deviation 10)

Some of the deviations implied the preparation of new OP, issued before the staggered test campaign (October 2010):

- Leak test duration (section 9.1.2, First phase, dev. 9)

### **9.3 Unresolved non conformances and deviations**

Most of the deviations were not corrected, as they imply the update of the protocol for batch cultures, proposed to be performed at the end of COO9.

Some of the deviations imply the update of the test protocol, proposed to be issued at the end of the present COO:

- Unforeseen volume of ethanol (section 9.1.2, First phase, dev. 1)
- Not using mask against vapours (section 9.1.2, First phase, dev. 2)
- Disinfection not performed as agreed with ESA (section 9.1.2, First phase, dev. 3, 5 and 8)
- Additional EC measurement (section 9.1.2, First phase, dev. 4 and 6)
- Rinsing with distilled water (section 9.1.2, First phase, dev. 7)
- Definition of validity period for instruments calibration (section 9.1.2, First phase, dev. 10; section 9.1.3, First phase, dev. 3; section 9.1.4, Second phase, dev. 7)
- Sterilization of tanks in advance, not recorded (Section 9.1.3, Second phase, deviation 1)
- Not critical tasks not performed when foreseen (section 9.1.2, Second phase, dev. 1; section 9.1.3, Third phase, dev. 2)
- No watering of seeds being wet (section 9.1.2, Second phase, dev. 2)
- Lights not switched on for cleaning (section 9.1.3, First phase, dev. 1)
- Not checking seedlings germination when foreseen (section 9.1.3, Second phase, dev. 3 and 4; Section 9.1.4, Second phase, dev. 2)
- pH of seedling solution not adjusted (section 9.1.4, Second phase, dev. 4)
- Sampling not from the top (section 9.1.4, Second phase, dev. 6)
- Placing only one scale for weighing (Section 9.1.4, Third phase, deviation 7)



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Another deviation implies the purchase of equipment, change of method, or implementation of new software, date to be defined:

- Ethylene analysis not performed for not available column and not adequate limit of detection (section 9.1.3, Third phase, dev. 1 and 3; section 9.1.4, third phase, dev. 1, 2, 3 and 4)
- Failure of chamber environmental conditions (section 9.1.4, Third phase, dev. 5)

## 10. Verification matrix

In the following Table, the summary of the executed tests vs planned, and the rationale about success of the tests are described, based on the objectives of the tests (Section 6.1 of this TN):

	<b>Objective/Planned tests</b>	<b>Executed tests</b>	<b>Successful tests</b>
1	Series of 2-3 batch culture experiments, demonstrating the capability to grow adequately lettuce biomass by control of the required culture conditions	4 batch experiments were executed	3 batch experiments were successful; the last experiment showed a failure wrt environmental control conditions
2	Optimisation of experimental variables	Experimental variables were controlled and recorded in all the tests	Conclusions about the maintenance of the variables and the impact of failure were drawn
3	Assure culture homogeneity	Culture homogeneity was foreseen by performing three replicates with the same duration and environmental conditions	3 batch experiments showed a good reproducibility of results, but the growth distribution along the chamber was non homogeneous; the last experiment, where the air flow distribution was modified, showed an improvement in growth homogeneity along the chamber
4	Demonstration of Cleanability of the chamber	Cleaning was performed as indicated in the protocols in all the tests	According to visual inspection and normal evolution of the culture, the cleaning was successful
5	Demonstration of tightness	Leak tests were performed	Tests were successful for the



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	for liquid and gas loops	both for liquid and gas loops	established criteria
6	Capability of growing lettuce seedlings under controlled conditions	Seedlings were grown as foreseed within the chamber	Germination was successful; confirmed by visual inspection and posterior evolution of the culture

## 11. Interpretation of the results

### 11.1 Experimental design

All experiments were performed with lettuce (*Lactuca sativa* L cv., Grand Rapids). Lettuce seedlings were 28-days old for the three first tests and 26 for the last . The 4 experiments were performed according to the following table:

Experiment (B-20 stands for Batch culture, 20 days)	Plant age at the moment of the transfer to the chamber	Total days in the chamber	Plant age at harvest
B-20/1	8 days	20 days	28 days
B-20/2	8 days	20 days	28 days
B-20/3	8 days	20 days	28 days
B-20/4	8 days	18 days	26 days

### 11.2 Results and discussion

#### 11.2.1 Biomass production

A previous lettuce batch culture of 38 days had been carried out and reported in RD1. This was followed by 3 replicates of lettuce grown in batch cultures for 20 days (plus 8 days employed for the growth and preparation of plants seedlings). In order to discuss the results obtained with these experiments, the previous results of 38 days are first described. Yield data for the edible biomass



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are expressed in two ways: 1) final dry matter per plant; and 2) rate of production or productivity (also called crop growth rate), incorporating both area and days inside the chamber. All data are expressed on a basis of 5m<sup>2</sup> available growing area.

## a) Lettuce harvest results of previous 38-days Batch culture

The following table summarizes the main harvest data for the 38 day batch experiment in a 5m<sup>2</sup> area containing 100 plants.

	Leaves	Roots	Total Plant
Total dry weight, DW (g dw)	1725	141.8	1866
Total dry weight per plant, DW (g dw.plant <sup>-1</sup> )	17.42	1.43	18.85
Plant growth rate (g dw.plant <sup>-1</sup> .d <sup>-1</sup> )	0.460	0.040	0.500
Total productivity (g dw.m <sup>-2</sup> .d <sup>-1</sup> )	9.08	0.75	9.82

**Table 1. Harvest data for lettuce grown in batch culture for 38 days**

At the end of batch culture one plant did not grow thus only 99 plants were harvested. The 99 plants were analyzed by plant parts (shoots and roots). The total productivity for the culture was 9.82 g dw m<sup>-2</sup> d<sup>-1</sup>. The productivity was 34% lower than of the 14.92 g dw m<sup>-2</sup> d<sup>-1</sup> reported by A. Massot (RD3) and 35% higher than the 7,3 g dw m<sup>-2</sup> d<sup>-1</sup> reported by Handford et al (RD2), which represent an average value from different tests performed in higher plant compartment with different conditions than those used in the current batch experiment. Indeed, the differences in seedling growth duration, photoperiod and test duration could explain these different values.

## b) Lettuce harvest results of 20-days Batch cultures



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## First 20-days test (04.02.10-01.03.10)

The main observations during the harvest are here below described:

- 1) After plants harvest and preliminary data analysis, a decrease in total lettuce leaves biomass per tray was observed from tray #6 to tray#16 (see data in Annex 1).
- 2) Starting from tray #2, slight leaves yellowing was observed, but for the plants of the trays #8 - tray #15 most of the lowest oldest leaves were yellow (pictures are attached, Fig.1-7).
- 3) It was noticed that all the plants located next to spigots had some contamination on their leaves and started to rot (Fig.8).
- 4) Contamination (considering like that the presence of undesirable microorganisms in the crops, understanding that some bacteria are always coexisting with the plants) also could be seen inside the plants on young leaves (black spots, Fig.9) and it was noticed not only for the plants located next to spigots (where solution can be partially sprayed on plants and make favourable conditions for rotting), but also for the plants located in the other parts of the trays.
- 5) At the end of the test it was noticed that there was some contamination of water and some organic particles inside condensate tank. Since it's rather difficult to disconnect condensate tank for cleaning, it was not cleaned along 4 crop tests. This time the tank was disconnected, samples of water and organic particles were taken for analyses. The tank was also cleaned with soap and disinfected with ethanol.
- 6) In the end of the test it was found out that a vessel with water was left next to heat exchanger, used previously for HVAC mapping in order to decrease humidity in the chamber. Water also contained some organic particles after the test.
- 7) Contamination of Rockwool cubes, roots and trays with algae was not high after the test, mostly for the plants situated next to the spigots and in the part where liquid from a tray goes down



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to main collector (Fig.10).



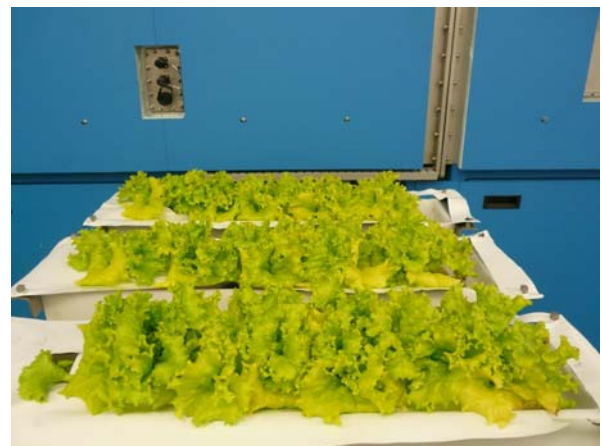
**Fig.1 tray1-3, from left to right**



**Fig. 2 tray4-6 from back to front**



**Fig.3. trays 7-9 from back to front**



**Fig.4. trays 10-12 from back to front**



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**Fig.5 trays 13-15 from back to front**



**Fig. 6. trays 16-18 from back to front**



**Fig.7. tray 19-20 from back to front**



**Fig.8 trays 5-6 from right to left**

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**Fig.9. leaves contamination**



**Fig.10 tray 17 roots**

The results regarding fresh and dry weight of the harvested plants along the chamber are shown in Figures 11 (average per module) and 12 (average per tray). As it can be deduced from these data, the productivity in module B was clearly lower than in the other two modules.

For the second crop test, it was proposed to perform the test with the same duration but in order to eliminate potential risks for plants growth inhibition, the following actions were considered:

- 1) To conduct disinfection of HPC1 by means of products that would not prejudice hardware of HPC1 and would not be toxic for the plants. For all accessible surfaces of HPC1 it was proposed to use soap and ethanol. For the liquid loop, it was considered to use KOH. Finally, after



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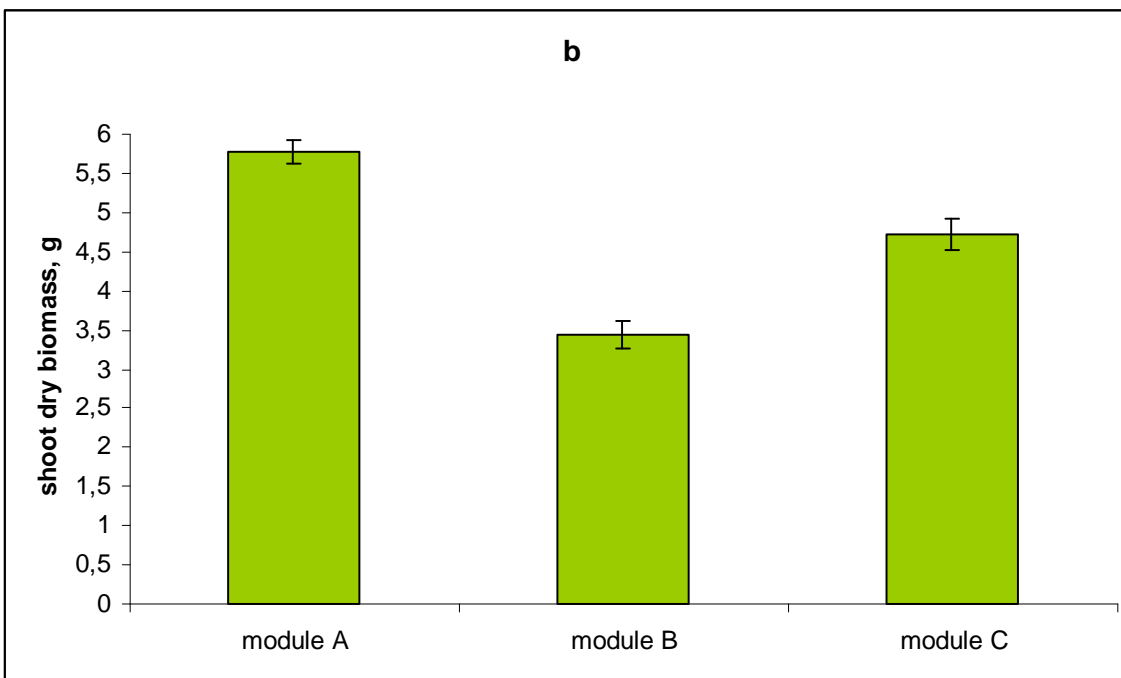
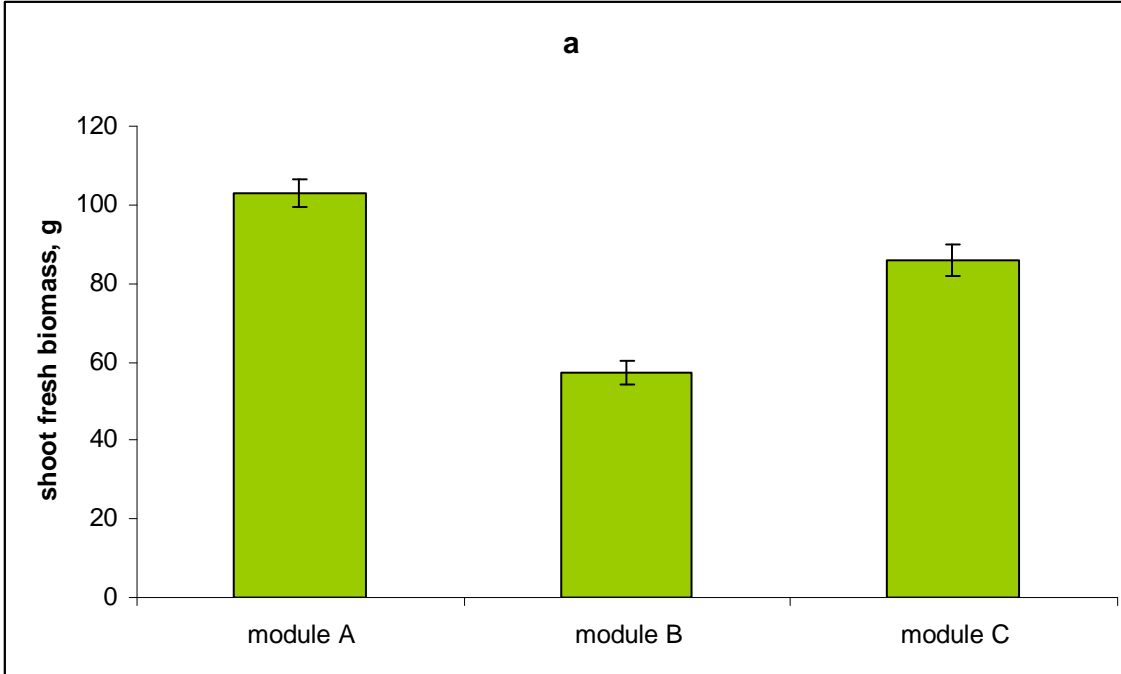


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discussion with ESA, the use of KOH was not implemented, considering that a previous validation was needed, so it was only rinsed with water.

2) In order to eliminate nutritive potential reasons for plants inhibition, nutrient solution would be changed every 7 days and all steps to be taken during the test would be stressed and followed in a formal test protocol.

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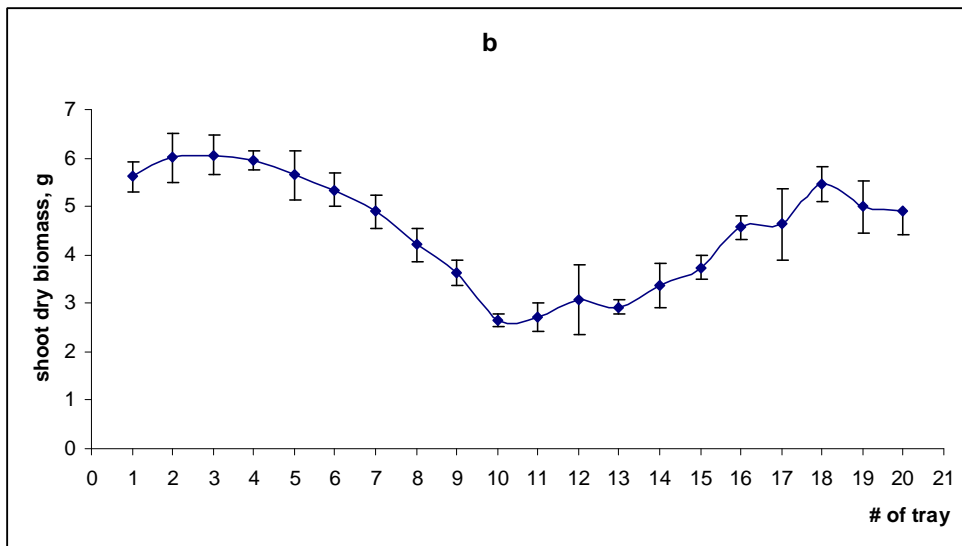
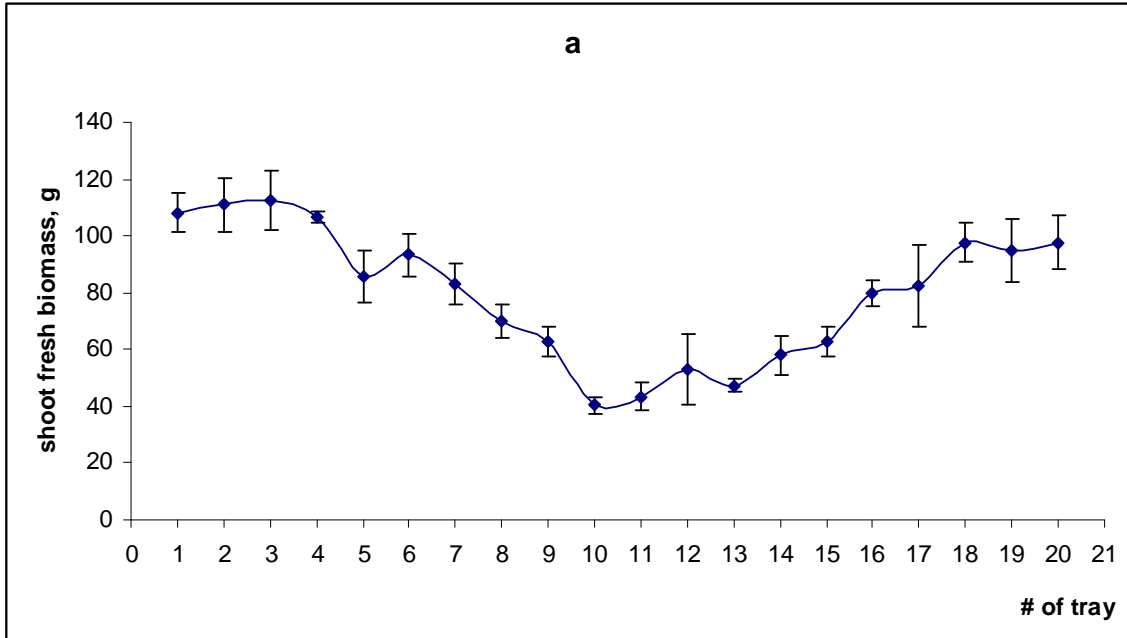


**Figure 11. Lettuce shoot biomass (g) of plants #1,2,3,4,5, average per module: a – fresh biomass; b – dry biomass (trays #1-6 – module A, trays #7-14 – module B, trays #15-20 – module C)**

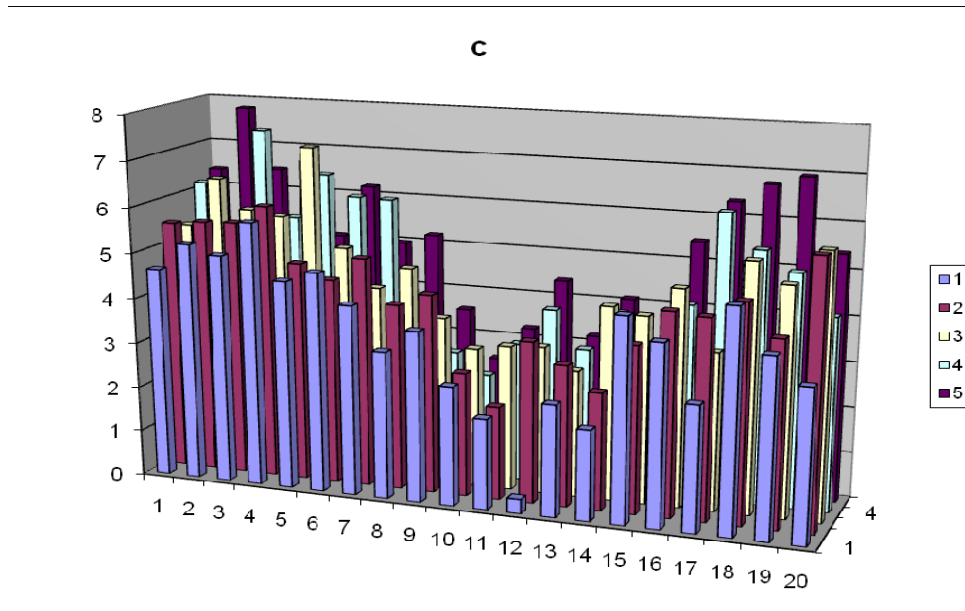


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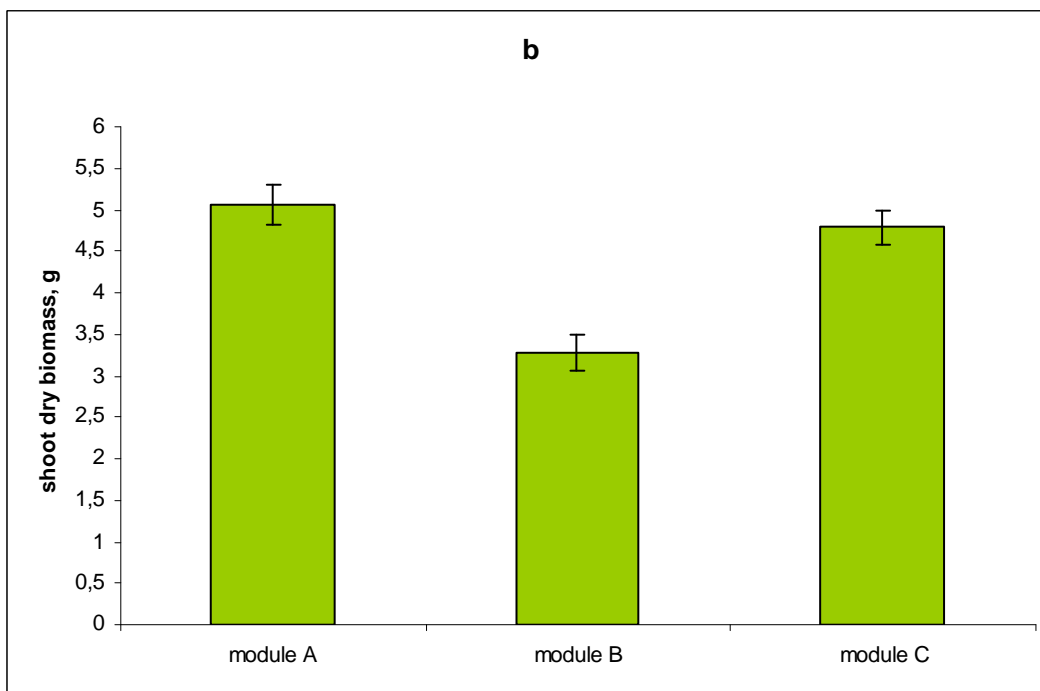
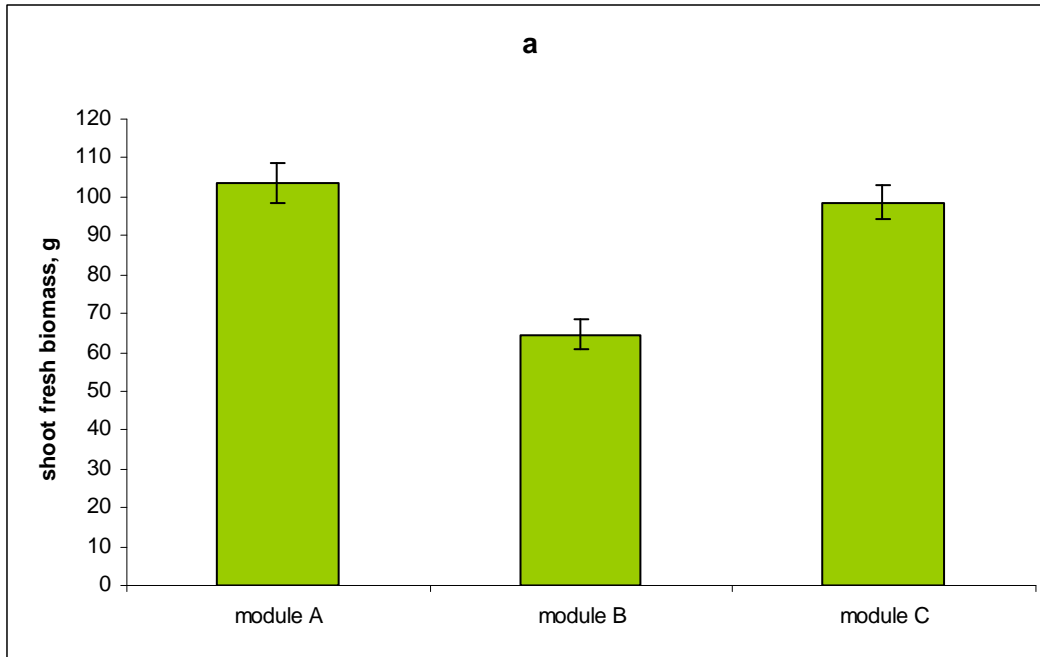


**Figure 12. Lettuce shoot biomass (g) of plants #1-5 average per tray: a – fresh biomass; b – dry biomass (trays #1-6 – module A, trays #7-14 – module B, trays #15-20 – module C); c – dry biomass, 3D representation**

### Second 20-days test (04.03.10-01.04.10)

The results regarding fresh and dry weight of the harvested plants along the chamber are shown in Figures 13 (average per module) and 14 (average per tray). As it can be deduced from these data, the productivity in module B was again lower than in the other two modules.

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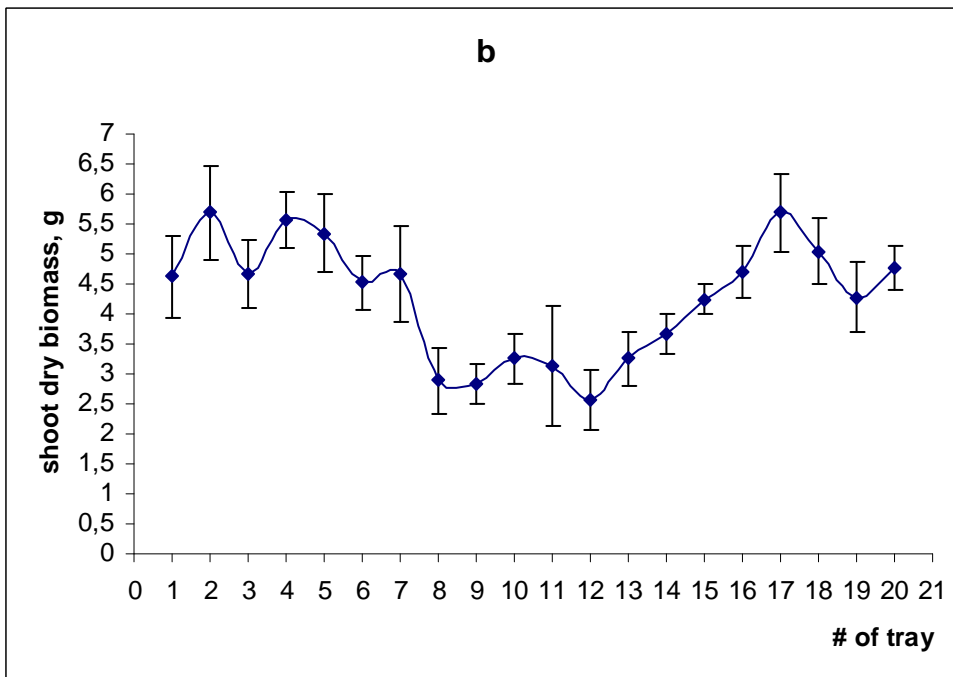
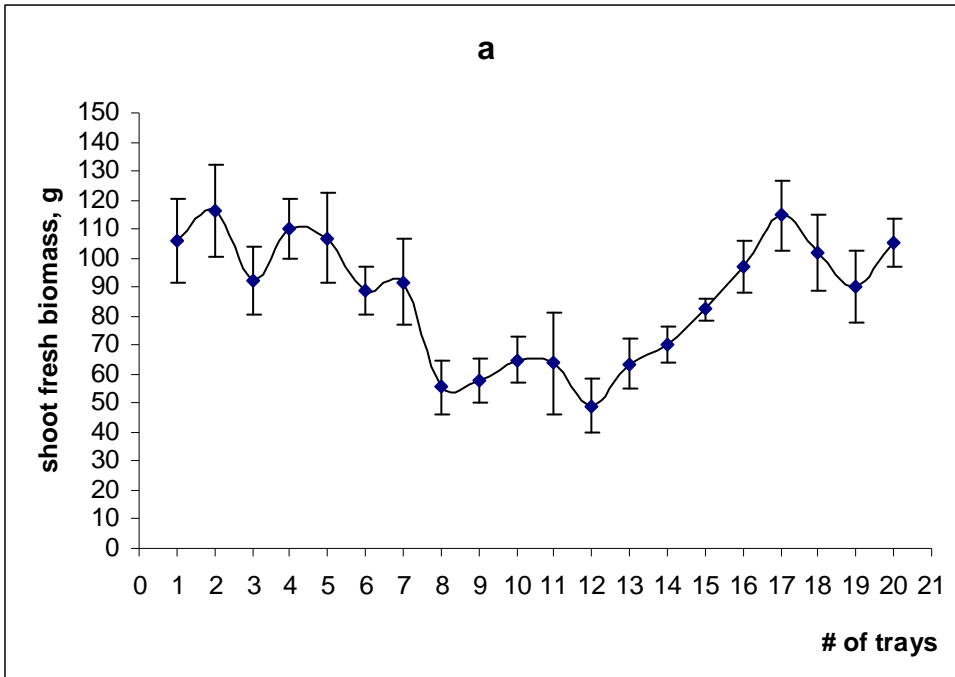
**Figure 13. Lettuce shoot biomass (g) of plants #1,2,3,4,5, average per module: a – fresh biomass; b – dry biomass (trays #1-6 – module A, trays #7-14 – module B, trays #15-20 – module C)**





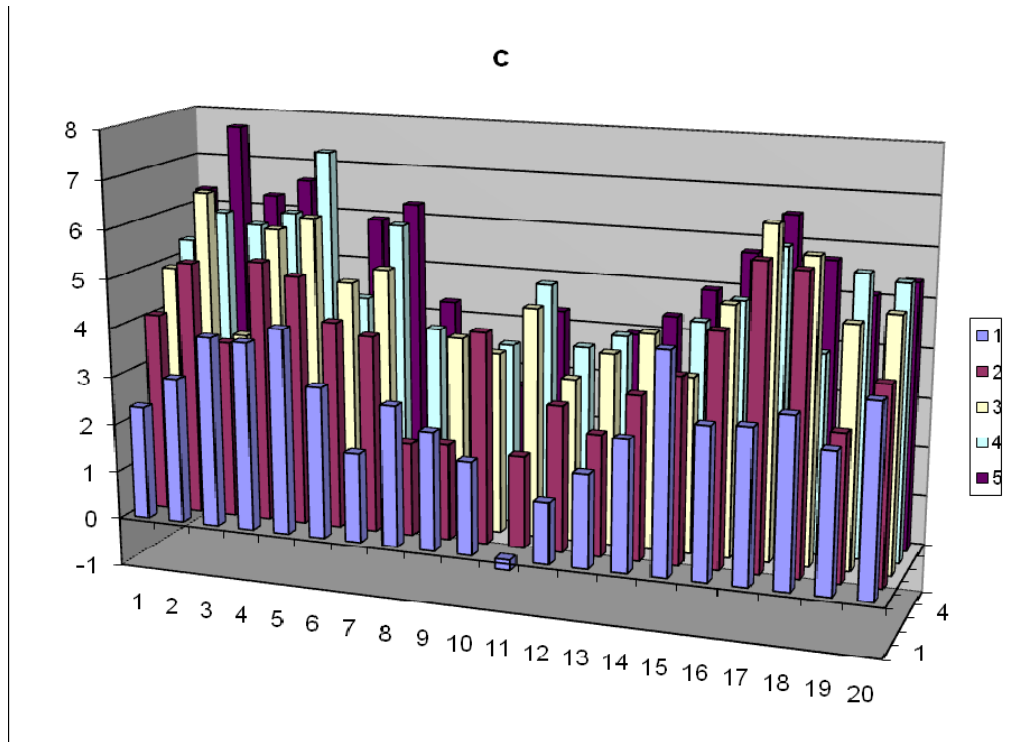
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**Figure 14. Lettuce shoot biomass (g) of plants #1-5 average per tray: a – fresh biomass; b – dry biomass (trays #1-6 – module A, trays #7-14 – module B, trays #15-20 – module C).**

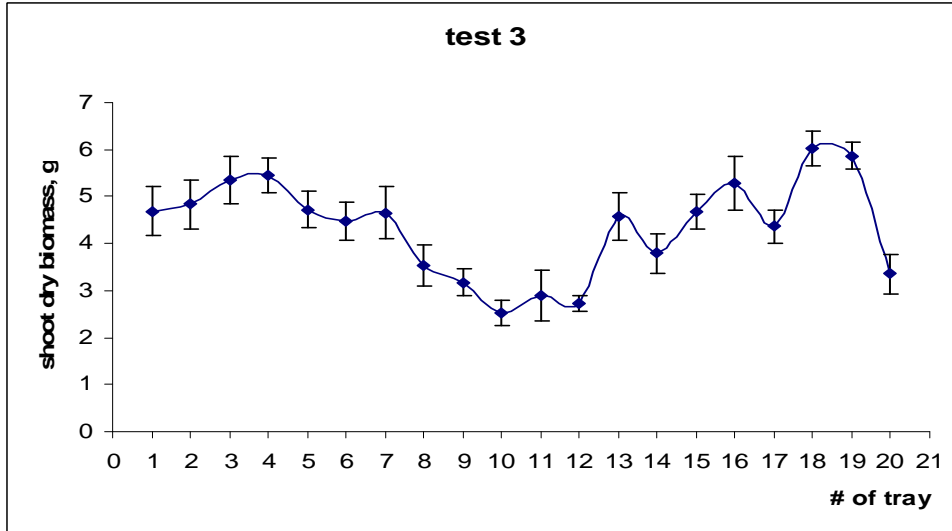
### Third 20-days test (20.05.10-11.06.10)

The third test performed showed the same pattern of growth along the chamber (see Figure 15). As potential explanations for the different behaviour of Module B, the heterogeneity on the temperature profile inside the chamber (Fig. 16) and in the air velocity distribution along the chamber (Fig. 17) were proposed. The confirmation of these hypothesis would require further investigation and eventually additional modifications in the chamber.

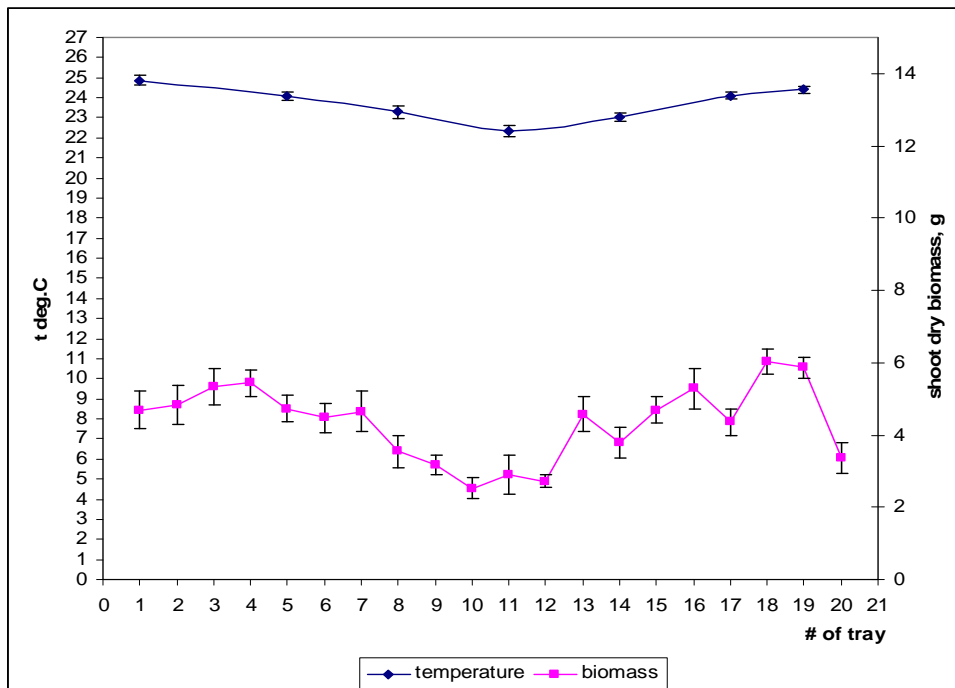


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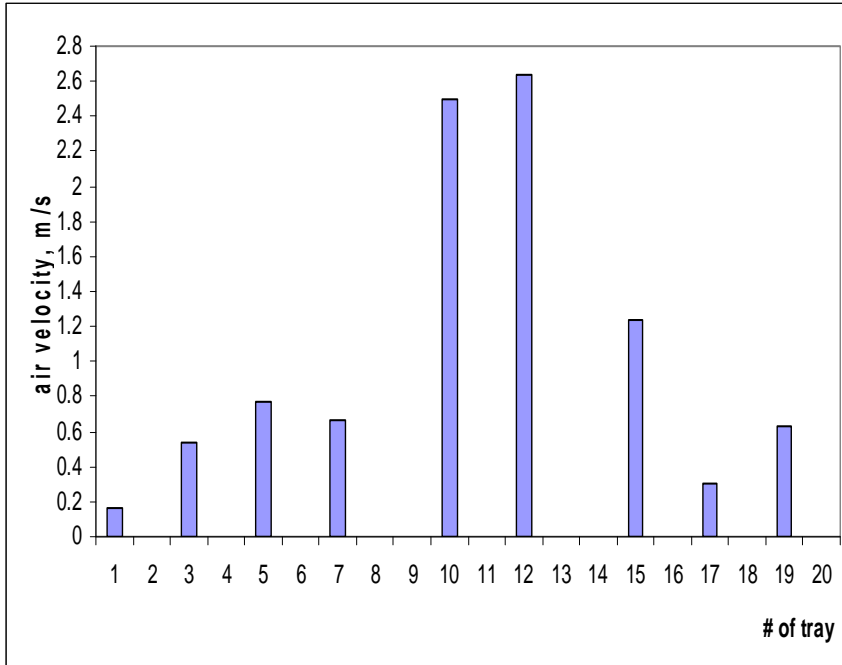


**Figure 15. Lettuce shoot biomass (g) of plants #1-5 average per tray – dry biomass (trays #1-6 – module A, trays #7-14 – module B, trays #15-20 – module C)**

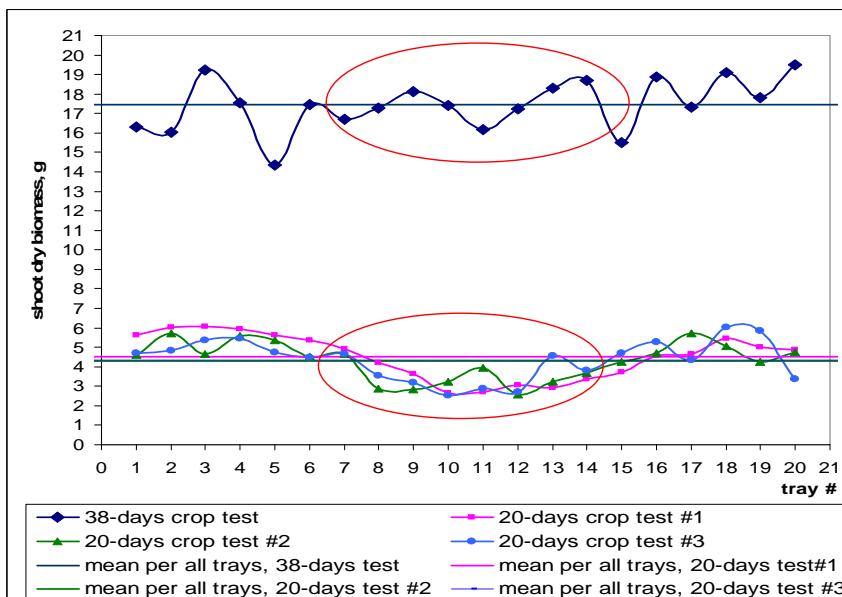


**Figure 16: Temperature values along the chamber (°C) and lettuce shoot dry biomass (g) during the third 20-days test**

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**Figure 17: Air velocity along the chamber (m/s) during the third 20-days test, measured at the outlet of the 9 louvers communicating the plenum with the cultivation area.**



**Figure 18. Lettuce shoot dry biomass (average per tray) after 4 crop tests with different duration of closure**



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The following table summarizes the harvest data and yield data collection for the three first lettuce batch experiments with 20-days duration.

<b>Lettuce Batch Harvest Data</b>	B-20/1	B-20/2	B-20/3	Mean	% Std. dev.
<b>Leaves</b>					
Dry Weight,DW (g dw)	452	427	427	435,3	3,3%
Dry Weight,DW (g dw.plant <sup>-1</sup> )	4.52	4.31	4.40	4.41	2,4%
Plant growth rate (g dw.plant <sup>-1</sup> .d <sup>-1</sup> )	0.23	0.22	0.22	0.22	2,6%
Days in the chamber	20	20	20	20	0
Total plants	100	99	97	99	1,5%
<b>Roots</b>					
Dry Weight,DW (g dw)	98.26	84.32	71.08	84.55	16,1%
Dry Weight,DW (g dw.plant <sup>-1</sup> )	0.98	0.85	0.73	0.850	20,7%
Plant growth rate (g dw.plant <sup>-1</sup> .d <sup>-1</sup> )	0.05	0.04	0.03	0.0400	25,0%
<b>Total</b>					
Dry Weight,DW (g dw.plant <sup>-1</sup> )	5.50	5.16	5.14	5.27	3,8%
Plant growth rate (g dw.plant <sup>-1</sup> .d <sup>-1</sup> )	0.28	0.26	0.26	0.27	4,3%
Total Dry weight (g dw)	550	511	498	519	5,2%
Area Chamber (m <sup>2</sup> )	5	5	5	5	0
Total productivity (g dwm <sup>-2</sup> d <sup>-1</sup> )	5.50	5.11	4.98	5.20	5,2%

**Table 2. Harvest data for lettuce grown in three first batch culture**

In Figure 18, the growth pattern obtained in all the three 20-days batch tests is compared with the growth profile of the 38-days one previously performed. Both Figure 18 and Table 2 evidence that no significant differences were found in lettuce plant growth rate among batch culture replicates. However in the frame of each replicate of 20 days batch culture, significant dispersion of plants biomass along the chamber was observed, showing a clear decrease within Module B, as previously discussed (see Figure 18). This dispersion could be caused by the fluctuation of air velocity along the chamber that varied between 0.2 and 2.7 m/s (measured at the level of the louvers).



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Nevertheless this irregular air flow pattern did not affect the global lettuce growth and the reproducibility of the experiment was rather good (see dispersion values in Table 2, mean data).

Average plant growth rate of lettuce obtained in B-20/1, B-20/2 and B-20/3 was  $0.27 \text{ g dw.plant}^{-1} \text{ d}^{-1}$  (see Table 2) and two times lower in comparison with the B-38 where average plant growth rate was  $0.5 \text{ g dw.plant}^{-1} \text{ d}^{-1}$ . This difference can be explained by the duration of the lettuce growth inside the chamber. Actually, the empirical model for plant growth is the exponential growth function (Massot. A, 2007) which can explain that at 20 days, lettuce are still in the early exponential growth phase whereas at 38 day they are at the late exponential growth phase. On top of that, the growth obtained with 38 days culture did not show any significant decrease within Module B, which has been previously explained by a progressive homogenization of the crops size in the last period of the culture, where the growth rate was probably slowed down in the bigger plants, although this hypothesis cannot be demonstrated.

The average total productivity obtained at 20-d test ( $5.20 \text{ g dw m}^{-2} \text{ d}^{-1}$ ) was also two times lower than the 38-d one ( $9.82 \text{ g dw m}^{-2} \text{ d}^{-1}$ ). However, in the later case, due to a large size of plants, crop density was high and air circulation between the plants was reduced, favouring the conditions for plants rotting. Plant rot led to a number of plants inedible biomass. Moreover, purging of excess oxygen accumulation may have influenced some test results. Reducing the duration of the test to 20-28 days, quite shorter than the 38-days test, allowed not to interrupt the test and gave a better estimation of the carbon balance of the crop which is relevant to characterize the HPC.

Taking into account the above described results with 20-days batch culture tests, a fourth test (B-20/4) with the same duration was performed, but in this case an additional restriction to the ventilation flow was installed in the louvers n° 5 and 6, the ones that provided a higher flow (see Fig. 17), so that the flow distribution was partially balanced.

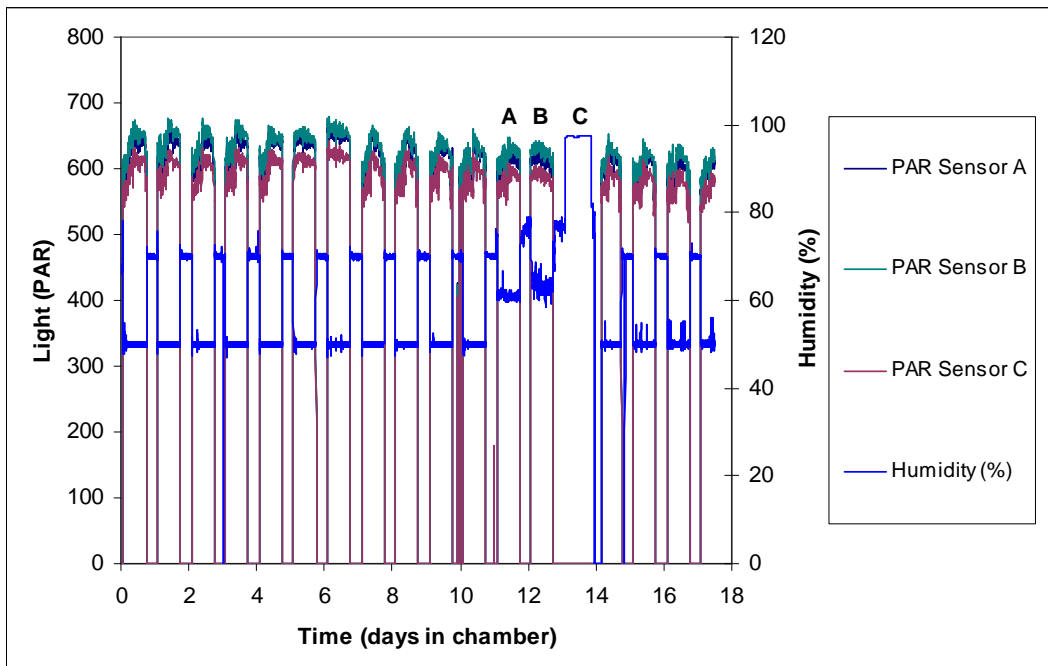
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c) Comparison between batch cultures B-20/1, B-20/2, B-20/3 and B-20/4 harvest data

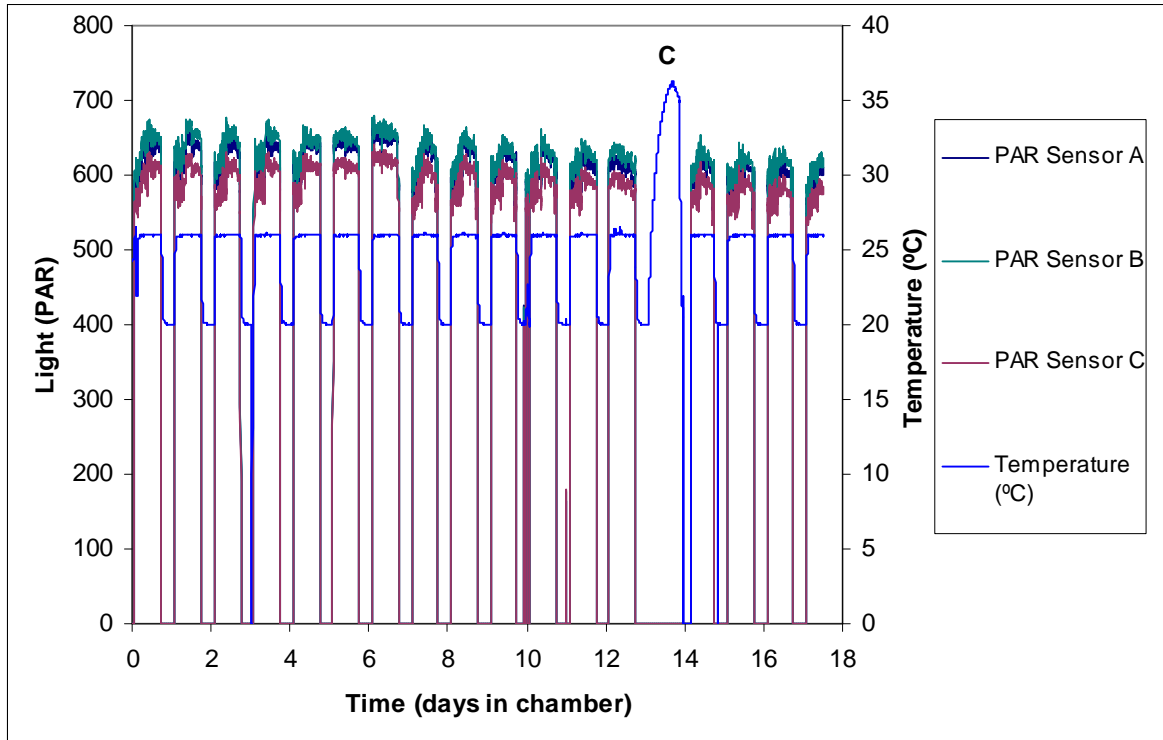
During test B-20/4, relevant anomalies took place between 11<sup>th</sup> and 14<sup>th</sup> days of culture:

- On days 11th and 12th, the cooling machine failed, which affected the proper control of humidity and temperature. Figure 19 (A and B) shows the loss of humidity control caused by this failure, and the corresponding effect on the chamber temperature is shown in Fig. 20.
- A power failure occurred on day 13<sup>th</sup>, as shown in Figure 19 and 20 (C), with the consequence of switching off the lamps, the blower and the cooling machine. During 17h, light was off and temperature and relative humidity in the chamber increased up to 36°C and 98% respectively. The main issue here is that the light was off so no photosynthesis or growth could occur and, on top of that, the stomata cannot open. As a consequence, since the stomata are closed, the plant has no way to transpire, photosynthesis and cool down, therefore heat could build up and bring irreversible tissue damages and/or protein denaturation.

**Figure 19 .Humidity control in the HPC over 18 day period of closure at daytime and nighttime**



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**Figure 20. Temperature control in the HPC over 18 day period of closure at daytime and nighttime**

Table 3 summarizes the harvest and yield data for the four 20 days lettuce batch cultures. The mean of the three 20 day tests is compared with the data corresponding to the test B-4. As it can be seen from Table 3, relevant differences in the dry weight, plant growth rate and productivity value of lettuce plants grown in the last crop test compared with the three others was observed. Productivity of plants grown in the three repetitive tests (B-20/1; B-20/2; B-20/3) was 1.3 times higher in comparison with the last test (B-20/4). This difference can be explained by the anomaly in the system that did not allow the plant to grow well (3.2.1). This test was stopped two days in advance (18 days) than the others (20 days) which is an additional factor to explain the low results obtained in B-20/4.



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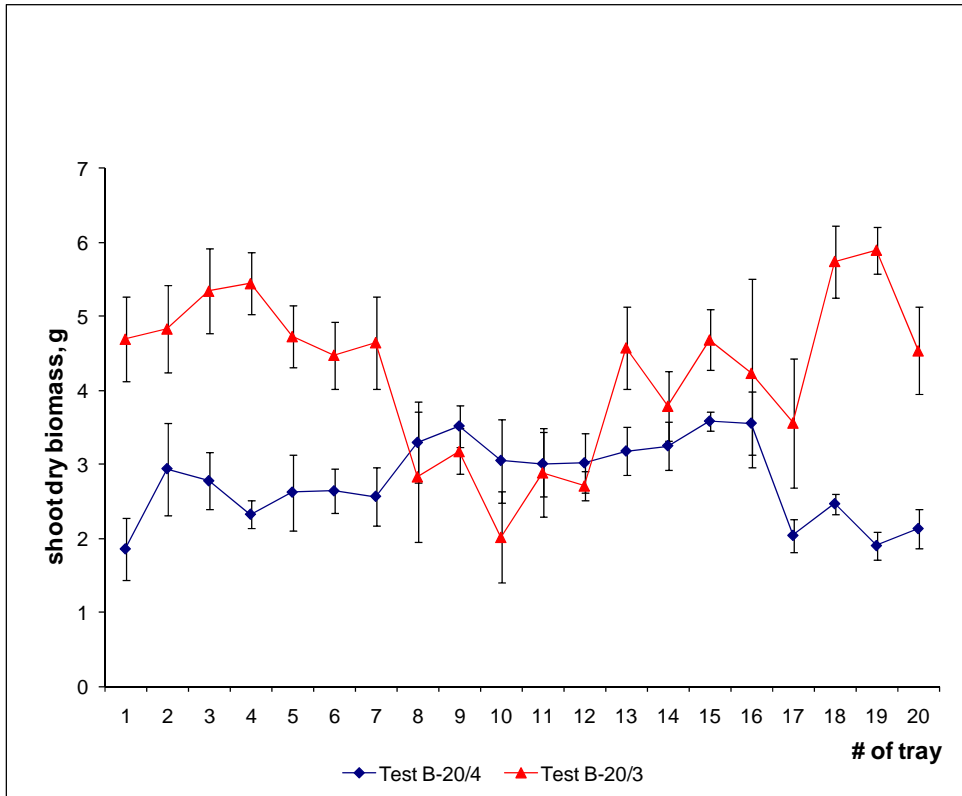
Lettuce Batch Harvest Data	Mean of B-20/1, 20/2 and 20/3	B-20/4
<b>Leaves</b>		
Dry Weight,DW (g dw)	435 ± 14	279
Dry Weight,DW (g dw.plant <sup>-1</sup> )	4.41 ± 0.1	2.79
Plant growth rate (g dw.plant <sup>-1</sup> .d <sup>-1</sup> )	0.22 ± 0.01	0.15
Days in the chamber	20	18
Total plants	99	100
<b>Roots</b>		
Dry Weight,DW (g dw)	84.55 ± 13.6	64.86
Dry Weight,DW (g dw.plant <sup>-1</sup> )	0.850 ± 0.1	0.65
Plant growth rate (g dw.plant <sup>-1</sup> .d <sup>-1</sup> )	0.0400 ± 0.01	0.04
<b>Total</b>		
Dry Weight,DW (g dw.plant <sup>-1</sup> )	5.27 ± 0.2	3.43
Plant growth rate (g dw.plant <sup>-1</sup> .d <sup>-1</sup> )	0.27 ± 0.01	0.19
Total Dry weight (g dw)	519 ± 27	343
Area Chamber (m <sup>2</sup> )	5	5
Total productivity (g dwm <sup>-2</sup> d <sup>-1</sup> )	5.20 ± 0.3	3.82

**Table 3. Harvest data for lettuce grown in batch culture B-20/1-3 and B-20/4**

However, the distribution of growth along the chamber in the test B-20/4 was clearly more homogeneous than the pattern obtained in the previous tests (see Figure 21), what is coherent with the fact that the ventilation flow pattern was improved by means of the modification of the louvers, as previously discussed.



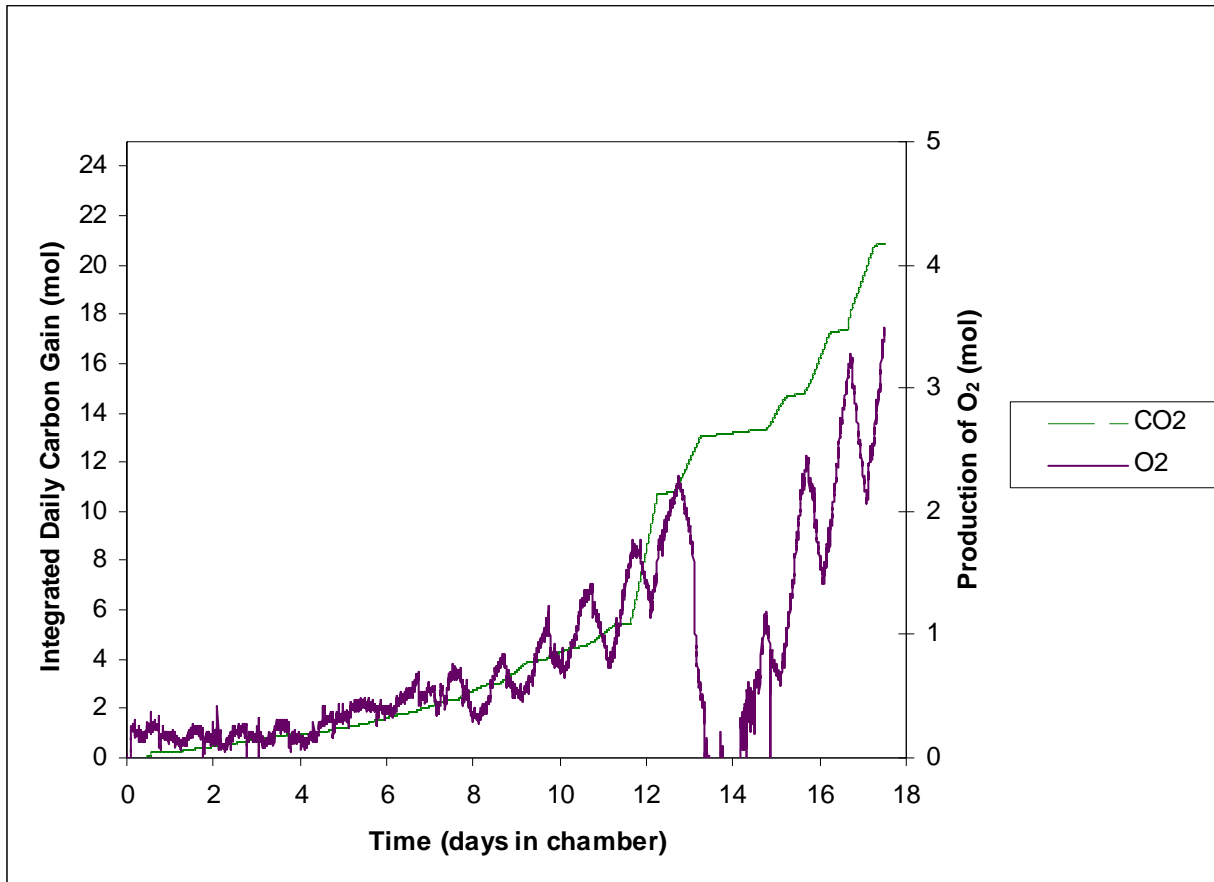
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**Figure 21. Shoot dry biomass distribution along the chamber in the tests performed before and after the louvers modification**

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## 11.2.2 CO<sub>2</sub>/O<sub>2</sub> balance of test B-20/4



**Figure 22. CO<sub>2</sub> and O<sub>2</sub> (IDCG and O<sub>2</sub> produced) in HPC over the 18 day period of closure at daytime and nighttime**

Figure 22 shows the Integrated Daily Carbon Gain (IDCG), which represents the accumulated CO<sub>2</sub> injected during the plant growth process and the accumulated O<sub>2</sub> produced by the plant above nominal condition (20,9%) versus days in the chamber. Results point well the anomalies between the thirteen and fourteen day of the crop. Since the dark period lasted 17 hour, the CO<sub>2</sub> concentration increased due to respiration since was not possible to remove CO<sub>2</sub> from the chamber, CO<sub>2</sub> measurement reached 2000 ppm inside the chamber, and the absence of light interrupted the photosynthetic process of plant that explain the significant decrease of the O<sub>2</sub> production.



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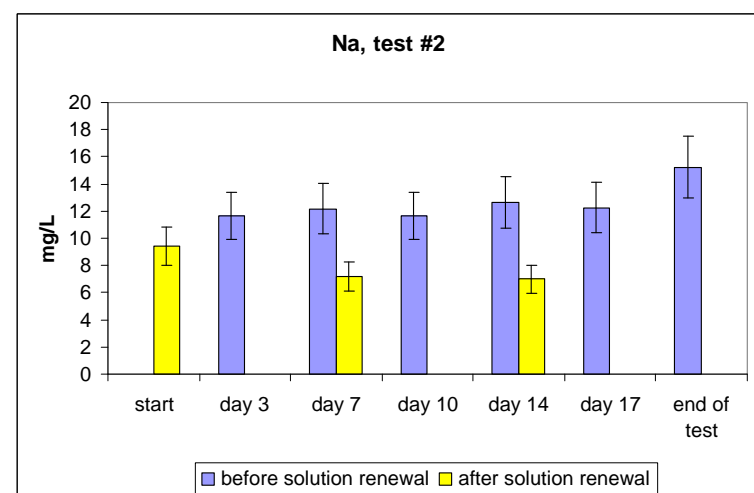
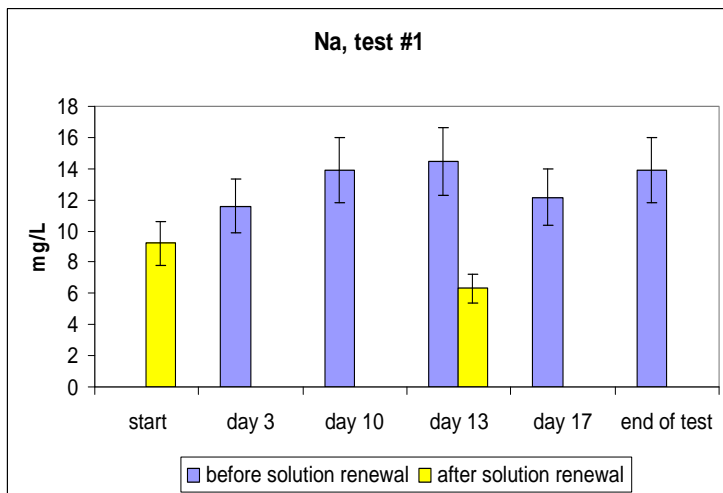
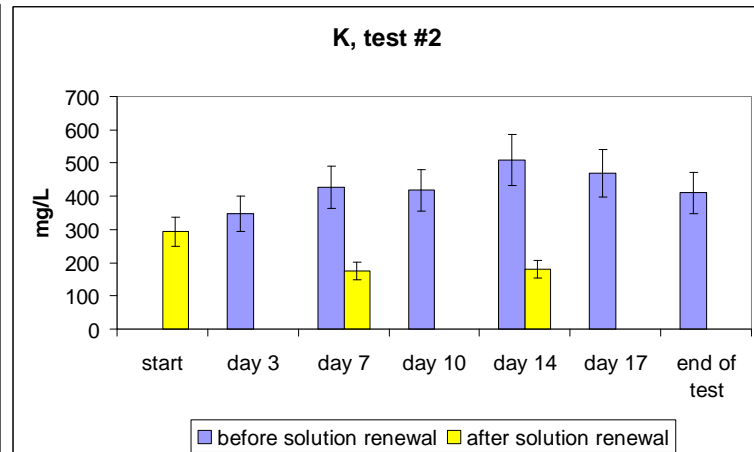
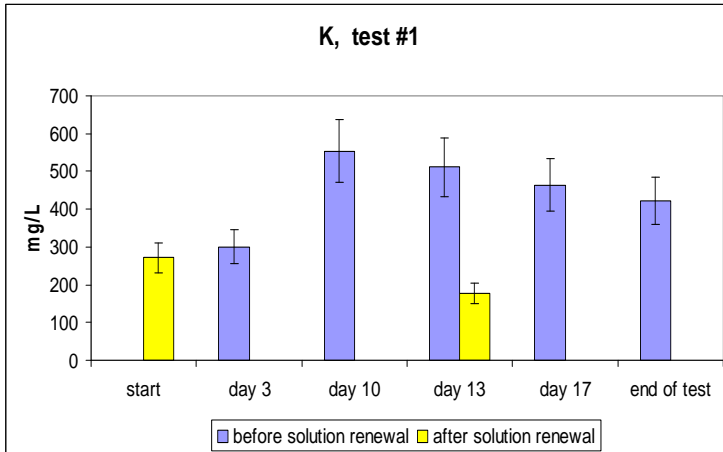
### 11.2.3 Nutrient solution composition and plant mineral content

The potential accumulation or depletion of nutrients in the hydroponic solution was evaluated along the 20-days culture. The results of mineral analysis regarding K and Na in the first two tests are shown in Figure 22.

As it can be deduced from this Figure, the concentration of K and Na measured at different moments after changing the solution are higher than the initial ones, indicating a certain accumulation of these ions. Therefore, the nutrients accumulation in the HPC1 nutrient solution would require an improvement of the nutrient solution feeding/control strategy in order to:

- Prevent plant growth inhibition (e.g. definition of limiting/inhibitory/toxicity levels)
- Decrease mineral accumulation (optimisation of the nutrient solution composition and composition control)
- Minimise operations

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**Figure 22: K and Na concentration in the nutrient solution at different periods of 20-days crop test (mg/L)**

**NOTE: Tests #1 and #2 stand for Tests B-20/1 and 20/2**



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Regarding the mineral composition found in the crops, the results of the analysis performed on the shoots are shown in Table 4. These results are in average quite similar to the ones already obtained for the 38-days batch culture (RD1) and are as well quite close to the ones obtained by Masot (RD3) and fall within the range between the values obtained in field cultures and the ones reported by other authors for hydroponic cultures (Table 5).

Treatment	Na	K	Ca	P	S	Mg	Fe	Si	B	Mn	Zn	Cu	Mo	N
Test #1	0.03	7.6	1.0	0.9	0.4	0.2	0.02	0.01	0.002	0.009	0.007	0.001	0.0003	5.0
Test #2	0.02	7.1	0.9	0.8	0.3	0.2	0.02	0.01	0.002	0.009	0.007	0.001	0.0002	5.3
<b>Average of 2 tests</b>	0.03	7.3	0.9	0.9	0.4	0.2	0.02	0.01	0.002	0.009	0.007	0.001	0.0003	5.2

**Table 4. Mineral composition of lettuce shoot (% dw), harvested at the end of vegetation**

	K	Ca	Mg	P	N
<b>HPC1, 20 days tests</b>	<b>7.3</b>	<b>0.9</b>	<b>0.2</b>	<b>0.9</b>	<b>5.2</b>
<b>McKeehen, 1994</b>	<b>8.2</b>	<b>0.6</b>	<b>0.2</b>	<b>0.6</b>	<b>4.5</b>
<b>Wheeler et al., 1994</b>	<b>17.0</b>	<b>0.9</b>	<b>0.3</b>	<b>0.4</b>	<b>4.8</b>
<b>Masot et al., 2007</b>	<b>7.9</b>	<b>1.2</b>	<b>0.3</b>	<b>1.0</b>	<b>5.5</b>
<b>Field</b>	<b>3.9</b>	<b>0.7</b>	<b>0.2</b>	<b>0.6</b>	<b>3.0</b>

**Table 5. Content of main minerals in lettuce leaves, (% dw) in filed and hydroponic cultures**

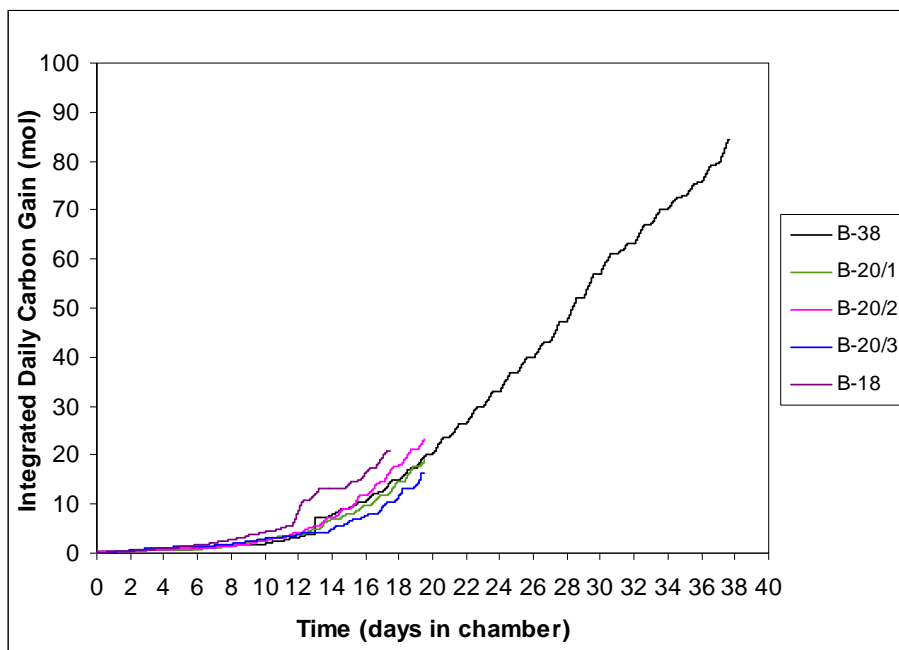


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## 11.2.4 MASS BALANCE

### a) Carbon balance

The photosynthetic response data give a prediction of the control of carbon exchange in the HPC which is relevant to know the good functionality of plant process (photosynthesis) and to estimate the O<sub>2</sub> production. Some authors have used an approximate photosynthetic quotient (mol O<sub>2</sub>/mol CO<sub>2</sub>) of 1 (RD2, RD4), however a quotient of 1 is generally only found under ideal conditions in isolated chloroplasts. Actual net oxygen production can be substantially reduced due to respiration requirements, cultivar differences, cultural practices, and microbial activity. As O<sub>2</sub> quantity is prominent for the survival of the crew in the loop, characterizing oxygen production for a variety of crops under various cultivation techniques is an important aspect of future study. The following graph shows the Integrated Daily Carbon Gain (IDCG) profile of the five lettuce batch experiments.



**Figure 23. Integrated Daily Carbon Gain (IDCG) for lettuce batch culture (B-38 – 38 days test-, B-20/1; B-20/2; B-20/3, and B-18 –equal to B-20/4-)**



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The IDCG which is also known as Net Carbon Exchange Rate (NCER) is the accumulation of CO<sub>2</sub> at the end of the batch culture. It contributes to estimation of biomass production (DW) with knowledge of carbon content and to the assessment of the CO<sub>2</sub> balances in the HPC.

Table 6 summarizes the carbon balance results obtained in the lettuce batch corrected by the estimated leak value (4.37 % per day or 2µmol in the HPC per day)

Lettuce Batch Harvest Data	B-38	B-20/1	B-20/2	B-20/3	B-20/4
CO <sub>2</sub> consumption (mol)	84	19	23	16	21
C from CO <sub>2</sub> consumption (g)	1008	228	276	192	252
Carbon content (%)	40	39	40	42	42
C from DW at harvest (g)	690	176	171	180	117
C Balances (%)	68	77	62	93	46

**Table 6. Comparison between estimated biomass from CO<sub>2</sub> injection and carbon content in biomass harvested**

The harvested dried biomass (DW) carbon value is in general lower than the potential C production based on the CO<sub>2</sub> injected. Some of the possible reasons are the lack of accuracy in the calibration of CO<sub>2</sub> metered injection in the beginning of the first batch culture and the insufficient tightness of the nutrient tank in the second batch culture after the nutrient solution change-over. B-20/3 shows a better result due to the improvement of calibration techniques and operation by the MPP personal.

B-20/4 presented some events in the evolution of the culture (already described) that made necessary to open the chamber in order to recover the nominal humidity and temperature conditions as soon as possible. This fact at least made not possible to obtain a correct value of the C balance.



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## b) Nitrogen balance

Nitrogen is the nutrient which is consumed by lettuce at the highest rate. That is why this nutrient was chosen for the mass balance analysis among the batch cultures. Besides it is the main nutrient produced by CIII in MELiSSA loop which feeds CIVa and CIVb. Nitrogen mass balance was determined by comparing the accumulated nitrogen (total nitrogen consumed by plant) as obtained through analysis of the hydroponics solution at every change over the period with the total nutrient content in biomass obtained from tissue analysis at the harvesting. The accumulated Nitrogen uptake in moles comprise also the Stock A/B and nitric acid volume injected at every change over.

<b>Lettuce Batch Harvest Data</b>	<b>B-20/3</b>	<b>B-20/4</b>
N total consumed by plant (mol)	2.1	1.2
N from DW at harvest (g) in roots	2.1	1.9
N from DW at harvest (g) in shoot	23.1	14.7
Nitrogen content (%) in roots	3	3
Nitrogen content (%) in shoot	5	5
N total measured in harvested plants (mol)	1.8	1.2
N Balances (%)	87	100

**Table 7. Comparison between estimated biomass from accumulated nitrogen uptake and nitrogen content in the harvested biomass**

Nitrogen mass balance of 20 days batch culture showed some deviation which could be explained by several factors that took place during the experiments such as some leaks in Acid and Base reservoirs. Besides it was quite difficult to separate completely lettuce roots from the Rockwool substrate. For this reason there was a loss of some data on nitrogen accumulation in lettuce roots. Moreover, it is necessary to mention that microorganisms and algae could consume some of the nutrient. Also not 100% mass balance of N could be connected with errors in analytical systems for analysis of nutrient solution composition or mineral composition of plants tissues. All those factors could lead to non-exact results.





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## 12. Conclusions

1. The first series of lettuce batch cultures in the HPC1 of the MPP served to acquire relevant knowledge on the operation of the system and to have a first set of data on its operation at two operating conditions, ie, short (20 days) and long (38 days) plant growing period within the chambers. Such a characterization was done for *Lactuca sativa* L. cv. Grand Rapids cultivation inside the HPC.
2. The test at 20 days was performed in triplicate to analyse the reproducibility of the tests, and the growth pattern in the three batches was quite similar.
3. Regarding gas exchange, it was observed that the IDCG evolution is a good method for on-line estimation of plant growth and dry weight production inside the chamber without using destructive analysis. However, it is stressed that a proper calibration of instruments, and the determination of the leakage rate are very important for achieving accurate data on plant biomass values based on CO<sub>2</sub> data.
4. Nitrogen uptake from nutrient solution has demonstrated the possibility to estimate biomass, since nitrogen nutrient content present in the harvest biomass matches with it.
5. In order to maintain life in a closed environmental system, the O<sub>2</sub> production should be relatively constant to be distributed uniformly to the crew. Staggered production systems should provide a more stable output of O<sub>2</sub>, H<sub>2</sub>O and food once a “steady state” is achieved. This is in contrast to batch production systems, where an entire crop is harvested at a single time. Nevertheless, most of the batch culture data obtained in the batch tests will be useful



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inputs for the characterization of the staggered production within the HPC, which is the aim of the future experiments in the HPC.

6. Environmental control parameters were validated for the batch cultures performed at 20 days of duration, and high air velocity in specific area of HPC1 was detected, that may have affected plant growth in this area. The improvement of air distribution in the chamber to equilibrate this area might increase plants production by approximately 20 % or more.
7. Crop test performance during 38 days resulted in relatively high biomass production of lettuce and oxygen production, and apparently there was a positive evolution with time of the canopy with respect to the unfavourable environmental conditions, still to be investigated; however, a decrease of plants edible biomass value was observed.
8. Increase of test duration from 20 to 30 days would allow a considerable increase in biomass and oxygen production and radiation conversion efficiency. Taking into account the results obtained with 20 and 38 days of culture, an intermediate duration like 28-30 days will probably be the optimal for the lettuce cultures in the HPC, providing a balance among the desired parameters: oxygen production, biomass production and quality, etc. so this will be proposed for the staggered tests. However, maybe some measures should be taken for oxygen removal from the chamber when higher concentration would be achieved, in order to avoid any potential risk.
9. The level of minerals accumulated in the lettuce grown in HPC1 was within the normal values compared to the published data from other work on lettuce plants grown in hydroponics solution.



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10. Nutrients accumulation in the HPC1 nutrient solution indicated that in order to prevent plant growth inhibition and optimize both composition and operations, it is required to renew regularly the nutrient solution or to modify the feeding strategy in order to correct the different nutrient levels.
  
11. Preliminary modification in the louvers to improve air distribution homogeneity along the chamber by balancing the air flow velocity in the modules resulted in increased crop growth homogeneity along the chamber, although the results should be confirmed with further measurements.



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## 13. Annexes

### Annex 1. Raw data of the crops weight and C content

Fresh and dry weight of lettuce, harvested on 1.02.2010

# of tray	# of plant	Fresh weight of shoots, g 1.02.2010	Dry weight of shoots, g 10.02.2010	Weight of dry roots*, g 10.02.2010
1	1	83,1	4,64	0,84
	2	110,32	5,52	0,98
	3	105,93	5,35	1,09
	4	120,56	6,19	1,31
	5	121,42	6,39	1,26
2	1	87,13	5,24	1,2
	2	101,65	5,59	0,79
	3	116,93	6,43	0,96
	4	106,33	4,97	1,17
	5	142,66	7,8	1,41
3	1	80,06	5,04	1,1
	2	106,17	5,62	1,14
	3	111,79	5,79	1,72
	4	144,92	7,42	1,21
	5	119,76	6,46	1,44
4	1	99,87	5,8	1,38
	2	108,85	6,03	0,7
	3	104,73	5,68	1,14



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	4	108,85	5,53	1
	5	111,76	6,68	1,57
5	1	73,94	4,59	1,21
	2	85,93	4,83	0,9
	3	70,23	7,23	1,32
	4	121,43	6,53	0,71
	5	77,96	5,04	1,02
6	1	77,35	4,83	1,4
	2	79,32	4,52	0,95
	3	91,4	5,08	0,91
	4	116,52	6,08	1,71
	5	102,12	6,19	1,63
7	1	63,5	4,18	1,22
	2	88,01	5,04	1,34
	3	77,14	4,25	0,73
	4	106,21	6,06	1
	5	80,36	4,97	0,9
8	1	50,26	3,24	0,64
	2	71,64	4,09	0,91
	3	78,86	4,74	0,4
	4	64,76	3,83	0,8
	5	84,66	5,19	1,5
9	1	60,11	3,75	0,53
	2	75,53	4,37	0,85
	3	69,03	3,7	0,64
	4	43,88	2,76	0,47
	5	64,64	3,56	0,9
10	1	42,85	2,62	0,88
	2	41,61	2,72	0,57



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	3	49,66	3,08	1,31
	4	35,02	2,31	0,63
	5	33,46	2,5	0,79
11	1	29,78	2	0,86
	2	33,07	2,05	0,66
	3	51,78	3,2	0,89
	4	50,78	3,07	0,76
	5	51,75	3,25	0,73
12	1	4,02	0,32	0,93
	2	62,25	3,54	0,68
	3	58,81	3,25	NA
	4	73,3	3,89	0,9
	5	67,23	4,38	0,92
13	1	39,2	2,45	0,83
	2	49,79	3,1	0,67
	3	45,86	2,79	0,69
	4	52,77	3,08	1,42
	5	48,57	3,2	0,71
14	1	34,91	1,98	0,65
	2	49,33	2,58	0,57
	3	73,49	4,25	0,89
	4	66,15	3,94	0,93
	5	65,76	4,09	0,62
15	1	75,14	4,45	1,19
	2	57,35	3,66	0,72
	3	74,88	4,11	0,76
	4	58,11	3,29	0,7
	5	48,2	3,2	0,78
16	1	63,11	3,96	0,83



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	2	76,18	4,43	0,98
	3	85,71	4,75	0,87
	4	83,43	4,24	1,03
	5	89,52	5,45	1,33
	17	1	38,15	2,74
2		77,01	4,37	0,95
3		69,83	3,46	1,22
4		116,4	6,25	1,09
5		110,63	6,35	1,4
18	1	80,66	4,83	1,23
	2	87,03	4,75	0,82
	3	100,46	5,43	1,2
	4	99,4	5,51	1,57
	5	121,26	6,75	1,41
19	1	66	3,89	0,76
	2	79	4,05	0,96
	3	99,46	4,99	0,82
	4	99,17	5,09	0,84
	5	131,26	6,95	1,38
20	1	67,14	3,32	0,97
	2	111,59	5,79	0,66
	3	122,13	5,75	0,75
	4	88,35	4,22	1,18
	5	99,04	5,39	1,02

\*includes some Rockwool particles



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Fresh and dry weight of lettuce, harvested on 1.04.2010

# of tray	# of plant	Fresh weight of shoots, g 1.04.2010	Dry weight of shoots, g	Dry weight of roots, g
1	1	52,56	2,35	-0,24
	2	102,24	4,1	0,11
	3	121,43	4,93	0,25
	4	120,76	5,38	0
	5	133	6,33	0,25
2	1	62,21	3,01	-0,17
	2	105,78	5,23	0,27
	3	130,44	6,54	0,4
	4	128,61	6,01	0,2
	5	155	7,68	0,19
3	1	71,02	3,93	0,24
	2	66,93	3,65	0,16
	3	83	3,63	-0,05
	4	121,86	5,8	0,17
	5	117,24	6,28	0,4
4	1	71,43	3,89	0,23
	2	105,91	5,36	0,24
	3	126,23	5,89	0,3
	4	123,96	6,09	0,44





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	5	122,57	6,64	0,02
5	1	81,39	4,23	0,46
	2	105,61	5,12	0,15
	3	124,84	6,17	0,31
	4	154,92	7,38	0,29
	5	67,89	3,81	0,08
6	1	63,37	3,11	-0,08
	2	82,52	4,25	0,33
	3	95,93	4,9	0,81
	4	89,94	4,43	0,51
	5	112,68	5,92	0,36
7	1	38,3	1,83	0,61
	2	80,5	4,03	0,16
	3	107,39	5,22	0,2
	4	114,63	5,98	0,34
	5	116,92	6,28	0,24
8	1	50,53	2,88	-0,14
	2	37,18	1,9	0,21
	3	33,98	1,46	0,29
	4	77,83	3,9	0,19
	5	77,58	4,3	0,67
9	1	47,31	2,43	0
	2	40,1	1,96	-0,14
	3	80,79	3,95	0,41
	4	69,29	3,25	0,1
	5	50,3	2,57	0,32
10	1	41,27	1,9	0,13
	2	82,89	4,3	0,06
	3	75,14	3,7	0,04



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	4	74,59	3,7	-0,09
	5	50,34	2,66	-0,07
11	1	6,89	-0,21	-0,49
	2	40,77	1,87	-0,17
	3	88,28	4,67	0,2
	4	101,81	4,99	0,07
	5	80,97	4,29	0,01
12	1	27,71	1,24	-0,29
	2	58,01	2,97	-0,06
	3	62,86	3,31	-0,05
	4	70,05	3,78	-0,03
	5	27,31	1,57	-0,31
13	1	37,61	1,9	-0,24
	2	49,36	2,47	0,03
	3	76,08	3,89	0,12
	4	83,6	4,07	-0,04
	5	71,56	3,93	0,18
14	1	51,66	2,66	-0,12
	2	66,1	3,32	-0,03
	3	86,96	4,35	0,11
	4	65,53	3,67	-0,08
	5	81,36	4,36	0,08
15	1	82,07	4,46	0,04
	2	77,26	3,77	0,18
	3	71,31	3,56	-0,15
	4	90,69	4,48	0,08
	5	91,09	4,95	0,04
16	1	62,62	3,08	-0,22
	2	97,23	4,7	-0,07



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	3	102,32	5,04	0,31
	4	110,91	4,96	0,54
	5	112,32	5,75	0,28
17	1	66,19	3,13	-0,19
	2	123,54	6,09	-0,13
	3	135,78	6,64	0,26
	4	123,72	6,07	0,5
	5	123,71	6,55	0,23
18	1	65,79	3,43	0,08
	2	121,4	5,96	0,11
	3	137,01	6,09	-0,27
	4	79,62	4,04	0,13
	5	106,35	5,71	0,25
19	1	62	2,82	-0,06
	2	63,76	2,96	0,05
	3	100,99	4,85	0,06
	4	126,02	5,66	0,05
	5	97,51	5,08	-0,09
20	1	80,06	3,84	0,14
	2	97,79	3,96	-0,01
	3	120,21	5,09	-0,1
	4	125,64	5,53	0,03
	5	103,69	5,39	-0,01



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Fresh and dry weight of lettuce, harvested on 11.06.2010

# of tray	# of plant		Fresh weight of shoots, g 11.06.2010	Dry weight of shoots, g	Dry weight of roots, g
1	1		74,96	4,15	0,94
	2		96,44	4,54	0,95
	3		104,22	4,49	1,41
	4		85,76	3,63	0,75
	5		126,9	6,65	0,88
2	1		114,06	5,7	0,81
	2		87,89	4,07	0,35
	3		98,06	3,46	0,64
	4		118,15	4,59	0,84
	5		120,11	6,33	1,33
3	1		81,75	4,23	1,05
	2		93,3	4,8	1,02
	3		109,93	5,07	0,72
	4		110,49	5,36	0,66
	5		138,79	7,24	1,49
4	1		81,08	4,6	1,13
	2		116,44	5,98	1,07
	3		120,01	6,12	1,16
	4		111,92	6,05	1,28
	5		89,43	4,46	0,81
5	1		83,7	4,41	1,46
	2		102,58	4,04	1,33



# MELiSSA Pilot Plant

**UAB**

Universitat Autònoma  
de Barcelona

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	3		87,88	3,95	0,75
	4		108,75	5,58	0,87
	5		95,06	5,66	1,4
6	1		88,83	3,72	0,97
	2		80,08	4,26	0,72
	3		89,03	3,57	0,60
	4		98,85	5,16	1,16
	5		103,96	5,67	1,36
7	1		67,58	3,51	0,66
	2		84,38	3,43	0,71
	3		88,88	4,6	1,01
	4		106,5	5,34	1,14
	5		108,71	6,35	1,47
8	1		62,64	2,54	0,52
	2		76,5	4	0,83
	3		89,15	3,12	0,91
	4		85,51	4,52	0,97
	5		0	0	0
9	1		63,06	3,39	0,77
	2		63,79	3,05	0,75
	3		79,3	3,97	1,53
	4		63,11	3,21	0,71
	5		44,39	2,26	0,86
10	1		50,23	2,56	0,53
	2		51,71	2,26	0,57
	3		59,53	1,98	0,74
	4		62,84	3,29	0,98
	5		0	0	0
11	1		24,20	0,91	0,24



# MELiSSA Pilot Plant

Document Identification : Test report for the characterization of lettuce batch cultures in HPC1	Type	Number	Issue	Page : 62 / 68
	TN	101.3	(0)	

	2		50,8	2,93	0,52
	3		55,86	3,1	0,66
	4		61,96	3,42	0,42
	5		70,02	4,09	0,45
12	1		41,15	2,28	0,67
	2		54,61	2,71	0,43
	3		50,84	2,56	0,52
	4		62,68	3,32	0,36
	5		45,85	2,69	0,28
13	1		60,52	3,11	0,63
	2		80,26	6,16	0,75
	3		78,63	4,21	0,38
	4		88,96	4,95	0,62
	5		90,82	4,45	0,92
14	1		33,4	2,48	0,37
	2		54,6	4,09	0,26
	3		72,95	3,18	0,5
	4		85,66	4,43	0,53
	5		85,88	4,76	0,31
15	1		69,07	4,16	0,49
	2		86,12	4,54	0,4
	3		81,36	4,36	0,36
	4		120,07	6,12	0,71
	5		82	4,23	0,64
16	1		0	0	0
	2		80,78	4,04	0,41
	3		100,14	5,18	0,59
	4		113,89	5,15	0,72
	5		128,68	6,79	1,01



# MELiSSA Pilot Plant

Document Identification : Test report for the characterization of lettuce batch cultures in HPC1	Type	Number	Issue	Page : 63 / 68
	TN	101.3	(0)	

17	1		81,27	4,19	0,7
	2		79,76	3,98	0,43
	3		95,43	5,51	0,76
	4: 2 plants in one cube	plant 1 = 24,78	<b>94,91</b>	0,76	0,51
		plant 2 = 70,13		3,37	
5		88,23	4,76	0,6	
18	1		100,03	5,15	0,66
	2		109,3	5,37	0,66
	3		143,86	7,19	0,64
	4		132,41	6,24	0,62
	5		126,53	6,2	0,49
19	1		95,98	4,89	0,5
	2		89,17	6,35	0,32
	3		129,98	6,39	0,53
	4		112,75	5,64	0,51
	5		105,33	6,05	0,32
20	1		72,65	3,03	0,38
	2		93,4	4,11	0,58
	3		96,29	4,13	0,33
	4		114,95	5,36	0,52
	5: 2 plants in one cube	plant 1 = 53,71	<b>76,89</b>	2,18	0,3
	plant 2 = 23,18	0,97			



# MELiSSA Pilot Plant

Document Identification : Test report for the characterization of lettuce batch cultures in HPC1	Type	Number	Issue	Page : 64 / 68
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Fresh and dry weight of lettuce, harvested on 13.07.2010

# of tray	# of plant	Fresh weight of shoots, g 23.07.2010	Dry weight of shoots, g	Dry weight of roots, g
1	1	59,72	0,5	0,1
	2	48,16	1,76	0,13
	3	50,77	2,2	0,09
	4	41,18	2,1	0,12
	5	31,14	2,72	0,02
2	1	58,49	1,17	0,12
	2	86,02	4,12	0,26
	3	48,07	2,18	0,12
	4	69,61	3,94	0,11
	5	27	3,29	0
3	1	55,09	2,64	0,27
	2	51,23	1,62	0,25
	3	68,35	3,41	0,2
	4	42,76	2,65	0,23
	5	54,49	3,56	0,18
4	1	46,02	2,93	0,08
	2	45,51	2,36	0,1
	3	45,52	2,18	0,12
	4	55,23	1,89	0,18
	5	53,14	2,26	0,29





# MELiSSA Pilot Plant

Document Identification : Test report for the characterization of lettuce batch cultures in HPC1	Type	Number	Issue	Page : 65 / 68
	TN	101.3	(0)	

5	1	71,08	1,57	0,28
	2	65,06	3,17	0,19
	3	70,21	1,47	0,25
	4	70,56	3,29	0,23
	5	34,17	3,61	0,26
6	1	56,76	2,11	0,33
	2	67,82	2,43	0,17
	3	39,69	2,16	0,21
	4	51,99	3,51	0,2
	5	43,6	2,99	0,16
7	1	55,15	1,91	0,15
	2	69,43	1,93	0,16
	3	48,5	2,13	0,16
	4	43,65	3,32	0,19
	5	40,08	3,54	0,13
8	1	51,46	2,78	0,15
	2	75,48	4,91	0,54
	3	45,93	2,19	0,12
	4	92,31	3,88	0,37
	5	50,92	2,71	0,17
9	1	73,88	3,51	0,23
	2	70	2,69	0,21
	3	73,56	3,94	0,39
	4	74,69	3,36	0,24
	5	67,71	4,08	0,3
10	1	67,13	1,84	0,14
	2	88,21	2,37	0,49
	3	64,33	2,96	0,14
	4	48,75	4,83	0,09



# MELiSSA Pilot Plant

Document Identification : Test report for the characterization of lettuce batch cultures in HPC1	Type	Number	Issue	Page : 66 / 68
	TN	101.3	(0)	

	5	34,28	3,25	0,07
11	1	77,60	2,3	0,45
	2	83,62	3	0,39
	3	64,97	1,98	0,31
	4	61,91	3,77	0,39
	5	47,86	3,98	0,18
12	1	72,38	2,25	0,08
	2	71,28	2,14	0,35
	3	62,27	3,1	0,24
	4	43,15	3,79	0,15
	5	58,22	3,81	0,4
13	1	67,15	3,74	0,21
	2	64,88	2,87	0,4
	3	76,04	3,8	0,2
	4	68,53	2,24	0,36
	5	70,78	3,24	0,15
14	1	59,7	3,51	0,24
	2	73,18	2,14	0,33
	3	68,4	3,43	0,2
	4	58,28	3,88	0,28
	5	66,75	3,29	0,26
15	1	71,04	3,62	0,5
	2	68,1	3,51	0,27
	3	64,55	3,27	0,27
	4	69,99	3,54	0,2
	5	67,01	3,97	0,48
16	1	43,61	2,22	0,28
	2	84,83	4,53	0,3
	3	69,63	3,49	0,2



# MELiSSA Pilot Plant

Document Identification : Test report for the characterization of lettuce batch cultures in HPC1	Type	Number	Issue	Page : 67 / 68
	TN	101.3	(0)	

	4	81,98	3,62	0,49
	5	85,05	3,91	0,14
17	1	49,25	2,14	0,25
	2	51,06	2,49	0,3
	3	39,74	1,52	0,22
	4	47,94	2,41	0,11
	5	35,41	1,63	0,14
18	1	49,43	2,72	0,2
	2	43,03	2,09	0,08
	3	45,88	2,25	0,19
	4	52,15	2,63	0,22
	5	54,08	2,64	0,1
19	1	31,62	1,43	0,1
	2	40,97	1,78	0,19
	3	46,38	1,76	0,2
	4	48,75	2,16	0,2
	5	51,82	2,37	0,12
20	1	53,22	2,78	0,18
	2	42,99	1,84	0,27
	3	47,93	1,86	0,22
	4	47,88	1,58	0,17
	5	50,28	2,59	0,21



# MELiSSA Pilot Plant

Document Identification : Test report for the characterization of lettuce batch cultures in HPC1	Type	Number	Issue	Page : 68 / 68
	TN	101.3	(0)	

## Annex 2: As-run annotated procedures



# MELISSA Pilot Plant

<b>TEST RECORD SHEET</b>	Type	Reference	Chrono	Page :
	MPP-REC	10 -4101(0)	0.1	EP 16 / 17

Compartment : CIVb      Test Phase : 1

Test title : Preparation of the chamber

Objectives:

Applicable test plan and test protocols    TN 101.2

Hardware: HPC1 compartment and control system

Person responsible for the test : Natalia Tikhomirova

Test prerequisites :

- clean with ethanol 70% all surfaces inside HPC1+HVAC
- clean with **KOH** liquid loop of HPC1

Step No.	Action description	Expected results / Nominal behaviour	Date / Hour	Observed results / calculated / remarks - ref. of Deviation	C/ NC	Initials
1	Switching on 1 MH lamp per module for manipulation inside the chamber	Lamps are switched on and environmental conditions in HPC1 allow to perform manipulations inside the chamber	01/03/10 14:50		C	CM
2	Putting on Personal Protective Equipment: goggles, lab coat, safety shoes, gloves, shoe covers	Personal Protective Equipment is put on operator is ready to perform cleaning manipulations inside the chamber	01/03/10 14:55		C	CM
3	Removal of aluminium air balancing panels out of HPC1	Aluminium air balancing panels are removed in order to enter inside the chamber for free manipulation	01/03/10 15:00		C	CM
4	1. Demounting of plastic tips of spigots for further cleaning ; 2. pictures are taken	1. Tips are removed and can be cleaned separately 2. Pictures available	01/03/10 15:15		C	CM
5	Cleaning of plastic tips of spigots with soap and distilled water and subsequent rinsing with distilled water	Plastic tips of spigots are cleaned and rinsed well	3/03/10 16:00		C	CM



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## TEST RECORD SHEET

Type

Chrono

Page: 2

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Compartment : CIVb

Test Phase : 1

6	Cleaning of the bottom inside the chamber with vacuum cleaner <i>OK</i>	No dust can be observed inside the chamber <i>OK</i>	02/03/2000 15.30	OK	C	CM
7	<i>DEVA</i> Preparation of 3 L of 70% ethanol, filling of bottles with sprayers for disinfection of surfaces in HPC1 <i>OK</i>	There is no problem with supply of ethanol in sufficient quantity from department, tools are ready for disinfection	01/03/2000 15.30	it was prepared 1 bottle of 500 ml (70% ethanol)	C	CM
8	Preparation of the chamber inside for disinfection: taking trays out of the chamber <i>OK</i>	Chamber is ready for entering inside and disinfection of the surfaces		Trays were already out of the chamber	C	CM
9	Putting on mask against vapours and disinfection of all accessible surfaces inside the chamber with ethanol	All accessible surfaces in the chamber are treated with ethanol	01/03/2000 16.00 02/03/2000 9.30	mask was not needed. <i>OK</i>	C	CM
10	Disinfection of HVAC surfaces	All accessible surfaces in HVAC of HPC1 are disinfected with ethanol	03/03/2000 18.30	OK	C	WT
11	Disinfection of aluminium air balancing panels with ethanol outside of the chamber	Panels are disinfected and can be placed back into the chamber	02/03/2000 11.00	Also with salts (for dishwasher) and product for inoxidable material But not all of them were removed	C	CM
12	Placement of aluminium air balancing panels on the bottom of the chamber to their nominal position preliminary putting on disposable shoes covers	Aluminium air balancing panels are placed in their nominal position	02/03/2000 12.00	OK	C	EM
13	Connecting of spigots plastic tips	Tips are connected to spigot at their nominal position	02/03/2000 12.30	OK	C	CM
14	Placement of trays, preliminary cleaned with soap, inside the chamber	Trays are placed in the chamber in their nominal position	03/03/2000 16.00	OK	C	CM
15	Cleaning of 120-L external tank with soap and rinsing with decalcified water	Tank is clean and ready for further manipulation	02/03/2000 8.30	Cleaned and rinsed with distilled water	C	CM



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## TEST RECORD SHEET

Type

Chrono

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# 3/A

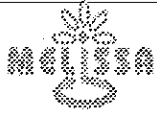
Compartment : CIVb

Test Phase : 1

16	Filling of external tank with decalcified water and 2 % NaOH, mixing in external tank	Tank is filled and solution is mixed, left for 1 hour		Not done only with soap and water	NC	CM
17	Taking of 3 samples of solution for pH, measurements of pH	pH is measured, data is considered as experimental point 1		Not done	NC	CM
18	Disinfection of external pump and flexible tubing, to be used for main nutrient tank filling, with solution of NaOH from external tank	Pump and flexible tubing are disinfected		Not done only with water	NC	CM
19	Emptying of the tank, rinsing with decalcified water, measurement of pH of water until it's equal to pH of decalcified water (control point)	Tank is rinsed well and pH of last sample from the tank is equal to pH of decalcified water		tank was cleaned with ethanol and water	NC	CM
20	Rinsing of external pump and tubing with decalcified water until pH of water, going out of the pump and tubing is equal to pH of decalcified water	Pump is rinsed well and pH of last sample from the tank is equal to pH of decalcified water		Not done	NC	CM
21	Rinsing of external tank, tubing and pump with distilled water	Tank, tubing and pump are rinsed and ready for further use		Not done	NC	CM
22	Emptying stock A, stock B, Acid and Base tanks by opening electro valves from HMI in manual mode for 4 minutes	Indicator of low level of liquid for all tanks can be seen from HMI	02/03/2010 8:45 03/03/2010 13:30	OK	C	CM NT
23	When level of water in the tanks is low, emptying of the rest of water manually with plastic cup and after with pipette	No liquid can be observed on the bottom of the tanks	02/03/2010 8:50 03/03/2010 15:00	OK	C	CM NT
24	Disinfection of stock A, stock B, Acid and Base tanks with ethanol and drying with paper towel	Tanks are disinfected and no ethanol can be observed in the tanks	02/03/2010 8:55 03/03/2010	Only acid and Base with water For stock A and stock B see no 34 CM will be done before	C	CM

DEV 3

EP



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## TEST RECORD SHEET

Type

Chrono

Page :

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EP  
4/7

Compartment : CIVb

Test Phase : 1

25	Cleaning of main nutrient tank with soap and rinse several times with decalcified water	Nutrient tank is cleaned with soap and ready for further manipulation	3.03.2010 15:00-16:00	Rinsed with distilled water once	C	CM
26	Filling of nutrient tank with decalcified water until top of the tank	Approximately 200 L	3.03.2010 16:00	Filled with distilled water	C	CM
27	Taking of 3 samples of water from nutrient tank for pH measurements, pH measuring <sup>and EC</sup>	pH is measured and will be a control point for further measurements	3.03.2010 17:35	sample 1 pH = 7.03 EC = 409 sample 2 pH = 7.12 EC = 396 sample 3 pH = 7.08 EC = 393 µS/cm	C <del>NT</del>	NT
28	Closing of sampling loop of HPC1 liquid loop before following manipulations	Sampling loop is closed, values of EC and pH are not correct	3.03.2010 17:35	Sampling loop is not closed as there is no any reagent in water (no KOH)	C <del>NT</del>	NT
29	Preparation of KOH solution using previously put on Personal Protective Equipment	4 kg of KOH for 200 L of water in nutrient tank		—	NC	CM
30	Using external pump mixing and dissolving of KOH in the nutrient tank	KOH is completely dissolved in the nutrient tank, no pallet can be noticed in the nutrient tank		—	NC	CM
31	Taking of 3 samples of solution from nutrient tank for pH measurement	pH is measured and data will be used as first experimental point		—	NC	CM
32	Closing of nutrient tank with nutrient tank lid	Nutrient tank is closed with screws	3.03.2010 17:30	OK	C	NT
33	Enabling of irrigation in manual mode, water flow is 15 L/min	Irrigation is enabled with given flow during 1 hour	3.03.2010 17:45	flow rate = 16 L/min	C	NT
34	Disabling of irrigation mode	Irrigation mode is off	3/03/2010 18:50	OK	C	NT

DEV. S  
EP





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## TEST RECORD SHEET

Type

Chrono

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*AT 5/A*

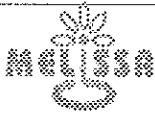
Compartment : CIVb

Test Phase : 1

35	Taking of 3 samples of solution from nutrient tank for pH measurements <i>DEV. 6 EP</i> <i>EC measurement</i>	pH is measured and data will be the <del>second</del> experimental point	<i>3/03/2010</i> <i>18:00</i>	<i>sample 1 pH=7.09 EC=108.9</i> <i>sample 2 pH=6.36 EC=110.3</i> <i>sample 3 pH=6.81 EC=110.3</i>	C	NT
36	Emptying of nutrient tank using drain valve	Nutrient tank is almost empty but some liquid is observed on the bottom of the tank due to design of the tank	<i>4/03/2010</i> <i>17:00</i>		C	CM
37	Rinsing of nutrient tank several times with <del>decalcified</del> <i>distilled</i> water and pH measurements after each rinse until pH value is equal to control point (see step No 27). <i>DEV. 7 EP</i>	pH of last sample taken from nutrient tank is equal to control point so tank is rinsed well	<i>4/03/2010</i> <i>17:30</i> <i>18:05</i>	<i>Before rinsing</i> <i>sample 1 pH=9.38 EC=27.81</i> <i>sample 2 pH=8.16 EC=27.80</i> <i>sample 3 pH=7.56 EC=3.02</i> <i>After rinsing</i> <i>sample 1 pH=6.58 EC=17.01</i> <i>sample 2 pH=6.42 EC=16.83</i> <i>sample 3 pH=6.39 EC=16.78</i>	C	NT
38	Filling of nutrient tank with decalcified water and enabling of irrigation mode for 15 minutes	Rinsing of liquid loop from residues of KOH			NC	NT
39	Taking of liquid samples from 3 extreme spigots of module A and module C (6 in total) and from nutrient tank drain valve for pH measurements	pH is measured and data will be considered as 3d, 4 <sup>th</sup> and 5 <sup>th</sup> experimental points			NC	NT
40	Disabling of irrigation mode and emptying of nutrient tank	Irrigation mode is off, tank is almost empty			NC	NT
41	Repetition of steps No 38-40 until pH of the solution is equal to control value	pH of solution is equal to control value, so liquid loop is rinsed well from KOH residues			NC	NT
42	Taking of 3 samples of distilled water and pH measurement	pH is measured and data will be the 2d control point			NC	NT
43	Filling of nutrient tank with distilled water and enabling of irrigation mode for 15 minutes	Final rinsing of liquid loop			NC	NT

*\* after pH meter calibration (was done before at 12:40, but necessary to do again) since values vary*  
*sample 1 pH=8.84*  
*sample 2 pH=8.23*  
*sample 3 pH=7.27*  
*\* EC of distilled water = 0.75 µs/cm*  
*EC of CM4 = 0.54 µs/cm*

*DEV. 8 EP*



# MELiSSA Pilot Plant

## TEST RECORD SHEET

Type	Reference	Chrono
MPP-REC	10 -4101(0)	0.1

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Compartment : CIVb Test Phase : 1

41	Repetition of steps No 38-40 until pH of the solution is equal to control value	pH of solution is equal to control value, so liquid loop is rinsed well from KOH residues				MC	NT
42	Taking of 3 samples of distilled water and pH measurement	pH is measured and data will be the 2d control point				MC	NT
43	Filling of nutrient tank with distilled water and enabling of irrigation mode for 15 minutes	Final rinsing of liquid loop				MC	NT
44	Taking of liquid samples from 3 extreme spigots of module A and module C (6 in total) and from nutrient tank drain valve for pH measurements	pH is measured and data will be considered as experimental points				MC	NT
45	Disabling of irrigation mode and emptying of nutrient tank	Irrigation mode is off, tank is almost empty				MC	NT
46	In case pH of solution is equal to pH of distilled water, rinsing of liquid loop is finished. If it's higher than 2d control point, repetition of steps No 43-45 until pH value is equal to control one.	pH of water is equal to 2d control point, rinsing of liquid loop is finished				MC	MC NT
47	Rinsing of external pump with decalcified water until pH of water, going out of the pump is equal to pH of decalcified water	Pump is rinsed well and pH of last sample from the tank is equal to pH of decalcified water				MC	NT
48	Rinsing of external tank and pump with distilled water, pH of water, going out of the pump is equal to pH of distilled water	Tank and pump are rinsed and pH of last sample from the pump is equal to pH of distilled water				MC	NT

*REV. 8*  
*28*

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Memorandum of Understanding 19071/05/NL/CP



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## TEST RECORD SHEET

Type	Reference	Chrono	Page : $\infty$
MPP-REC	10-4101(0)	--	16 7/7

Compartment : CIVb Test Phase : 1

49	Cleaning of pH and EC probes from sampling loop with distilled water and paper towel	pH and EC probes are rinsed and dried					
50	Chamber Shell Integrity Leakage Test. CO <sub>2</sub> is injected into the chamber in a closed configuration (all sub-systems off, main centrifugal blower excepted) to a set-point of 1500 ppm. Allowing the system to equilibrate at 1500 ppm for 2 hours to allow time for equilibration with the passive air pressure compensation bags. CO <sub>2</sub> is allowed to passively decay through the chamber shell over a 24 hour period. The rate of leakage is calculated as the slope of a tangent to a 24 hour CO <sub>2</sub> curve, expressed as % Leakage of CO <sub>2</sub> (relative to initial value) per day	% Leakage of CO <sub>2</sub> (relative to initial value) per day is less than 7% per day	3/03/2010 19:41 19:30 (starting injection) 4/03/10 10:00	CO <sub>2</sub> (ppm) = 1570,8 CO <sub>2</sub> (ppm) = 1419,08			
				There is small leakage as compartment with blow and hood color exchangers are not closed well. 11:23 chamber is opened and closed again with blower, 12:00 CO <sub>2</sub> (ppm) concn = 2222,81 16:34 2215,38 CO <sub>2</sub> (ppm) Chamber was open in order to place trays with sown seeds.			

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Which sensors are calibrated?  
Where are the certificates?

Microbiological control of some locations?

Leakage for 5 hours is of 3%, then for 24hs should be 1,4%

DEN.9

MPP-REC 10-4101 (0)-01

Conclusion for the Test	Name E. PEIRO	Signature Eunju Peiro	Date 30.04.10
<input checked="" type="checkbox"/> Passed <input type="checkbox"/> Failed			
- Number of deviations attached to the document : 10 - All deviations have been justified or corrected ? YES / <b>(NO)</b>			
Comments			
Checked by MELISSA Pilot Plant	Name A. FOSTEN	Signature A. FOSTEN	Date 2/5/10

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Appendix 1 for MPP-REC 10-4101 (0)-01

Appendix 1 - record of implied personnel

Name	ORGANIZATION	Function	Initials
Natalia Tikhomirova	Visiting Researcher MPP	Plant Scientist	NT
Cynthia Munganga	MPP	Analysis Technician	CM
Enrique Peiro	MPP	Technical manager	EP

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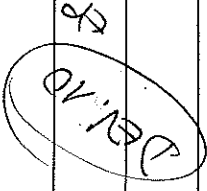
Memorandum of Understanding 19071/05/NL/CP

Appendix 2 for MPP-REC 10-4101 (a) -01

Appendix 2 - record of calibration certificates for the test instruments

Instrument description	HPCI TAG	Inv. number	Calibration record reference	Date of calibration	Calibration valid until	Signature
pH sensor	AT_4107_01		NT Logbook	01.03.10	Next test	
Electrical Conductivity of nutrient	AT_4108_01		NT Logbook	01.03.10	Next test	
Humidity A1 associated with temp A1	AT_4112_01		Manufacturer certificate	26.09.07		
Humidity B1 associated with temp B1	AT_4112_02		Manufacturer certificate	26.09.07		
Humidity C1 associated with temp C1	AT_4112_03		Manufacturer certificate	26.09.07		
CO2 Analyser	AT_4113_01		TN 96.11	23.07.09		
O2 Analyser	AT_4113_02		TN 96.11	23.07.09		
CO2 Mass Flow	FQRC_4113_01		Manufacturer certificate	16.08.07		
Outlet nutrient flow sensor	FT_4106_01		Manufacturer certificate			
Air velocity sensor	FT_4111_01		Manufacturer certificate			
Pressure sensor for airlock A	PT_4102_01		Manufacturer certificate	26.06.07		
Pressure sensor for airlock C --> Reaffected to External Pressure	PT_4103_01		Manufacturer certificate	26.06.07		NT / EP

Growing Area Pressure	PT_4114_01		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_02		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_03		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_04		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_05		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_06		Manufacturer certificate	26.06.07		
PAR Sensor - A	RT_4104_01		NTE-HPC_HVAC-RP-004	July 2009		
PAR Sensor - B	RT_4104_02		NTE-HPC_HVAC-RP-004	July 2009		
PAR Sensor - C	RT_4104_03		NTE-HPC_HVAC-RP-004	July 2009		
Light Loft Temperature sensor A	TT_4105_01		Manufacturer certificate	26.09.07		
Light Loft Temperature sensor B	TT_4105_02		Manufacturer certificate	26.09.07		
Light Loft Temperature sensor C	TT_4105_03		Manufacturer certificate	26.09.07		
Temperature sensor for solution reservoir	TT_4109_01		Manufacturer certificate	26.09.07		


  
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 NY / EP

Appendix 2 for MPP-REC 10-4181 (0)-01

Temperature A1 associated with humidity	TT_4112_01	Manufacturer certificate	26.09.07		
Temperature B1 associated with humidity	TT_4112_02	Manufacturer certificate	26.09.07		
Temperature C1 associated with humidity	TT_4112_03	Manufacturer certificate	26.09.07		
Temperature A2	TT_4112_04	Manufacturer certificate	26.09.07		
Temperature A3	TT_4112_05	Manufacturer certificate	26.09.07		
Temperature ambient	TT_4112_06	Manufacturer certificate	26.09.07	DEL 10	
Temperature B2	TT_4112_07	Manufacturer certificate	26.09.07		
Temperature B3	TT_4112_08	Manufacturer certificate	26.09.07		
Temperature B4	TT_4112_09	Manufacturer certificate	26.09.07		
Temperature C2	TT_4112_10	Manufacturer certificate	26.09.07		
Temperature C3	TT_4112_11	Manufacturer certificate	26.09.07		
Temperature ambient	TT_4112_12	Manufacturer certificate	26.09.07		
Temperature for facility chilled water	TT_4112_13	Manufacturer certificate	26.09.07		NT
Temperature	TT_4112_14	Manufacturer	26.09.07		ex



for facility hot water line			certificate		
Chilled coil surface temperature	TT_4112_15		Manufacturer certificate	26.09.07	
Heating coil surface temperature	TT_4112_16		Manufacturer certificate	26.09.07	26/10/07
Outlet Air, chilled exchanger	TT_4112_19		Manufacturer certificate	26.09.07	
Outlet Air, hot exchanger	TT_4112_20		Manufacturer certificate	26.09.07	
Inlet water Chilled Exchanger	TT_4112_21		Manufacturer certificate	26.09.07	NT / 08
Inlet water Hot Exchanger	TT_4112_22		Manufacturer certificate	26.09.07	

Appendix 3 for MPP-REC 10-4101(0)-01

Appendix 3 - deviations list

DEV. FORM # 1	Deviation: Preparation of 1.5L Ethanol in stead of 3L	Criticality Low Medium High	
	Corrective action: Take into account in the new version of the protocol (End C009)	Resp. EP	Due date End of C009
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date
DEV. FORM # 2	Deviation: Mask against vapours was not used as the good ventilation of the room allowed air removal	Criticality Low Medium High	
	Corrective action: Reminder in safety meetings; reconsider the need in new version of the protocol (End C009)	Resp. EP	Due date End of C009
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date
DEV. FORM # 3	Deviation: Disinfection with NaOH / KOH was not performed, according to was agreed with ESD (Ref. MOH-10-4101-AF-20100302)	Criticality Low Medium High	
	Corrective action: Take into account in the new edition of the protocol (End C009)	Resp. EP	Due date End of C009
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date

Appendix 3 for UPP-REC 10-4101 (10) - 01

Appendix 3 - deviations list

<b>DEV. FORM #</b> 4	<b>Deviation:</b> Not only pH but also EC measurement was taken.	<b>Criticality</b> <input checked="" type="radio"/> Low <input type="radio"/> Medium <input type="radio"/> High	
	<b>Corrective action:</b> Take into account the potential need of this EC meas. in the new version of the protocol (End 0009)	<b>Resp.</b> EP	<b>Due date</b> End of 0009
	<b>Corrective action performed and checked:</b> <b>Ref. of retests:</b>	<b>Checked / approved by</b>	<b>Closing Date</b>
<b>DEV. FORM #</b> 5	<b>Deviation:</b> See deviation 3	<b>Criticality</b> <input type="radio"/> Low <input checked="" type="radio"/> Medium <input type="radio"/> High	
	<b>Corrective action:</b> N.A.	<b>Resp.</b>	<b>Due date</b>
	<b>Corrective action performed and checked:</b> <b>Ref. of retests:</b>	<b>Checked / approved by</b>	<b>Closing Date</b>
<b>DEV. FORM #</b> 6	<b>Deviation:</b> See deviation 4	<b>Criticality</b> <input checked="" type="radio"/> Low <input type="radio"/> Medium <input type="radio"/> High	
	<b>Corrective action:</b> N.A.	<b>Resp.</b>	<b>Due date</b>
	<b>Corrective action performed and checked:</b> <b>Ref. of retests:</b>	<b>Checked / approved by</b>	<b>Closing Date</b>

Appendix 3 for MPP-REC 10-4101 (0) - 01

Appendix 3 - deviations list

DEV. FORM # 7	Deviation: Rinsing of nutrient tank with distilled water in stead of decalcified water	Criticality Low Medium High	
	Corrective action: Take into account in the new version of the protocol (End of 0009)	Resp. EP	Due date End of 0009
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date

DEV. FORM # 8	Deviation: See deviation 3	Criticality Low Medium High	
	Corrective action: N.A.	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date

DEV. FORM # 9	Deviation: The leak test was performed during 5 hours in stead of the 24 h foreseen. (can be acceptable if the measurement and rate are stable)	Criticality Low Medium High	
	Corrective action: Write an operating procedure defining in detail the conditions for the leak test, before the staggered culture campaign	Resp. EP	Due date October 2010 EP
	Corrective action performed and checked: Ref. of retests: Operating procedure issued on 27 February 2011 (MPP-OP-11-4103) January	Checked / approved by EP	Closing Date 01.02.11

Appendix 3 for MPP-REC 10-4101 (0)-01

Appendix 3 - deviations list

DEV. FORM # 10	Deviation: The validity period for the calibration of instruments is not defined	Criticality Low Medium High	
	Corrective action: To establish an HPC1 maintenance plan, including the validity period for each calibration performed on the sensors.	Resp. RM /EP	Due date Before the staggered cultures
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date
DEV. FORM #	Deviation:	Criticality Low Medium High	
	Corrective action:	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date
DEV. FORM #	Deviation:	Criticality Low Medium High	
	Corrective action:	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date



# MELISSA Pilot Plant

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## TEST RECORD SHEET

Type

Reference

Chrono

Page :

MPP-REC

10 -4101(0)

02

1 / 6

Compartment : CIVb

Test Phase : 1

Test title : Preparation of the chamber

Objectives:

Applicable test plan and test protocols

TN-101.2

Hardware: HPC1 compartment and control system

Person responsible for the test : Natalia Tikhomirova

Test prerequisites :

-clean with ethanol 70% all surfaces inside HPC1+HVAC

-clean with distilled water liquid loop of HPC1

Step No.	Action description	Expected results / Nominal behaviour	Date / Hour	Observed results / calculated / remarks - ref. of Deviation	C/ NC	Initials
1	Switching on 1 MH lamp per module for manipulation inside the chamber	Lamps are switched on and environmental conditions in HPC1 allow to perform manipulations inside the chamber	15:30 05.05.10 (1min)	Lamps were not switched on because with the lamps in the HPC1 room were enough. <b>DEVI</b>	C	CM
2	Putting on Personal Protective Equipment: goggles, clean lab coat, safety shoes, gloves, shoe covers, hairnet, clean lab trousers	Personal Protective Equipment is put on. Operator is ready to perform cleaning manipulations inside the chamber	15:30 05.05.10 (5 min)		C	CM
3	Removal of aluminium air balancing panels out of HPC1	Aluminium air balancing panels are removed in order to enter inside the chamber for free manipulation	15:50 05.05.10 (15 min)		C	CM
4	1. Demounting of plastic tips of spigots for further cleaning ; 2. pictures are taken	1. Tips are removed and can be cleaned separately 2. Pictures available	16:30 05.05.10 (2 min)		C	CM
5	Cleaning of plastic tips of spigots with soap and distilled water and subsequent rinsing with distilled water	Plastic tips of spigots are cleaned and rinsed well	11:00 06.05.10		C	CM



# MELISSA Pilot Plant

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## TEST RECORD SHEET

Type	Reference	Chrono	Page :
MPP-REC	10 -4101(0)	02	2/6

Compartment : CIVb Test Phase : 1

6	Cleaning of the bottom inside the chamber with vacuum cleaner	No dust can be observed inside the chamber	15:35 05.05.10 (1h)		C	CM
7	Preparation of 1.5 L of 70% ethanol, filling of bottles with sprayers for disinfection of surfaces in HPC1	There is no problem with supply of ethanol in sufficient quantity from department, tools are ready for disinfection	10:00 06.05.10 (5min)		C	CM
8	Preparation of the chamber inside for disinfection: taking trays out of the chamber	Chamber is ready for entering inside and disinfection of the surfaces	10:05 06.05.10	Trays were taking out on first of April 10.05.10 → 12h - 12h30	C	CM
9	Putting on mask against vapours and disinfection of all accessible surfaces inside the chamber with ethanol	All accessible surfaces in the chamber are treated with ethanol	10:15 06.05.10 (1h)	↓	C	CM
10	Disinfection of HVAC surfaces <i>stainless steel</i>	All accessible surfaces in HVAC of HPC1 are disinfected with ethanol	11h15 06.05.10 (25min)		C	CM
11	Disinfection of <i>stainless steel</i> air balancing panels with ethanol outside of the chamber	Panels are disinfected and can be placed back into the chamber	11h40 06.05.10 (20min)			
12	Placement of aluminium air balancing panels on the bottom of the chamber to their nominal position preliminary putting on disposable shoes covers	Aluminium air balancing panels are placed in their nominal position	17h00 06.05.10		C	RM
13	Connecting of spigots plastic tips	Tips are connected to spigot at their nominal position	16:50 06.05.10		C	RM
14	Placement of trays, preliminary cleaned with soap, inside the chamber	Trays are placed in the chamber in their nominal position	<del>12:45</del> 12:45 10.05.10	Trays were preliminary cleaned on first of April.	C	CM



# MELISSA Pilot Plant

## TEST RECORD SHEET

Type

Reference

Chrono

Page :

MPP-REC

10 -4101(0)

02

3 / 6

Compartment : CIVb

Test Phase : 1

14	Emptying stock A, stock B, Acid and Base tanks by opening electro valves from HMI in manual mode for 4 minutes	Indicator of low level of liquid for all tanks can be seen from HMI	16:00 06.05.10 (4 min)			C	CM
15	When level of water in the tanks is low, emptying of the rest of water manually with plastic cup and after with pipette	No liquid can be observed on the bottom of the tanks	16:10 06.05.10 (10 min)			C	CM
16	Disinfection of stock A, stock B, Acid and Base tanks with ethanol and drying with paper towel	Tanks are disinfected and no ethanol can be observed in the tanks	<del>06</del> <del>07</del> .05.10 <del>13:45</del> 16:15 (2 min)			C	CM
17	Cleaning of main nutrient tank with soap and rinse several times with decalcified water	Nutrient tank is cleaned with soap and ready for further manipulation	06.05.10 16:20 (20 min)			C	CM
18	Filling of nutrient tank with distilled water until top of the tank	Approximately 200 L	16:45 06.05.10 (20 min)			C	CM
19	Taking 3 samples of distilled water and pH, EC measurements	Done	17:00 06.05.10 (1 min)	$\text{pH}_1 = 8,37$ $\text{Ec}_1 = 7,24 \mu\text{S/cm}$ $\text{pH}_2 = 8,01$ $\text{Ec}_2 = 1,36 \mu\text{S/cm}$ $\text{pH}_3 = 7,84$ $\text{Ec}_3 = 1,03 \mu\text{S/cm}$		C	CM
20	Taking of 3 samples of water from nutrient tank, pH, EC measurements	Done	17:02 06.05.10 (1 min)	$\text{pH}_1 = 7,98$ $\text{Ec}_1 = 52 \mu\text{S/cm}$ $\text{pH}_2 = 7,91$ $\text{Ec}_2 = 18,44 \mu\text{S/cm}$ $\text{pH}_3 = 7,91$ $\text{Ec}_3 = 9,21 \mu\text{S/cm}$		C	CM
21	Closing of nutrient tank with nutrient tank lid	Nutrient tank is closed with screws	17:07 06.05.10 (3 min)			C	CM
22	Putting on Personal Protective Equipment: goggles, clean lab coat, safety shoes, gloves, shoe covers, hairnet, clean lab trousers and entering into the chamber with several	Done	10:00 12:50 07.05.10 (1,5 min)	At 10'o'clock water escape from the main collector because of a bag installation of the hole of the main collector		C	CM RM





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## TEST RECORD SHEET

Type	Reference	Chrono	Page :
MPP-REC	10-4101(0)	02	4 / 6

Compartment : CIVb Test Phase : 1

	sheets of paper, placement of paper under connection of main collector	10:10 / 13:11 07.05.10 (2min)	→			C	RM
23	Enabling of irrigation in manual mode, water flow is 15 L/min	Irrigation enabled flow = 15L/min Duration : 1hour	10:17 07.05.10 (1h)	13:45 / 17:00 07.05.10 / 10.05.10 18:08		C	CM
24	Disabling of irrigation mode	Irrigation mode is off	15:11 07.05.10			C	CM
25	Taking of 3 samples of solution from nutrient tank for pH and EC measurements	pH is measured and data will be the first experimental point	15:15 07.05.10	PH <sub>1</sub> = 6,54 EC <sub>1</sub> = 51,4 µS/cm PH <sub>2</sub> = 6,79 EC <sub>2</sub> = 58,4 µS/cm PH <sub>3</sub> = 6,79 EC <sub>3</sub> = 59,6 µS/cm			CM
26	Emptying of nutrient tank using drain valve	Nutrient tank is almost empty but some liquid is observed on the	15:15 07.05.10			C	CM
		bottom of the tank due to design of the tank	15:35				
27	Rinsing of nutrient tank several times with distilled water and pH, EC measurements after each rinse until EC value is not higher than 20 µS/cm	Done	15:45 07.05.10 17:15 10/05/10 18:20	PH <sub>1</sub> = 8,79 EC <sub>1</sub> = 20,6 µS/cm PH <sub>2</sub> = 6,92 EC <sub>2</sub> = 61,8 µS/cm PH <sub>3</sub> = 7,25 EC <sub>3</sub> = 16,54 µS/cm EC <sub>1</sub> = 5,21 EC <sub>2</sub> = 6,95 EC <sub>3</sub> = 5,87			CM
28	Entering into the chamber and checking the paper under the main collector whether it is dry or wet.	Done	13:00 07.05.10 18:00			C	CM
29	In case the paper is wet, performance of main collector sealing and check the leak again until no leaks can be observed	No leaks	11:00 10.05.10 12:00	It was a leak, the paper was wet and there was water down the main collector. RM sealed and checked.		C	RM
30	Cleaning of 120-L external tank with soap and rinsing with distilled water	Tank is clean and ready for further manipulation	5/05/2010 20:30			e	MT



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## TEST RECORD SHEET

Type	Reference	Chrono	Page :
MPP-REC	10 -4101(0)	02	5 / 6

Compartment : CIVb Test Phase : 1

31	Taking samples from several locations in the chamber for further microbiological analysis	Done	13:00 10.05.10 13:30		C	CM
32	Chamber Shell Integrity Leakage Test. CO <sub>2</sub> is injected into the chamber in a closed configuration (all sub-systems off, main centrifugal blower excepted) to a set-point of 1500 ppm. Allowing the system to equilibrate at 1500 ppm for 2 hours to allow time for equilibration with the passive air pressure compensation bags. CO <sub>2</sub> is allowed to passively decay through the chamber shell over a 24 hour period. The rate of leakage is calculated as the slope of a tangent to a 24 hour CO <sub>2</sub> curve, expressed as % Leakage of CO <sub>2</sub> (relative to initial value) per day	% Leakage of CO <sub>2</sub> (relative to initial value) per day is less than 7% per day	18:50 01.05.10 19:15	<p>The door of module c was not closed at all - we the CO<sub>2</sub> decreased</p> <p>An other test should be done. CO<sub>2</sub> should added in manual mode to 1500 ppm during 2h and after to stop the manual mode and see the stability of CO<sub>2</sub>.</p>	C	CM D12 #P
			9:25 11.05.10 11:25 12.05.10 11:25-12:31	<p>Switch on CO<sub>2</sub> = 1500ppm Auto + blower = Auto</p> <p>Switch off of CO<sub>2</sub> The test was a success only 4,4% per 25 hours.</p>	C	CM
33	Disconnection and cleaning of condensate tank with soap and water, after with ethanol. After mounting back of the condensate tank	Done	15:30 07.05.10		C	RM

MPP-REC 10-4101 (0) - 02

Conclusion for the Test	Name E. PEIRO	Signature <u>Eugene Peiro</u>	Date 30.06.10
<input checked="" type="checkbox"/> Passed <input type="checkbox"/> Failed			
- Number of deviations attached to the document : <u>3</u> AF 30/6/10			
- All deviations have been justified or corrected? YES <u>(NO)</u>			
Comments			
Checked by MELISSA Pilot Plant	Name A. FOSSEN	Signature <u>A. Fossen</u>	Date 30/6/10

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Memorandum of Understanding 19071/05/NL/CP

Appendix 1 for <sup>20</sup> MPP-REC 10-4101(0)-02

Appendix 1 - record of implied personnel

Name	ORGANIZATION	Function	Initials
Natalia Tikhomirova	Visiting Researcher MPP	Plant Scientist	NT
Cynthia Munganga	MPP	Analysis Technician	CM
Enrique Peiro	MPP	Technical manager	EP

Appendix 2 for MPP-REC 10-4101 (0) - 02

Appendix 2 - record of calibration certificates for the test instruments

Instrument description	HPC1 TAG	inv. number	Calibration record reference	Date of calibration	Calibration valid until	Signature
pH sensor	AT_4107_01		NT Logbook	05.05.10	Next test	
Electrical Conductivity of nutrient	AT_4108_01		NT Logbook	05.05.10	Next test	
Humidity A1 associated with temp A1	AT_4112_01		Manufacturer certificate	26.09.07		
Humidity B1 associated with temp B1	AT_4112_02		Manufacturer certificate	26.09.07		
Humidity C1 associated with temp C1	AT_4112_03		Manufacturer certificate	26.09.07		
CO2 Analyser	AT_4113_01		TN 96.11	23.07.09		
O2 Analyser	AT_4113_02		TN 96.11	23.07.09		
CO2 Mass Flow	FQRC_4113_01		Manufacturer certificate	16.08.07		
Outlet nutrient flow sensor	FT_4106_01		Manufacturer certificate			
Air velocity sensor	FT_4111_01		Manufacturer certificate			
Pressure sensor for airlock A	PT_4102_01		Manufacturer certificate	26.06.07		
Pressure sensor for airlock C --> Reaffected to External Pressure	PT_4103_01		Manufacturer certificate	26.06.07		<del>NT</del> EP

Growing Area Pressure	PT_4114_01		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_02		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_03		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_04		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_05		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_06		Manufacturer certificate	26.06.07		
PAR Sensor - A	RT_4104_01		NTE-HPC_HVAC-RP-004	July 2009		
PAR Sensor - B	RT_4104_02		NTE-HPC_HVAC-RP-004	July 2009		
PAR Sensor - C	RT_4104_03		NTE-HPC_HVAC-RP-004	July 2009		
Light Loft Temperature sensor A	TT_4105_01		Manufacturer certificate	26.09.07		
Light Loft Temperature sensor B	TT_4105_02		Manufacturer certificate	26.09.07		
Light Loft Temperature sensor C	TT_4105_03		Manufacturer certificate	26.09.07		
Temperature sensor for solution reservoir	TT_4109_01		Manufacturer certificate	26.09.07		NT/28

Appendix 2 for MAP-REC 10-4101(0)-02

Temperature A1 associated with humidity	TT_4112_01	Manufacturer certificate	26.09.07		
Temperature B1 associated with humidity	TT_4112_02	Manufacturer certificate	26.09.07		
Temperature C1 associated with humidity	TT_4112_03	Manufacturer certificate	26.09.07		
Temperature A2	TT_4112_04	Manufacturer certificate	26.09.07		
Temperature A3	TT_4112_05	Manufacturer certificate	26.09.07		
Temperature ambient	TT_4112_06	Manufacturer certificate	26.09.07		
Temperature B2	TT_4112_07	Manufacturer certificate	26.09.07		
Temperature B3	TT_4112_08	Manufacturer certificate	26.09.07		
Temperature B4	TT_4112_09	Manufacturer certificate	26.09.07		
Temperature C2	TT_4112_10	Manufacturer certificate	26.09.07		
Temperature C3	TT_4112_11	Manufacturer certificate	26.09.07		
Temperature ambient	TT_4112_12	Manufacturer certificate	26.09.07		
Temperature for facility chilled water	TT_4112_13	Manufacturer certificate	26.09.07		
Temperature	TT_4112_14	Manufacturer certificate	26.09.07		

DEL. 3

MT  
EF

for facility hot water line			certificate		
Chilled coil surface temperature	TT_4112_15		Manufacturer certificate	26.09.07	
Heating coil surface temperature	TT_4112_16		Manufacturer certificate	26.09.07	
Outlet Air, chilled exchanger	TT_4112_19		Manufacturer certificate	26.09.07	
Outlet Air, hot exchanger	TT_4112_20		Manufacturer certificate	26.09.07	
Inlet water Chilled Exchanger	TT_4112_21		Manufacturer certificate	26.09.07	
Inlet water Hot Exchanger	TT_4112_22		Manufacturer certificate	26.09.07	

22/5/12

MT  
~~EP~~



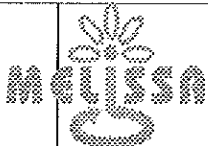
Appendix 3 for MPR REC 10-4101(0) - 02

Appendix 3 - deviations list

DEV. FORM # 1	Deviation: Lights not switched on for cleaning as natural illumination was enough from the room, and the ventilation was not started, so to prevent hot conditions	Criticality Low Medium High	
Corrective action: Take it into account in the new edition of the protocol to have ventilation ON when lamps are used (end of 0009)		Resp. EP	Due date End of 0009
Corrective action performed and checked: Ref. of retests:		Checked / approved by	Closing Date
DEV. FORM # 2	Deviation: Door of module C not well closed, so the leak test failed.	Criticality Low Medium High	
Corrective action: Close tight the door of air-lock C, then perform a new leak test.		Resp. NT	Due date 07.05.10
Corrective action performed and checked: Ref. of retests: Action performed on the spot.		Checked / approved by EP	Closing Date 28.06.10
DEV. FORM # 3	Deviation: The validity period for the calibration of instruments is not defined	Criticality Low Medium High	
Corrective action: To establish an HPC maintenance plan, including the validity period for each calibration performed on the sensors		Resp. RM EP	Due date Before the staggered cultures
Corrective action performed and checked: Ref. of retests:		Checked / approved by	Closing Date

3

Taula formatada



**MELISSA Pilot Plant**



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<b>TEST RECORD SHEET</b>	Type	Reference	Chrono :	Page :
	MPP-REC	10 -4102(0)	01	1 / 7

Compartment : CIVb Test Phase : 2

Test title : Seedlings phase

Objectives:

Applicable test plan and test protocols **TN 101.2**

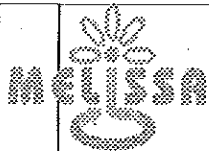
Hardware:

Person responsible for the test : Natalia Tikhomirova

Test prerequisites : phase 1 nominally executed

Step No.	Day No.	Action description	Expected results / Nominal behaviour	Date / Hour	Observed results / calculated / remarks - ref. of Deviation	C/N C	Initials
1	0	Sterilization of 2 flats of rockwool small cubes - Grodan AO 36/40 6/15W using following procedure for Rockwool safe manipulation: OP-10-4101 (0)	Rockwool flats are sterilised and ready for seeds sowing	3/03/10 16:20	OK	C	CM
2	0	Sterilization of 1 litre of distilled water in autoclave	30 minutes at 120°C	3/03/10 16:20	OK	C	CM
3	0	Sterilization of 10-litres tanks for stock A and stock B (2), 20-litres tank for seedlings solution in autoclave	30 minutes at 120°C	02/03/10 17:00	OK	C	CM
4	0	Preparation of 10 L of stock A and 10 L of stock B solutions and 10 L of seedling solution according to TN 96.3.	Solutions must be kept in a dark place in order to prevent algae growth	04/03/10 11:00	OK	C	CM
5	0	Taking a bag with lettuce seeds from the fridge and placement about 200 seeds into a glass. Addition of 5% hypochlorite (bleach) to the glass with the seeds	Sterilization during 15 minutes	4/03/10 15:51		C	MT

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## TEST RECORD SHEET

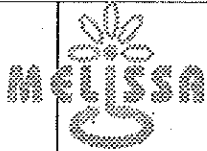
Type	Reference	Chrono :	Page :
MPP-REC	10 -4102(0)	01	2 / 7

Compartment : CIVb Test Phase : 2

		(until bleach covers all the seeds)					
6	0	Placement of the seeds into a sieve and rinse with 1 L of distilled sterile water	Seeds are sterilized but some lost of colour can be observed due to treatment with bleach	4/03/10 16:06		C	NT
7	0	Sterilization 2 seed germination trays with 70% ethanol and paper towel	No ethanol can be observed on the trays	4/03/10 15:52- -16:06		C	NT
8	0	Placement of 2 flats of rockwool small cubes - Grodan AO 36/40 6/15W into 2 seed germination trays	Each tray must contain 98 cubes	4/03/10 16:06- 16:36		C	NT
9	0	Using tweezers, placement a single lettuce seed in each hole of Rockwool cubes	Attention should be paid during process of seeds sowing in order not to sow more than 1 seed per cube and not miss any cube	4/03/10 16:37- -17:13		C	NT
10	0	Measurement of pH of irrigation solution for seedlings, in case it's 5.8-5.9, watering of Rockwool cubes with this solution. If pH is higher than 6.0 or lower than 5.8 addition of about 1 mL of 0.5M HNO <sub>3</sub> or 1mL of 0.5M KOH (depending on pH if it's low or high) to the 10L of the solution, mixing well and checking of pH again. Repetition if necessary until pH is about 5.8-5.9.	pH of seedlings solution is 5.8-5.9, Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	4/03/10 5:42 AM 12:40	pH = 6, 0 AM	C	NT 0 AM
11	0	Taking photos of the trays.	Day <sub>0</sub> of crop test (Rockwool sheets without seedlings)	4/03/10 17:17		C	NT
12	0	Addition of a plastic tray cover to each tray	Trays are covered in order to maintain humidity inside the tray and enhance seeds germination	4/03/10 17:17		C	NT

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## TEST RECORD SHEET

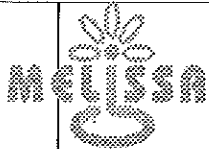
Type	Reference	Chrono :	Page :
MPP-REC	10 -4102(0)	01	3 17

Compartment : CIVb Test Phase : 2

13	0	Activation of Schneider controller of HPC1. Light mode: auto, 1 MH and 1 HPS lamps per module on. Day time - 16 hours, night time - 8 hours. Fan Mode: auto. Temperature and humidity mode: auto. T day and night set points = 20°C, day Rh =50%, night Rh=70%. Air Blower Mode: auto.	Schneider controller is activated with environmental conditions set points used for seeds germination and seedlings growth  Expected measurements on PAR sensors? Expected values for PAR sensors are around 300µE	4/03/10 17:08	When light is off, value of PAR for module A = 125 µE for module B and C = ONE when 2 lamps per module are on, PAR in mod. B = 231 mod. C = 286	C	NT	Suprimir: controller Suprimir: controller
14	0	Placement of the germination trays into HPC1 and closing of the doors from both sides of HPC1.	Chamber is closed and all conditions in the chamber correspond to conditions for seeds germination	4/03/10 18:25	mod. A = 430 Day start: 06:00 Day end: 22:00	C	NT	
15	1	Opening of the chamber, taking photos of the trays with plastic cover and after without the cover.	Day <sub>1</sub> of crop test	5/03/10 10:36- -10:47	OK	C	NT	
16	1	In case not more than 60% of seedlings in each tray can be observed, addition of plastic cover and closing the chamber	Check of seedlings germination, repeat in the afternoon	5/03/10 10:47	No seedlings can be seen	C	NT	
17	1	In the afternoon checking of the seeds germination again. If more than 60% of seedlings are observed opening of the trays. If not the trays are left covered until next day	Check of seedlings germination	5/03/10 18:39	No seedlings can be seen	C	NT	
18	2	Opening of the chamber, taking photos of the trays with plastic cover and after without the cover.	Day <sub>2</sub> of crop test	7/03/10 20:30	70% of seedling can be seen I couldn't take photos	C	CM	Mistake no n° 22 DEN 1
19	2	In case not more than 60% of seedlings in each tray can be observed, addition of plastic cover and closing the chamber	Check of seedlings germination, repeat in the afternoon	6/03/10	No check as it is Saturday and too early for germination	NC		EP

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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10 -4102(0)	01	417

Compartment : CIVb Test Phase : 2

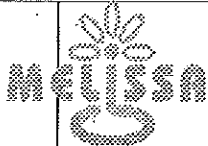
20	2	In the afternoon checking of the seeds germination again. If more than 60% of seedlings are observed opening of the trays. If not the trays are left covered until next day	Check of seedlings germination			NC	CM
21	3	Checking the seeds germination and if more than 60% of seedlings are observed in each tray opening of the trays (taking out the plastic covers).	By this day usually germination percentage is more than 60% per tray and plastic covers can be removed from the trays	7/3/10 20:30	70% of seedlings could be seen	C	CM
22	3	Taking photos of both trays	Days of crop test	7.03.10	see n°18	C	CM
23	3	Checking of pH of the seedlings nutrient solution and in case it's 5.8-5.9, watering Rockwool cubes with this solution, approximately 900 mL per each tray. Regulation of pH with acid or base in case it is higher than 6.0 or lower than 5.7 (see step No.10)	pH of seedlings solution is 5.8-5.9, Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)		Rockwool is wet and no need to water (since plastic covers were removed today)	NC	CM
24	4	Taking photos of both trays	Day <sub>4</sub> of crop test	8/03/10 11:28	OK	C	NT
25	4	In the afternoon watering of the plants with the solution if necessary, approximately 500 mL per tray.	Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	8/03/10 14:30	300 mL of seedlings solution per each tray	C	NT
26	5	Taking photos of both trays. Watering of the plants with the solution if necessary	Day <sub>5</sub> of crop test. Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	9/03/10 13:31	pH of seedling solution = 6.02 watered right tray with 500 mL of sol-n, 74 seedlings are observed, left tray - 1100 mL, 73 seedlings	C	NT
27	6	Cutting of polypropylene film for trays covering against algae growth at maturity phase	DB stripes using template	10/03/10 10:00 - 14:20		C	NT

Dev.1

Dev.2

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## TEST RECORD SHEET

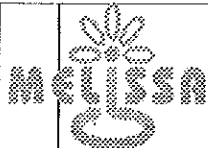
Type	Reference	Chrono :	Page :
MPP-REC	10 -4102(0)	01	5 / 7

Compartment : CIVb Test Phase : 2

28	6	Taking photos of both trays. Watering of the plants with the solution if necessary	Day <sub>6</sub> of crop test. Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	10/03/10 15:24	Right tray is wet, not watered, left one is watered with 900mL of seedlings solution	C	NT
29	7	Checking of pH of the seedlings nutrient solution and in case it's 5.8-5.9, watering Rockwool cubes with this solution, approximately 900 mL per each tray. Regulation of pH with acid or base in case it is higher than 6.0 or lower than 5.7 (see step No.10)	pH of seedlings solution is 5.8-5.9, Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	11/03/10 14:03	pH = 5,97 Watered right tray with 900mL of seedlings solution, 73 seedlings are observed, watered left tray with 650 mL of nutrient solution, 73 seedlings are observed. Solution is finished	C	NT
30	7	Taking photos of both trays.	Day <sub>7</sub> of crop test.	11/03/10 14:00		C	NT
31	7	Addition of stock A, stock B, acid and base solutions into the appropriate tanks until a mark on each tank	Tanks are filled for maturity test start up	12/03/10 9:30		C	NT
32	7	Addition of 1.3 litres of stock A and 1.3 litres of stock B into the 120L external tank, mixing thoroughly solution in the 120L external tank using a pump	Solution is mixed and ready for transfer into HPC1 nutrient tank	11.03.10	1,3L of A 1,3L of B were added in the external tank and mixed	C	CM
33	7	Transfer of 120 liters of the nutrient solution from external tank into HPC1 nutrient tank using a pump	Solution is transferred without any leak <u>Expected measurements on level sensor?</u>	11.03.10	120 L was transferred in the nutrient tank (no more 40 in the lab) we have to wait for the day after	C	CM
34	7	Addition of 40 liters of distilled water into 120L external tank and transfer of 40 liters of distilled water from external tank into HPC1 nutrient tank using a pump	Water is transferred without any leak <u>Level?</u>	12.03.10 8:15 8:45	40L of water was transferred in the nutrient tank Disinfection of stock A and B with water (sats were in the bottom of the tanks)		
35	7	Taking the trays with seedlings out of the chamber	During 1 hour the trays are in HPC room under ambient conditions	11.03.10 15:00		C	VG

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## TEST RECORD SHEET

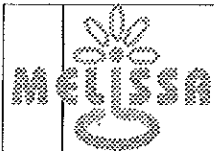
Type	Reference	Chrono :	Page :
MPP-REC	10 -4102(0)	01	6 / 7

Compartment : CIVb Test Phase : 2

36	7	Taking 20 trays out of the chamber and wiping 20 trays with paper towel wetted with ethanol	Sterilization of the trays	11/03/10 11:00 13:45		C	NS
37	7	Placement of 20 trays into the chamber	Trays are inside the chamber in their nominal position	11/03/10 11:00 - 11:45		C	NS
38	7	Activation of irrigation system from HMI in auto mode in order to adjust pH and EC of the nutrient solution	Irrigation system is activated, water flow is 10-11 L/min	12/03/10 10:54	Flow reading is 10,0 - 10,28 L/min	C	NS
39	7	Performance of leakage test of the main nutrient solution collector in the chamber	No leaks	12/03/10 10:55	OK	C	NS
40	7	When pH is equal to 5.9 and EC to 1.9, disabling of irrigation system	Irrigation system is off, no liquid circulation in the HPC1	12/03/10 11:48	OK	C	NS
41	7	Taking of 6 samples of nutrient solution from nutrient tank: a. Irrigation system is off. b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this solution but empty it into sewerage. c. After taking 3 samples continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge. d. Taking 3 samples manually from the top of the nutrient tank and placement them into the fridge f. Closing nutrient tank with plastic cover and screws, making sure it is airtight	Samples of the solution are taken according to the written procedure, nutrient tank is tightly closed	12/03/10 12:00 13:30	Samples are taken Nutrient tank is closed	C	NS

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<b>TEST RECORD SHEET</b>		Type	Reference	Chrono :	Page :
		MPP-REC	10 -4102(0)	01	7 / 7
		Compartment : CIVb		Test Phase : 2	
42	8		Day <sub>8</sub> of crop test.		

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MPP-REC 10-4102 (0)-01

Conclusion for the Test	Name E. PERO	Signature <i>E. PERO</i>	Date 30.04.10
<input checked="" type="checkbox"/> Passed <input type="checkbox"/> Failed			
- Number of deviations attached to the document : 2 - All deviations have been justified or corrected? YES / <b>NO</b>			
Comments			
Checked by MELISSA Pilot Plant	Name A. Foster	Signature <i>A. Foster</i>	Date 2/5/10

Appendix 1 for MPP-REC 10-4102 (0)-01

Appendix 1 - record of implied personnel

Name	ORGANIZATION	Function	Initials
Natalia Tikhomirova	Visiting Researcher MPP	Plant Scientist	NT
Cynthia Munganga	MPP	Analysis Technician	CM
Enrique Peiro	MPP	Technical manager	EP

Appendix 2 : equal to Appendix 2 for MPP-REC  
10-4101 (0)-01

EP

# Appendix 3 for MPP - REC 10-4102(0) -01

## Appendix 3 - deviations list

<b>DEV. FORM #</b>	<b>Deviation:</b> Some tasks were not performed as they coincided with the weekend but they were not critical (take pictures and check seeds), as they could be performed later	<b>Criticality</b> Low Medium High
1	<b>Corrective action:</b> Take into account potential higher flexibility in the new version of the protocol for tasks not critical (End 009)	Resp. Due date EP End of 009
	<b>Corrective action performed and checked:</b> Ref. of retests:	Checked / approved by Closing Date
<b>DEV. FORM #</b>	<b>Deviation:</b> No watering of seedlings in their rockwool cubes as the cubes were still wet No impact on the culture	<b>Criticality</b> Low Medium High
2	<b>Corrective action:</b> Reword the task to leave flexibility	Resp. Due date EP End of 009
	<b>Corrective action performed and checked:</b> Ref. of retests:	Checked / approved by Closing Date
<b>DEV. FORM #</b>	<b>Deviation:</b>	<b>Criticality</b> Low Medium High
	<b>Corrective action:</b>	Resp. Due date
	<b>Corrective action performed and checked:</b> Ref. of retests:	Checked / approved by Closing Date



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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10 -4102(0)	01/02	1 / 7

Compartment : CIVb      Test Phase : 2

AP  
20/5/10

Test title : Seedlings phase

Objectives:

Applicable test plan and test protocols      TN - 101 / 2

Hardware:

Person responsible for the test : Natalia Tikhomirova

Test prerequisites : phase 1 nominally executed

Step No.	Day No.	Action description	Expected results / Nominal behaviour	Date / Hour	Observed results / calculated / remarks - ref. of Deviation	C/N C	Initials
1	0	Sterilization of 2 flats of rockwool small cubes - Grodan AO 36/40 6/15W using following procedure for Rockwool safe manipulation: MPP-OP-10-4101 (0)	Rockwool flats are sterilised and ready for seeds sowing	12.05.10 13:15 15:55		C	CM
2	0	Sterilization of 1 litre of distilled water in autoclave	30 minutes at 120°C	12.05.10 9:00 13:00		C	CM
3	0	Sterilization of 10-litres tanks for stock A and stock B (2), 20-litres tank for seedlings solution in autoclave	30 minutes at 120°C	03.05.10 04.05.10	DEV. 1 EP	NC	CM
4	0	Preparation of 10 L of stock A and 10 L of stock B solutions and 10 L of seedling solution according to TN 96.3. Solutions must be kept in a dark place in order to prevent algae growth	Solutions are prepared	03.05.10 04.05.10	DEV. 2 EP	NC	CM
5	0	Taking a bag with lettuce seeds <i>Lactuca sativa</i> L. cultivar Grand Rapids from the fridge and	Done	12.05.10 17:15- 17:30	OK	C	MT

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Page :

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10 -4102(0)

01

2 / 7

Compartment : CIVb

Test Phase : 2

02 AF 20/5/10

		placement about 200 seeds into a glass. Addition of 5% hypochlorite to the glass with the seeds (until bleach covers all the seeds). Sterilization during 15 minutes					
6	0	Placement of the seeds into a sieve and rinse with 1 L of distilled sterile water	Seeds are sterilized but some lost of colour can be observed due to treatment with bleach	12.05.10 17:30	OK	C	NT
7	0	Disinfection of 2 seed germination trays with 70% ethanol and paper towel	The trays are disinfected and no ethanol can be observed on the trays	16:05 12.05.10		C	NT
8	0	Placement of 2 flats of rockwool small cubes - Grodan AO 36/40 6/15W into 2 seed germination trays	Each tray must contain 98 cubes	12.05.10 16:30		C	NT
9	0	Using tweezers, placement of a single lettuce seed in each hole of Rockwool cubes	Attention should be paid during process of seeds sowing in order not to sow more than 1 seed per cube and not miss any cube	12.05.10 17:40 -18:00		C	NT
10	0	Measurement of pH of irrigation solution for seedlings, in case it's 5.8-5.9, watering of Rockwool cubes with this solution.	pH of seedlings solution is 5.8-5.9, Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	12.05.10 17:25	pH = 4,45 3 ml of Base (0,5M)  pH = 5,98 Rinsing of 2 trays with 3 L of seedlings solution	C	NT
11	0	If pH is higher than 6.0 or lower than 5.8 addition of about 1 mL of 0.5M HNO <sub>3</sub> or 1mL of 0.5M KOH (depending on pH if it's low or high) to the 10L of the solution, mixing well and checking of pH again. Repetition if necessary until pH is about 5.8-5.9.	pH of seedlings solution is 5.8-5.9				



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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10 -4102(0)	01	3 / 7

Compartment : CIVb

Test Phase : 2

02 AF 6/5/10

12	0	Taking photos of the trays.	Day <sub>0</sub> of crop test (Rockwool sheets without seedlings)	12/05/10 18:10	OK	C	NT
13	0	Addition of a plastic tray cover to each tray	Trays are covered in order to maintain humidity inside the tray and enhance seeds germination	12/05/10 18:15	OK	C	NT
14	0	Activation of Schneider controller of HPC1.	Schneider controller is activated with environmental conditions set points used for seeds germination and seedlings growth: Light mode: auto, 1 MH and 1 HPS lamps per module on. Fan Mode: auto. Temperature and humidity mode: auto. Day time - 16 hours, night time - 8 hours, T day and night set points = 20°C, day Rh =50%, night Rh=70%, PAR sensors measurements:270-300 µE	12/05/10 18:20 (not HMI time, nor mail)	Light Mode! start at 18:00 day, night start at 04:00! Tunnel hum. mode: OK RAS sensors measurements: RT-4104-01 (module A) 284, 5 µE, RT-4104-02 (module B) 230, 5 µE, RT-4104-03 (module C) 266, 6 µE	C	NT
15	0	Placement of the germination trays into HPC1 and closing of the doors from both sides of HPC1.	Chamber is closed and all conditions in the chamber correspond to conditions for seeds germination	12/05/10 18:32	OK	C	NT
16	1	Opening of the chamber, taking photos of the trays with plastic cover and after without the cover.	Day <sub>1</sub> of crop test	13/05/10 18:05	OK	C	NT
17	1	Check of seedlings germination, repeat in the afternoon	In case not more than 60% of seedlings in each tray can be observed, addition of plastic cover and closing the chamber	13/05/10 18:18	Almost no germination is observed	C	NT



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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10 -4102(0)	01	4 / 7

Compartment : CIVb

Test Phase : 2

02 AF 2015/10

18	1	In the afternoon checking of the seeds germination again. If more than 60% of seedlings are observed opening of the trays. If not the trays are left covered until next day	Check of seedlings germination			DEV.3 EP	MC	
19	2	Opening of the chamber, taking photos of the trays with plastic cover and after without the cover.	Day <sub>2</sub> of crop test	14/05/10 18:20		OK	C	NT
20	2	In case not more than 60% of seedlings in each tray can be observed, addition of plastic cover and closing the chamber	Check of seedlings germination, repeat in the afternoon			DEV.4 EP	MC	
21	2	In the afternoon checking of the seeds germination again. If more than 60% of seedlings are observed opening of the trays. If not the trays are left covered until next day	Check of seedlings germination	14/05/10 18:20		No germination at most, ~10%, trays are left covered	C	NT
22	3	Checking the seeds germination and if more than 60% of seedlings are observed in each tray opening of the trays (taking out the plastic covers).	By this day usually germination percentage is more than 60% per tray and plastic covers can be removed from the trays	15/05/10 9:40		~60% of germination can be observed plastic covers are removed from trays.	C	CM
23	3	Taking photos of both trays	Day <sub>3</sub> of crop test	15/05/10		No taken as camera was not available	C	CM
24	3	Checking of pH of the seedlings nutrient solution and in case it's 5.8-5.9, watering Rockwool cubes with this solution, approximately 900 mL per each tray.	pH of seedlings solution is 5.8-5.9, Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	15/05/10		High humidity in the trays, no necessary to water	C	CM
25	3	Regulation of pH with acid or base in case it is higher than 6.0 or lower than 5.7 (see step No.10)	pH of seedlings solution is 5.7-6.0	15/05/10		Not necessary	C	CM



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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10 -4102(0)	0102	5 / 7

Compartment : CIVb

Test Phase : 2

AF 20/5/10

26	4	Taking photos of both trays	Day <sub>4</sub> of crop test		Sunday, not performed	C	NT
27	4	In the afternoon watering of the plants with the solution if necessary, approximately 500 mL per tray.	Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)		Sunday, not performed	C	NT
28	5	Taking photos of both trays. Watering of the plants with the solution if necessary	Day <sub>5</sub> of crop test. Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	17/05/10 18:25	Pictures are taken. Left tray - 51% of germination, right tray 63% of germination per tray. 1000 ml of solution	C	NT
29	6	Cutting of polypropylene film for trays covering against algae growth at maturity phase	100 stripes using template	20/05/10 11:00	OK Was not possible to do before, but it's not critical	C	NT
30	6	Taking photos of both trays. Watering of the plants with the solution if necessary	Day <sub>6</sub> of crop test. Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	18/05/10 18:05	Pictures are taken. Germination: left tray: 51%, right - 68%. Not watered as not necessary	C	NT
31	7	Checking of pH of the seedlings nutrient solution and in case it's 5.8-5.9, watering Rockwool cubes with this solution, approximately 900 mL per each tray.)	pH of seedlings solution is 5.8-5.9, Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	19/05/10 18:50	pH of nutrient sol'n is 6.0. Watered with 1L of the sol'n left tray and 800ml - right tray.	C	NT
32	7	Regulation of pH with acid or base in case it is higher than 6.0 or lower than 5.7 (see step No.10	pH of seedlings solution is 5.7-6.0			Dev. 5 EP	NC NT
33	7	Taking photos of both trays.	Day <sub>7</sub> of crop test.	19/05/10 18:25	Pictures are taken, 62% of germination - left tray, 73% - right tray	C	NT
34	7	Addition of stock A, stock B, acid and base solutions into the appropriate tanks until a mark on each tank	Tank are filled for maturity test start up	16:20 20.05.10		C	CM
35	7	Addition of 1.3 litres of stock A and 1.3 litres of stock B into the 120L external tank, mixing thoroughly solution in the 120L external tank using a pump	Solution is mixed and ready for transfer into HPC1 nutrient tank	16:30 20.05.10		C	CM

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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10-4102(0)	01/02	6/7

Compartment : CIVb Test Phase : 2 AF 20/5/10

34	7	Transfer of 120 liters of the nutrient solution from external tank into HPC1 nutrient tank using a pump	Solution is transferred without any leak. Level sensor does not indicate that the level is low or high	16:20 19:20 20.05.10			C	CM
35	7	Addition of 40 liters of distilled water into 120L external tank and transfer of 40 liters of distilled water from external tank into HPC1 nutrient tank using a pump	Water is transferred without any leak. Level sensor does not indicate that the level is low or high	16:50 19:20 20.05.10			C	CM
36	7	Taking the trays with seedlings out of the chamber	During 1 hour the trays are in HPC room under ambient conditions					
37	7	Taking 20 trays out of the chamber and wiping 20 trays with paper towel wetted with ethanol	Sterilization of the trays	12:10 20.05.10			C	CM
38	7	Placement of 20 trays into the chamber	Trays are inside the chamber in their nominal position	15:15 21.05.10			C	RM
39	7	Activation of irrigation system from HMI in auto mode in order to adjust pH and EC of the nutrient solution approximately for 30 minutes	Irrigation system is activated, water flow is 10-11 L/min	15:30 21.05.10	pH: 5,05 EC: 1,57		C	RM
40	7	Performance of leakage test of the main nutrient solution collector in the chamber by putting under the connection of the collector several sheets of paper. When the irrigation pump is off, take the paper and check if it is dry.	No leaks  ~ a little leak under connection.	15:15 21/05/10		DEV.6 EP	NC	RM
41	7	When pH is equal to 5.9 and EC to 1.9, disabling of irrigation system	Irrigation system is off, no liquid circulation in the HPC1	16:00 21/05/10	pH: 5,93 EC: 1,90		C	RM
42	7	Taking of 6 samples of nutrient solution from nutrient tank:	Samples of the solution are taken according to the written procedure,	16:15 21/05/10	inside the fridge		C	RM

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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10 -4102(0)	0102	7 / 7

Compartment : CIVb      Test Phase : 2      AF 20/5/10

		<p>a. Irrigation system is off.</p> <p>b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this solution but empty it into sewerage.</p> <p>c. After taking 3 samples continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge.</p> <p>d. Taking 3 samples manually from the top of the nutrient tank and placement them into the fridge</p> <p>f. Closing nutrient tank with plastic cover and screws, making sure it is airtight</p>	<p>nutrient tank is tightly closed</p>	<p>AG:AS</p> <p>21/05/10</p>						
43	8		Day <sub>s</sub> of crop test.							

MPP-REC 10-4102 (0)-02

Conclusion for the Test	Name E. PERRO	Signature <u>Eugene Perro</u>	Date 30.06.10
<input checked="" type="checkbox"/> Passed <input type="checkbox"/> Failed			
- Number of deviations attached to the document : 6 - All deviations have been justified or corrected ? YES / <u>NO</u>			
Comments			
Checked by MELISSA Pilot Plant	Name A. FOSSEN	Signature <u>A. Fossen</u>	Date 30/6/10

Appendix 1 for MPP-REC 10-4102(0)-02

Appendix 1 - record of implied personnel

Name	ORGANIZATION	Function	Initials
Natalia Tikhomirova	Visiting Researcher MPP	Plant Scientist	NT
Cynthia Munganga	MPP	Analysis Technician	CM
Enrique Peiro	MPP	Technical manager	EP

Appendix 2: equal to Appendix 2 for MPP-REC-10-4101(0)-02  
EP

Appendix 3 for MPP-REC 10-4102(0) - 02

Appendix 3 - deviations list

DEV. FORM # 1	Deviation: 10-litre tanks for stock A and B not sterilised when foreseen as they had been sterilised in advance (not recorded)	Criticality Low Medium High
	Corrective action: N.A. (the action was performed although recording should be improved)	Resp. Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by Closing Date
DEV. FORM # 2	Deviation: Preparation of 10L stock A and B and 10L of seeding solution not performed when foreseen as they had been prepared in advance (not recorded)	Criticality Low Medium High
	Corrective action: N.A. (the action was performed although recording should be improved)	Resp. Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by Closing Date
DEV. FORM # 3	Deviation: Not checking seedlings germination as foreseen in the protocol (during the afternoon), but done during the morning	Criticality Low Medium High
	Corrective action: Let more flexible tasks in the new version of the protocol when it's not critical the moment of checking (end COO9)	Resp. Due date EP end of COO9
	Corrective action performed and checked: Ref. of retests:	Checked / approved by Closing Date

# Appendix 3 for MPP-REC-10-4102(o)-02

## Appendix 3 - deviations list

DEV. FORM # <b>4</b>	Deviation:  Same as Dev. 3	Criticality <u>Low</u> Medium High	
	Corrective action:  N.A.	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date

DEV. FORM # <b>5</b>	Deviation:  Regulation of pH with acid or base was not needed as the pH was in good levels (it's not a non compliance)	Criticality <u>Low</u> Medium High	
	Corrective action:  N.A.	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date

DEV. FORM # <b>6</b>	Deviation:  There was found a leak in the nutrient solution collector	Criticality Low <u>Medium</u> High	
	Corrective action:  The leak should be repaired	Resp. RM	Due date 21.05.10
	Corrective action performed and checked: Ref. of retests:  Leak repaired by using the appropriate glue.	Checked / approved by  EP	Closing Date  28.06.10

No test Phase 1 because of the visit, no cleaning

DEV 1  
EP

CMJ 25.06.10



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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10 -4102(0)	03	1 / 7

Compartment : CIVb      Test Phase : 2

Test title : Seedlings phase

Objectives: Test oriented to communication events (ICES conference)

Applicable test plan and test protocols      TN 101.2

Hardware:

Person responsible for the test : Natalia Tikhomirova

Test prerequisites : phase 1 nominally executed

Step No.	Day No.	Action description	Expected results / Nominal behaviour	Date / Hour	Observed results / calculated / remarks - ref. of Deviation	C/N C	Initials
1	0	Sterilization of 2 flats of rockwool small cubes - Grodan AO 36/40 6/15W using following procedure for Rockwool safe manipulation: MPP-OP-10-4101 (0)	Rockwool flats are sterilised and ready for seeds sowing	25.06.10 7:50		NE C	CM NT
2	0	Sterilization of 1 litre of distilled water in autoclave	30 minutes at 120°C	23.06.10 18:50		NE C	CM NT
3	0	Sterilization of 10-litres tanks for stock A and stock B (2), 20-litres tank for seedlings solution in autoclave	30 minutes at 120°C	23.06.10 18:50		NE C	CM NT
4	0	Preparation of 10 L of stock A and 10 L of stock B solutions and 10 L of seedling solution according to TN 96.3. Solutions must be kept in a dark place in order to prevent algae growth	Solutions are prepared	25.06.10 8:15		NE C	CM NT
5	0	Taking a bag with lettuce seeds <i>Lactuca sativa</i> L. cultivar Grand Rapids from the fridge and	Done	25.06.10		NE C	CM NT



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## TEST RECORD SHEET

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03

2 / 7

Compartment : CIVb

Test Phase : 2

		placement about 200 seeds into a glass. Addition of 5% hypochlorite to the glass with the seeds (until bleach covers all the seeds). Sterilization during 15 minutes		14:23 26.05.10		<del>AK</del> C	CM NT
6	0	Placement of the seeds into a sieve and rinse with 1 L of distilled sterile water	Seeds are sterilized but some lost of colour can be observed due to treatment with bleach	14:38 26.05.10		<del>AK</del> C	CM NT
7	0	Disinfection of 2 seed germination trays with 70% ethanol and paper towel	The trays are disinfected and no ethanol can be observed on the trays	14:00 26.05.10		<del>AK</del> C	CM NT
8	0	Placement of 2 flats of rockwool small cubes - Grodan AO 36/40 6/15W into 2 seed-germination trays	Each tray must contain 98 cubes	14:05 26.06.10		<del>AK</del> C	CM NT
9	0	Using tweezers, placement of a single lettuce seed in each hole of Rockwool cubes	Attention should be paid during process of seeds sowing in order not to sow more than 1 seed per cube and not miss any cube	14:40 26.06.10		<del>AK</del> C	CM NT
10	0	Measurement of pH of irrigation solution for seedlings, in case it's 5.8-5.9, watering of Rockwool cubes with this solution.	pH of seedlings solution is 5.8-5.9, Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	14:23 26.06.10	pH=5.80	<del>AK</del> C	CM NT
11	0	If pH is higher than 6.0 or lower than 5.8 addition of about 1 mL of 0.5M HNO <sub>3</sub> or 1mL of 0.5M KOH (depending on pH if it's low or high) to the 10L of the solution, mixing well and checking of pH again. Repetition if necessary until pH is about 5.8-5.9.	pH of seedlings solution is 5.8-5.9				





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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10 -4102(0)	03	3 / 7

Compartment : CIVb Test Phase : 2

12	0	Taking photos of the trays.	Day <sub>0</sub> of crop test (Rockwool sheets without seedlings)	14:50 25.06.10		AV C	CM NT
13	0	Addition of a plastic tray cover to each tray	Trays are covered in order to maintain humidity inside the tray and enhance seeds germination	15:00 25.06.10		AV C	CM NT
14	0	Activation of Schneider controller of HPC1.	Schneider controller is activated with environmental conditions set points used for seeds germination and seedlings growth: Light mode: auto, 1 MH and 1 HPS lamps per module on. Fan Mode: auto. Temperature and humidity mode: auto. Day time - 16 hours, night time - 8 hours, T day and night set points = 20°C, day Rh =50%, night Rh=70%, PAR sensors measurements:270-300 µE	15:20 25.06.10	<p>switch on water (killed) → see behind and do next the fridge -</p> <p>switch ON hot water (bottom)</p> <p>Activate Pump 5b Activate Pump H</p> <p>PAR sensors measurements: <u>348 - 372 µE</u></p>	C	NT
15	0	Placement of the germination trays into HPC1 and closing of the doors from both sides of HPC1.	Chamber is closed and all conditions in the chamber correspond to conditions for seeds germination	15:25 25.06.10		AV C	CM NT
16	(1) 3	Opening of the chamber, taking photos of the trays with plastic cover and after without the cover.	Day <sub>1</sub> of crop test Day 3. NT	12:00 26.06.10		AV C	UG NT
17	(1) 3	Check of seedlings germination, repeat in the afternoon	In case not more than 60% of seedlings in each tray can be observed, addition of plastic cover and closing the chamber	12:00 26.06.10		AV C	UG NT



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## TEST RECORD SHEET

Type

Reference

Chrono :

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03

4 / 7

Compartment : CIVb

Test Phase : 2

18	(1) 3	In the afternoon checking of the seeds germination again. If more than 60% of seedlings are observed opening of the trays. If not the trays are left covered until next day	Check of seedlings germination	16:10 28.06.10		<del>NE</del> C	<del>NT</del> NT
19	2	Opening of the chamber, taking photos of the trays with plastic cover and after without the cover.	Day <sub>2</sub> of crop test		Sunday, not performed	NE	NT
20	2	In case not more than 60% of seedlings in each tray can be observed, addition of plastic cover and closing the chamber	Check of seedlings germination, repeat in the afternoon	Dev.2	Sunday, not performed	NE	NT
21	2	In the afternoon checking of the seeds germination again. If more than 60% of seedlings are observed opening of the trays. If not the trays are left covered until next day	Check of seedlings germination	EP	Sunday, not performed	NE	NT
22	3	Checking the seeds germination and if more than 60% of seedlings are observed in each tray opening of the trays (taking out the plastic covers).	By this day usually germination percentage is more than 60% per tray and plastic covers can be removed from the trays		See step 17	C	NT
23	3	Taking photos of both trays	Day <sub>3</sub> of crop test		See step 16	C	NT
24	3	Checking of pH of the seedlings nutrient solution and in case it's 5.8-5.9, watering Rockwool cubes with this solution, approximately 900 mL per each tray.	pH of seedlings solution is 5.8-5.9, Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	Dev.3	Not performed as there was a confusion between day 1 and day 3	NE	NT
25	3	Regulation of pH with acid or base in case it is higher than 6.0 or lower than 5.7 (see step No.10)	pH of seedlings solution is 5.7-6.0	EP	Not performed as there was a confusion between days and days	NE	NT



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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10 -4102(0)	03	5 / 7

Compartment : CIVb Test Phase : 2

26	4	Taking photos of both trays	Day <sub>4</sub> of crop test	25/06/10 10:40 16:25	Plastic covers are taken out Photos are taken	C	NT
27	4	In the afternoon watering of the plants with the solution if necessary, approximately 500 mL per tray.	Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	28/06/10 16:30	pH of seedlings solution = 5.87. No pH correction is required (see step 25). Each tray is watered with 500 mL of seedlings solution	C	NT
28	5	Taking photos of both trays. Watering of the plants with the solution if necessary	Day <sub>5</sub> of crop test. Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	30/06/10 17:25	The photos were taken No watering as the substrate is wet.	C	NT
29	6	Cutting of polypropylene film for trays covering against algae growth at maturity phase	100 stripes using template	1/07/10	Will be performed on 2/07/10	C	NT
30	6	Taking photos of both trays. Watering of the plants with the solution if necessary	Day <sub>6</sub> of crop test. Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	1/07/10 17:15 17:20	The photos are taken The trays are watered, left tray - 1400 ml of seed. sol'n, right tray - 1100 ml.	C	NT
31	7	Checking of pH of the seedlings nutrient solution and in case it's 5.8-5.9, watering Rockwool cubes with this solution, approximately 900 mL per each tray.)	pH of seedlings solution is 5.8-5.9, Rockwool cubes are wet, but not overwatered (no liquid beneath Rockwool)	2/07/10 18:50	The plants are watered with nutrient solution, 900 mL per each tray.	C	NT
32	7	Regulation of pH with acid or base in case it is higher than 6.0 or lower than 5.7 (see step No.10)	pH of seedlings solution is 5.7-6.0	2/07/10	Was not necessary to check and regulate pH as it was done 3 days ago	NO	NT
33	7	Taking photos of both trays.	Day <sub>7</sub> of crop test.	2/07/10 18:00	The photos are taken.	C	NT
34	(7) 10	Addition of stock A, stock B, acid and base solutions into the appropriate tanks until a mark on each tank	Tank are filled for maturity test start up	5/07/10 11:25 Day 10	OK	C	NT
35	(7) 10	Addition of 1.3 litres of stock A and 1.3 litres of stock B into the 120L external tank, mixing thoroughly solution in the 120L external tank using a pump	Solution is mixed and ready for transfer into HPC1 nutrient tank	5/07/10 11:25 Day 10	OK	C	NT

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TEST RECORD SHEET		Type	Reference	Chrono :	Page :	
		MPP-REC	10 -4102(0)	03	6 / 7	
Compartment : CIVb		Test Phase : 2				
34	(7) 10	Transfer of 120 liters of the nutrient solution from external tank into HPC1 nutrient tank using a pump	Solution is transferred without any leak. Level sensor does not indicate that the level is low or high	5/06/10 11:50 07 EP	OK	C NT
35	(7) 10	Addition of 40 liters of distilled water into 120L external tank and transfer of 40 liters of distilled water from external tank into HPC1 nutrient tank using a pump	Water is transferred without any leak. Level sensor does not indicate that the level is low or high	5/06/10 13:00 07 EP	OK	C NT
36	(7) 10	Taking the trays with seedlings out of the chamber	During 1 hour the trays are in HPC room under ambient conditions	5/06/10 8:30 07 EP	OK	C NT
37	(7) 10	Taking 20 trays out of the chamber and wiping 20 trays with paper towel wetted with ethanol	Sterilization of the trays	5/06/10 10:00 07 EP	OK	C NT
38	7	Placement of 20 trays into the chamber	Trays are inside the chamber in their nominal position	07 EP		NE NT
39	(7) 10	Activation of irrigation system from HMI in auto mode in order to adjust pH and EC of the nutrient solution approximatey for 30 minutes	Irrigation system is activated, water flow is 10-11 L/min	5/06/10 14:00	OK	C NT
40	7	Performance of leakage test of the main nutrient solution collector in the chamber by putting under the connection of the collector several sheets of paper. When the irrigation pump is off, take the paper and check if it is dry.	No leaks	5/07/10 15:30	Leakage test with distilled water. Before closing of polypropylen film and nutrient tank cleaning was performed (10:40-13:00)	C NT
41	7	When pH is equal to 5.9 and EC to 1.9, disabling of irrigation system	Irrigation system is off, no liquid circulation in the HPC1	07 EP	Dev. 5	NE NT
42	7	Taking of 6 samples of nutrient solution from nutrient tank:	Samples of the solution are taken according to the written procedure,	5/06/10 15:00	3 samples are taken from drain valve, not necessary to take samples from the tap	C NT

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Dev. 6  
EP



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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10 -4102(0)	03	7 / 7

Compartment : CIVb      Test Phase : 2

		<p>a. Irrigation system is off.</p> <p>b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this solution but empty it into sewerage.</p> <p>c. After taking 3 samples continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge.</p> <p>d. Taking 3 samples manually from the top of the nutrient tank and placement them into the fridge</p> <p>f. Closing nutrient tank with plastic cover and screws, making sure it is airtight</p>	<p>nutrient tank is tightly closed</p>		<p><i>as solution is homogenous in the nutrient tank</i></p>		
				07 EP			
43	(8) 10		Day <sub>8</sub> of crop test.	5/06/10 14:00			C NT

MPP-REC 10-4102(0) - 03

Conclusion for the Test	Name E. REIRO	Signature <u>Enrique Peñu</u>	Date 30.07.10
<input checked="" type="checkbox"/> Passed <input type="checkbox"/> Failed			
- Number of deviations attached to the document : 7 - All deviations have been justified or corrected? YES / <u>NO</u>			
Comments			
Checked by MELISSA Pilot Plant	Name A. FERRER	Signature <u>A Ferrer</u>	Date 30/7/10

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Appendix 1 to MPP-REC 10-4102 (0)-03

Appendix 1 - record of implied personnel

Name	ORGANIZATION	Function	Initials
Natalia Tikhomirova	Visiting Researcher MPP	Plant Scientist	NT
Cynthia Munganga	MPP	Analysis Technician	CM
Enrique Peiro	MPP	Technical manager	EP

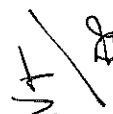
Appendix 2 for MPP-REC

10-4102(0) -03

Appendix 2 - record of calibration certificates for the test instruments

Instrument description	HPC1 TAG	Inv. number	Calibration record reference	Date of calibration	Calibration valid until	Signature
pH sensor	AT_4107_01		NT Logbook	25.06.10	Next test	
Electrical Conductivity of nutrient	AT_4108_01		NT Logbook	25.06.10	Next test	
Humidity A1 associated with temp A1	AT_4112_01		Manufacturer certificate	26.09.07		
Humidity B1 associated with temp B1	AT_4112_02		Manufacturer certificate	26.09.07		
Humidity C1 associated with temp C1	AT_4112_03		CIFA calibration report	08.06.10		
CO2 Analyser	AT_4113_01		TN 96.11	23.07.09		
O2 Analyser	AT_4113_02		TN 96.11	23.07.09		
CO2 Mass Flow	FQRC_4113_01		Manufacturer certificate	16.08.07		
Outlet nutrient flow sensor	FT_4106_01		Manufacturer certificate			
Air velocity sensor	FT_4111_01		Manufacturer certificate			
Pressure sensor for airlock A	PT_4102_01		Manufacturer certificate	26.06.07		
Pressure sensor for airlock C --> Reaffected to External Pressure	PT_4103_01		Manufacturer certificate	26.06.07		NT / EP



Growing Area Pressure	PT_4114_01		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_02		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_03		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_04		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_05		Manufacturer certificate	26.06.07		
Growing Area Pressure	PT_4114_06		Manufacturer certificate	26.06.07		
PAR Sensor - A	RT_4104_01		NTE-HPC_HVAC-RP-004	July 2009		
PAR Sensor - B	RT_4104_02		NTE-HPC_HVAC-RP-004	July 2009		
PAR Sensor - C	RT_4104_03		NTE-HPC_HVAC-RP-004	July 2009		
Light Loft Temperature sensor A	TT_4105_01		Manufacturer certificate	26.09.07		
Light Loft Temperature sensor B	TT_4105_02		CIFA calibration report	08.06.10		
Light Loft Temperature sensor C	TT_4105_03		CIFA calibration report	08.06.10		
Temperature sensor for solution reservoir	TT_4109_01		CIFA calibration report	08.06.10		NT / 

Appendix 2 for MPP REC 50-4102 (0) -03

Light Loft Temperature sensor B	TT_4105_02	Manufacturer certificate	26.09.07	
Light Loft Temperature sensor C	TT_4105_03	Manufacturer certificate	26.09.07	
Temperature sensor for solution reservoir	TT_4109_01	Manufacturer certificate	26.09.07	
Temperature A1 associated with humidity	TT_4112_01	Manufacturer certificate	26.09.07	
Temperature B1 associated with humidity	TT_4112_02	Manufacturer certificate	26.09.07	
Temperature C1 associated with humidity	TT_4112_03	Manufacturer certificate	26.09.07	Dev. 2
Temperature A2	TT_4112_04	Manufacturer certificate	26.09.07	
Temperature A3	TT_4112_05	Manufacturer certificate	26.09.07	
Temperature ambient	TT_4112_06	Manufacturer certificate	26.09.07	
Temperature B2	TT_4112_07	Manufacturer certificate	26.09.07	
Temperature B3	TT_4112_08	Manufacturer certificate	26.09.07	
Temperature B4	TT_4112_09	Manufacturer certificate	26.09.07	NT/EP

Temperature C2	TT_4112_10		Manufacturer certificate	26.09.07		
Temperature C3	TT_4112_11		Manufacturer certificate	26.09.07		
Temperature ambient	TT_4112_12		Manufacturer certificate	26.09.07		
Temperature for facility chilled water	TT_4112_13		Manufacturer certificate	26.09.07		
Temperature for facility hot water line	TT_4112_14		Manufacturer certificate	26.09.07		
Chilled coil surface temperature	TT_4112_15		Manufacturer certificate	26.09.07		
Heating coil surface temperature	TT_4112_16		Manufacturer certificate	26.09.07		
Outlet Air, chilled exchanger	TT_4112_19		Manufacturer certificate	26.09.07		
Outlet Air, hot exchanger	TT_4112_20		Manufacturer certificate	26.09.07		
Inlet water Chilled Exchanger	TT_4112_21		Manufacturer certificate	26.09.07		
Inlet water Hot Exchanger	TT_4112_22		Manufacturer certificate	26.09.07		NT/8

26.09.07

# Appendix 3 to MPRREC 10-4102(o)-03

## Appendix 3 - deviations list

DEV. FORM # 1	Deviation: Test phase 1 was not performed: no cleaning was performed and leak test as well. (Test oriented to communication)	Criticality Low Medium <del>High</del> EP	
	Corrective action: Not necessary as the test is intended to demonstrate operation for communication events in July.	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date
DEV. FORM # 2	Deviation: Check of seedlings and pictures not performed during the weekend (performed daily when working days)	Criticality Low Medium High	
	Corrective action: To increase flexibility for non critical tasks not date-dependant in the new edition of the protocol (End 005)	Resp. EP	Due date End of 009
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date
DEV. FORM # 3	Deviation: pH was not checked in the nutrient solution and watering, but watering was performed on day 4, not affecting the plants	Criticality Low Medium High	
	Corrective action: N.D.	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date

Appendix 3 to MPP-REC 10-4102(0) -03

Appendix 3 - deviations list

<p>DEV. FORM # 4</p>	<p>Deviation: pH of seedling solution was not adjusted.</p>	<p>Criticality Low Medium High</p>
	<p>Corrective action: To define better in the new version of the protocol the flexibility possible for pH adjustment or the criticality (end of CO09)</p>	<p>Resp. Due date EP End of CO09</p>
	<p>Corrective action performed and checked: Ref. of retests:</p>	<p>Checked / approved by Closing Date</p>
<p>DEV. FORM # 5</p>	<p>Deviation: Impaction system was not stopped before taking the samples.</p>	<p>Criticality Low Medium High</p>
	<p>Corrective action: (IT is not critical for the process) N.A.</p>	<p>Resp. Due date</p>
	<p>Corrective action performed and checked: Ref. of retests:</p>	<p>Checked / approved by Closing Date</p>
<p>DEV. FORM # 6</p>	<p>Deviation: Samples were not taken from the top as solution is homogeneous in the nutrient tank (previous results)</p>	<p>Criticality Low Medium High</p>
	<p>Corrective action: To define differently the sampling procedure in the next edition of the protocol or corresponding OP (end of CO09)</p>	<p>Resp. Due date EP End of CO09</p>
	<p>Corrective action performed and checked: Ref. of retests:</p>	<p>Checked / approved by Closing Date</p>

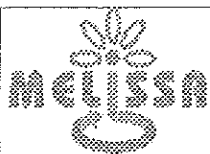
Appendix 3 to MAP-REC 10-4102 (0)-03

Appendix 3 - deviations list

DEV. FORM # 7	Deviation: The validity of <sup>of</sup> period for the calibration of instruments is not defined	Criticality Low Medium High	
	Corrective action: To establish an HPC-1 maintenance plan, including the validity period for each calibrated performed on the sensors.	Resp. RM / <sup>of</sup>	Due date Before the stopped cultures
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date
DEV. FORM #	Deviation:	Criticality Low Medium High	
	Corrective action:	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date
DEV. FORM #	Deviation:	Criticality Low Medium High	
	Corrective action:	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date

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# MELISSA Pilot Plant

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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10 -4103(0)	01	1 / 11

Compartment : CIVb      Test Phase : 3

Test title : Maturity phase

Objectives:

Applicable test plan and test protocols      TN 101.2

Hardware:

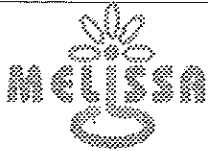
Person responsible for the test : Natalia Tikhomirova

Test prerequisites :

Step No.	Day No.	Action description	Expected results / Nominal behaviour	Date / Hour	Observed results / calculated / remarks - ref. of Deviation	C/N C	Initials
1	8	Use of procedure for Rockwool safe manipulation: OP-10-4101 (0) in order to prepare rockwool large cubes – Grodan Delta 4G 42/40 for seedlings planting	Rockwool large cubes – Grodan Delta 4G 42/40 are dampened and can be used for the following planting	12/03/10 10:00	OK	C	CM
2	8	Taking trays with seedlings out of the chamber. Taking photos of the seedlings before starting transfer to bigger rockwool cubes.	t <sub>8</sub> of crop test	12/03/10 10:20		C	MT
3	8	Selection of 100 small rockwool cubes with seedlings that look similar (height of the plants, size and quantity of leaves). Taking photos of these plants.	Selected plants are practically the same in their morphology and will be used for transfer to bigger rockwool cubes	12/03/10 11:40		C	MT
4	8	Taking 20 trays out of the chamber	Trays are prepared for planting	12/03/10 11:45		C	VG

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# MELISSA Pilot Plant

## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10 -4103(0)	01	2 / 11

Compartment : CIVb Test Phase : 3

5	8	Placement of 5 big Rockwool cubes into each tray, dampen with distilled water if necessary	Cubes are prepared for planting	12/03/10 12:00		C	NT VG CM
6	8	Covering of each tray with polypropylene nutrient film. Placement of small rockwool cubes with seedlings into each hole of big rockwool cubes	Attention should be paid during transfer process. Seedlings are not to be under the nutrient film. Size of the holes in the nutrient film can be increased if necessary.	12/03/10 12:00- 13:20		C	NT VG CM
7	8	Taking of photos of several trays with transferred seedlings. Placement of 20 trays with transferred seedlings into HPC1	Beginning of maturity phase of crop test	12/03/10 12:00- 13:10		C	NT
8	8	Activation of control system: Light Mode: auto, fan mode: auto, all lamps on, day/night=16hours/8hours Temp. and Hum.mode: auto, air blower mode: auto, day average temperature =26°C, night average temperature=20°C, day average humidity=50%, night average humidity=70%. Irrigation mode: auto, pH mode: auto, EC mode: auto, pH set point=5.9, EC set point=1.9 mS/cm, Condensate level: auto.	All activated loops are running	12/03/10 13:00- -13:37		C	NT
9	8	When all lamps are on taking photos of all trays with seedlings inside the chamber before closing the chamber	View of the plants inside the chamber before tightening of the system	12/03/10 13:45		C	NT

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# MELISSA Pilot Plant

## TEST RECORD SHEET

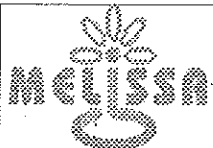
Type	Reference	Chrono :	Page :
MPP-REC	10 -4103(0)	01	3 / 11

Compartment : CIVb Test Phase : 3

10	8	Closing of the inner airlock doors (curtains) and latching of the outer doors. Activating of CO <sub>2</sub> mode: auto, CO <sub>2</sub> setpoint= 1000 ppm	Chamber is tightly closed and crop test is started	12/03/10 13:50		C	NT
11	8	Addition of stock A, stock B, acid and base solutions into the appropriate tanks until a mark on each tank	Tanks are filled until initial volume (2.5 L)	12/03/10 16 <sup>00</sup>		C	NT
12	9	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants from 3 windows	Results are studied and kept together with test protocol	15/03/10 11:00	History graphs were printed later as it was Saturday. Photos not taken.	C	NT
13	10	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants from 3 windows	Results are studied and kept together with test protocol	15/03/10 12:00	History graphs were printed later as it was Sunday. Photos not taken	C	NT
14	11	Disabling of CO <sub>2</sub> control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO <sub>2</sub> control	Use of peristaltic pump for taking a sample	15/03/10 18:17- -18:23	OK	C	NT
15	11	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants from 3 windows	Results are studied and kept together with test protocol	15/03/10 13:00 18:50	OK Photos were taken	C	NT
16	11	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics Max value?	15/03/10 17:00- 18:30	Not stable repetitive results.	C	NT OM
17	11	Taking of 3 samples of nutrient solution from nutrient tank: a. Irrigation system is off. b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the	Samples of the solution are taken according to the written procedure, nutrient tank is tightly closed	15/03/10 19:38- -19:57	OK	C	NT

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# MELISSA Pilot Plant

## TEST RECORD SHEET

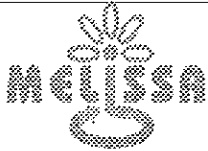
Type	Reference	Chrono :	Page :
MPP-REC	10 -4103(0)	01	4 / 11

Compartment : CIVb Test Phase : 3

		valve. Don't keep this solution but empty it into sewerage. c. After taking 3 samples continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge. d. Enabling of irrigation system in auto mode					
18	12	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	16/03/10 12:30- 13:30- 17:30- 17:35	Printing history graphs, results are nominal taking photos	C	NT
19	13	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	17/03/10 12:30- 13:40 18:45- 18:50	Printing history graphs, results are nominal taking photos	C	NT
20	14	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	18/03/10 15:00- 16:00 18:15	Printing history graphs, results are nominal Photos taking	C	NT
21	15	Changeover of the nutrient solution: disabling of irrigation mode, closing with manual valves inlet and outlet of the liquid in the chamber. Opening nutrient tank and taking 3 samples of nutrient solution from the top of the tank (manually) and from the bottom using drain valve (after outpouring first 3 samples into sewerage). Emptying of nutrient tank using drain valve. Rinsing of nutrient tank with distilled water. Preparation of nutrient solution in external tank (see TN 96.3) and transfer into nutrient tank. Taking 6 samples of nutrient solution: 3 from	Solution is changed and samples of solution before and after changeover are taken.	19/03/10 11:05- 12:25	Solution is changed and samples are taken.	C	NT VG

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# MELISSA Pilot Plant

## TEST RECORD SHEET

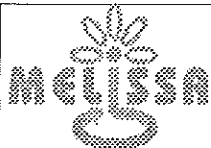
Type	Reference	Chrono :	Page :
MPP-REC	10 -4103(0)	01	5 / 11

Compartment : CIVb Test Phase : 3

		the top and 3 from the bottom. Sealing of nutrient tank. Opening of inlet and outlet of liquid in the liquid loop. Enabling of CO <sub>2</sub> control.			17:00-18:30 - ethylene measure-ments, results are not repeatable	C	CM
22	15	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	19/03/10 17:23- 18:23 18:25	Printing history, results are nominal  Taking pictures	C	NT
23	16	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	22/03/10 10:10- 10:43	Since solution was changed on 18/03, some distortion in data; irrigation mode could be observed. Air flow sensor doesn't work.	C	NT
24	17	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	22/03/10 10:46- 11:21	Air flow sensor doesn't work.	C	NT
25	18	Disabling of CO <sub>2</sub> control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO <sub>2</sub> control	Use of peristaltic pump for taking a sample	22/03/10 17:57- 18:05	For atmospheric control : CO <sub>2</sub> min-max values? O <sub>2</sub> min-max values? <del>et</del>	C	CM
26	18	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics	22/03/10 18:10- 18:45	results are not repeatable	C	CM
27	18	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	22/03/10 11:24- 11:43 16:45	Air flow sensor doesn't work  Photos taking	C	NT
28	18	Taking of 3 samples of nutrient solution from nutrient tank: a. Irrigation system is off. b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this	Samples of the solution are taken according to the written procedure, nutrient tank is tightly closed	22/03/10 17:19- 17:44	3 samples are taken	C	NT

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# MELISSA Pilot Plant

## TEST RECORD SHEET

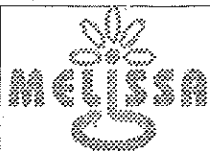
Type	Reference	Chrono :	Page :
MPP-REC	10 -4103(0)	01	6 / 11

Compartment : CIVb Test Phase : 3

		solution but empty it into sewerage. c. After taking 3 samples continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge. d. Enabling of irrigation system in auto mode		26.			
29	19	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	23/03/10 18:16 18:27	Printing history graphs, air velocity sensor doesn't work. Taking photos	C	NT
30	20	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	24/03/10 20:00 20:30	Taking photos Printing history graphs, air velocity sensor doesn't work	C	NT
31	21	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	25/03/10 18:10 18:30	Printing history, air velocity sensor doesn't work Taking photos	C	NT
32	22	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics				
33	22	Changeover of the nutrient solution: disabling of irrigation mode, closing with manual valves inlet and outlet of the liquid in the chamber. Opening nutrient tank and taking 3 samples of nutrient solution from the top of the tank (manually) and from the bottom using drain valve (after outpouring first 3 samples into sewerage). Emptying of nutrient tank using drain valve. Rinsing of nutrient tank with distilled water. Preparation of nutrient	Solution is changed and samples of solution before and after changeover are taken.	26.03.10 11:00 11:20	3 samples are taken from the top and 3 others from the drain valve. preparation of 8 120 L of nutrient solution Probleme with water there is not more water in the department	C	CM

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<b>TEST RECORD SHEET</b>	Type	Reference	Chrono :	Page :
	MPP-REC	10 -4103(0)	01	7 / 11

Compartment : CIVb Test Phase : 3

		solution in external tank (see TN 96.3) and transfer into nutrient tank. Taking 6 samples of nutrient solution: 3 from the top and 3 from the bottom. Sealing of nutrient tank. Opening of inlet and outlet of liquid in the liquid loop. Enabling of CO <sub>2</sub> control.	13:40 40L was added in the nutrient tank 14:00 IRRIGATION Node : Auto fill to have 10L/min			C	CM
34	22	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	16/03/10 18:30 19:45	Air velocity sensor doesn't work Photos	C	NT
35	23	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				
36	24	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol				
37	25	Disabling of CO <sub>2</sub> control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO <sub>2</sub> control	Use of peristaltic pump for taking a sample	29.03.10 19:30	OK	C	CM
38	25	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics	29/03/10 19:30	Calibration curve of ethylene was done and gas sample was taken for GC	C	CM
39	25	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	23/03/10 16:30	Taking photos	C	NT
40	25	Taking of 3 samples of nutrient solution from nutrient tank: a. Irrigation system is off.	Samples of the solution are taken according to the written procedure, nutrient tank is tightly closed	23/03/10 16:44		C	NT

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# MELISSA Pilot Plant

## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10 -4103(0)	01	8 / 11

Compartment : CIVb Test Phase : 3

		b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this solution but empty it into sewerage. c. After taking 3 samples continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge. d. Enabling of irrigation system in auto mode					
41	26	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	30/03/10 18:00	Taking pictures	C	NT
42	26	Labeling of 100 paper bags for drying lettuce leaves, 100 paper bags for drying lettuce roots, 100 plastic bags for storing rockwool roots with roots inside in a fridge	Done	26.03.10 29.03.10 30.03.10 30.03.10 10:59	Beginning of labelling Finishing of labelling weighing of paper bags. See 46		
43	27	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	31/03/10 10:45 - 11:15 18:20	Printing history graphs, then, add on's date to power cut off on 30.03 7am-3am Taking pictures	C	NT
44	27	Labeling 6 plastic cups for taking samples of nutrient solution in the end of the test.	Done	31.03.10 17:32	OK	C	CM
45	27	Placing of 2 scales to HPC room from the department and their adjustment.	Done	31.03.10 17:15	OK	C	CM

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## TEST RECORD SHEET

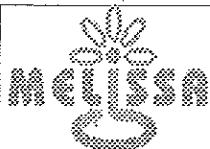
Type	Reference	Chrono :	Page :
MPP-REC	10 -4103(0)	01	9 / 11

Compartment : CIVb Test Phase : 3

46	27	Weighing of each paper bag for leaves and roots drying and recording its weight (special template is ready for that)	Done	30.03 10:59	OK	C	NT
43	28	Disabling of CO <sub>2</sub> control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO <sub>2</sub> control	Use of peristaltic pump for taking a sample	1/04/10 8:52	Sample is taken, but CO <sub>2</sub> is not enabled as crop test is finished	C	NT
44	28	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics				
45	28	Disabling of all control loops. Opening the nutrient tank and taking 6 samples of nutrient solution: 3 from the top and 3 from the bottom of the tank. Opening the chamber and taking pictures of the plants. Harvesting of the plants using procedure for rockwool safe manipulation. Taking pictures of plants in each tray. Writing down fresh weight of lettuce shoots (TN 96.4). Putting of lettuce shoots into preliminary weighed labeled paper bags. Weighing of paper bags for roots drying.	Plants are harvested, control system is disabled	1/04/10 8:57- -11:13	OK	C	NT
46	28	Delivery of paper bags with lettuce shoots into Veterinary School and plants drying in the oven at 70°C with preliminary	Beginning of lettuce shoots drying	1/04/10 18:00- 20:00	OK	C	NT

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# MELISSA Pilot Plant

<b>TEST RECORD SHEET</b>	Type	Reference	Chrono :	Page :
	MPP-REC	10 -4103(0)	01	10 / 11

Compartment : CIVb      Test Phase : 3

		fixation at 103°C during 10 minutes. Rockwool cubes are stored in a cold cabinet at 4 °C.				
47	29	Delivery of rockwool cubes with roots inside into Veterinary School and roots separation from rockwool using Procedure for Rockwool safe manipulation	Roots are separated and put into the oven for drying at 70°C	8/04/10 8:30 - -16:30		C MT VG
48	30	Cleaning of the trays with soap and water	Trays are clean and stored in the chamber	11/04/10 12:30		C VG CM
49	32	Checking of shoots and roots dry weight in Veterinary School	Weight of 3 samples of shoots and 3 samples of roots from each level in the oven, it total 24 samples	7/04/10 16:00 - 17:30		C MT
50	35	Checking of shoots and roots dry weight in Veterinary School	Weight of 3 samples of shoots and 3 samples of roots from each level in the oven, it total 24 samples			
51	36	Checking of shoots and roots dry weight in Veterinary School. In case weights of the samples don't change, final records of dry biomass should be done next day.	Weight of 3 samples of shoots and 3 samples of roots from each level in the oven, it total 24 samples			
52	37	Recording of dry weight of lettuce shoots and roots	Dry weights are recorded, samples are taken back to MPP	12/04/10 8:30 - 12:00	OK	C MT
53	38	Mixing of dry samples of lettuce shoots in order to have 1 average sample per tray, filling of plastic cups with samples	Samples are prepared for further milling	13/04/10 12:00 - 14:00	OK	C CM
54	39	Mixing of dry samples of lettuce roots in the extraction cabinet of the Department in order to have 1 average sample per tray, filling of plastic	Samples are prepared for storage	16/04/10 9:00 - 10:00	OK	C CM

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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10 -4103(0)	01	11/11

Compartment : CIVb      Test Phase : 3

		cups with samples					
55	40	Delivery of shoots samples to Veterinary School for milling					C NT

Conclusion for the Test	Name E. PEIRO	Signature <i>E. Peiro</i>	Date 30.04.10
<input checked="" type="checkbox"/> Passed <input type="checkbox"/> Failed			
- Number of deviations attached to the document : 0 - All deviations have been justified or corrected ? <input checked="" type="radio"/> YES / NO			
Comments			
Checked by TechnoMembranes EP MELISSA Pilot Plant	Name A. FOSSER	Signature <i>A. Fossier</i>	Date 2/5/10

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Appendix 1 for MPP-REC 10-4103(0)-01

Appendix 1 - record of implied personnel

Name	ORGANIZATION	Function	Initials
Natalia Tikhomirova	Visiting Researcher MPP	Plant Scientist	NT
Cynthia Munganga	MPP	Analysis Technician	CM
Enrique Peiro	MPP	Technical manager	EP

Appendix 2: equal to Appendix 2 for MPP-REC 10-4101(0)-01

EP

Appendix 3 for MPP REC 10-4103(o)-01

Appendix 3 - deviations list

<b>DEV. FORM #</b>	Deviation:			Criticality Low Medium High	
	Corrective action:	<i>EP</i>		Resp.	Due date
	Corrective action performed and checked: Ref. of retests:			Checked / approved by	Closing Date
<b>DEV. FORM #</b>	Deviation:			Criticality Low Medium High	
	Corrective action:	<i>EP</i>		Resp.	Due date
	Corrective action performed and checked: Ref. of retests:			Checked / approved by	Closing Date
<b>DEV. FORM #</b>	Deviation:			Criticality Low Medium High	
	Corrective action:	<i>EP</i>		Resp.	Due date
	Corrective action performed and checked: Ref. of retests:			Checked / approved by	Closing Date



# MELISSA Pilot Plant

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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10-4103(0)	01 02	1 / 11

Compartment : CIVb      Test Phase : 3      AP, 20/5/10

Test title : Maturity phase

Objectives:

Applicable test plan and test protocols      TN 101.2

Hardware:

Person responsible for the test : Natalia Tikhomirova

Test prerequisites :

Step No.	Day No.	Action description	Expected results / Nominal behaviour	Date / Hour	Observed results / calculated / remarks - ref. of Deviation	C/N C	Initials
1	8	Use of procedure for Rockwool safe manipulation: OP-10-4101 (0) in order to prepare rockwool large cubes – Grodan Delta 4G 42/40 for seedlings planting	Rockwool large cubes – Grodan Delta 4G 42/40 are dampened and can be used for the following planting	20/05/10 15:10	OK	C	NT
2	8	Taking trays with seedlings out of the chamber. Taking photos of the seedlings before starting transfer to bigger rockwool cubes.	t <sub>8</sub> of crop test	20/05/10 11:00	OK	C	NT
3	8	Selection of 100 small rockwool cubes with seedlings that look similar (height of the plants, size and quantity of leaves). Taking photos of these plants.	Selected plants are practically the same in their morphology and will be used for transfer to bigger rockwool cubes	20/05/10 15:20		C	NT
4	8	Taking 20 trays out of the chamber	Trays are prepared for planting	<del>12:00</del> 20-05-10		C	NT

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Type	Reference	Chrono :	Page :
MPP-REC	10 -4103(0)	07 02	2 / 11

Compartment : CIVb Test Phase : 3 AF 20/5/10

5	8	Placement of 5 big Rockwool cubes into each tray, dampen with distilled water if necessary	Cubes are prepared for planting	20/05/10 15:30	OK	e	NT VG
6	8	Covering of each tray with polypropylene nutrient film. Placement of small rockwool cubes with seedlings into each hole of big rockwool cubes	Attention should be paid during transfer process. Seedlings are not to be under the nutrient film. Size of the holes in the nutrient film can be increased if necessary.	20/05/10 15:35- 17:30		e	NT VG
7	8	Taking of photos of several trays with transferred seedlings. Placement of 20 trays with transferred seedlings into HPC1	Beginning of maturity phase of crop test	20/05/10 15:35- 17:30		e	NT VG
8	8	Activation of control system: Light Mode: auto, fan mode: auto, all lamps on, day/night=16hours/8hours Temp. and Hum.mode: auto, air blower mode: auto, day average temperature =26°C, night average temperature=20°C, day average humidity=50%, night average humidity=70%. Irrigation mode: auto, pH mode: auto, EC mode: auto, pH set point=5.9, EC set point=1.9 mS/cm, Condensate level: auto.	All activated loops are running	20/05/10 17:45- 18:10	Control system is activated, but leak of irrigation system inside the chamber was detected. So after almost 3 hours it was decided to leave the plants inside but at night conditions with preliminary watering with distilled water until next morning.	c	NT
9	8	When all lamps are on taking photos of all trays with seedlings inside the chamber before closing the chamber	View of the plants inside the chamber before tightening of the system	20/05/10 17:30	Pictures are taken	e	NT

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Type	Reference	Chrono :	Page :
MPP-REC	10-4103(0)	0102	3 / 11

Compartment : CIVb

Test Phase : 3

At 20/5/10

10	8	Closing of the inner airlock doors (curtains) and latching of the outer doors. Activating of CO <sub>2</sub> mode: auto, CO <sub>2</sub> setpoint= 1000 ppm	Chamber is tightly closed and crop test is started	21/05/10 16:00	The test was started with 1 day delay as there was a problem with a leak in the HPC, until that time no light for the plants	C	RM
11	8	Addition of stock A, stock B, acid and base solutions into the appropriate tanks until a mark on each tank	Tanks are filled until initial volume (2.5 L)	16:20 20:50	OK	C	GA
12	9	Disabling of CO <sub>2</sub> control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO <sub>2</sub> control	Use of peristaltic pump for taking a sample	22/05/10	Not taken as there is no equipment for analysis (no column, and with cis of the department not possible to do it)	NE	NT DEL. 1
13	9	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants from 3 windows	Results are studied and kept together with test protocol	22/05/10	Not performed as it's Saturday	C	NT
14	9	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics	22/05/10	Not performed as it's Saturday and no equipment available	C	NT
15	10	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants from 3 windows	Results are studied and kept together with test protocol	23/05/10	Not performed as it's Sunday. Will be done on Tuesday	C	NT
16	11	Disabling of CO <sub>2</sub> control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO <sub>2</sub> control	Use of peristaltic pump for taking a sample	24/05/10	Not performed as no equipment available for analysis (see point 12) step	C	NT DEL. 2
17	11	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants from 3 windows	Results are studied and kept together with test protocol	24/05/10	Not performed as it's a holiday, will be done on Tuesday	C	NT
18	11	Analysis of ethylene concentration in the air sample with GC of the	Results of analyses are presented as graphics	24/05/10	Not performed as no equipment (see step 12)	C	NT DEL. 2

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The foreseen dates are correct, so the changes are not valid.  
Checked by EP. 28.06.10



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Compartment : CIVb

Test Phase : 3

AF 2015/10

		department at least with 5 replications					
19	11 <sup>1/2</sup>	Taking of 3 samples of nutrient solution from nutrient tank: a. Irrigation system is off. b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this solution but empty it into sewerage. c. After taking 3 samples continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge. d. Enabling of irrigation system in auto mode	Samples of the solution are taken according to the written procedure, nutrient tank is tightly closed	24/05/10	Not performed as it is a holiday, will be done on Tuesday	C	NT
20	12 <sup>1/2</sup>	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	25/05/10 18:32	Photos are taken. History graphs for 21.05.10 are saved. 13 samples of nutrient sol-n are taken.	C	NT
21	13 <sup>1/2</sup>	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	26/05/10 15:15	Pictures are taken. History graphs for 22.05-25.05.10 are saved.	C	NT
22	14 <sup>1/2</sup>	Disabling of CO <sub>2</sub> control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO <sub>2</sub> control	Use of peristaltic pump for taking a sample	27/05/10	Not performed as an equipment for analysis is not available	C	NT
23	14 <sup>1/2</sup>	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics	27/05/10	See step 22.	C	NT
24	14 <sup>1/2</sup>	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	27/05/10 17:30 18:00	History graphs from 25.05-26.05.10 are saved. The pictures are taken	C	NT

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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10-4103(0)	0102	5 / 11

Compartment : CIVb Test Phase : 3 Af 20/11/10

25	14/6 15	Changeover of the nutrient solution: disabling of irrigation mode, closing with manual valves inlet and outlet of the liquid in the chamber. Opening nutrient tank and taking 3 samples of nutrient solution from the top of the tank (manually) and from the bottom using drain valve (after outpouring first 3 samples into sewerage). Emptying of nutrient tank using drain valve. Rinsing of nutrient tank with distilled water. Preparation of nutrient solution in external tank (see TN 96.3) and transfer into nutrient tank. Taking 6 samples of nutrient solution: 3 from the top and 3 from the bottom. Sealing of nutrient tank. Opening of inlet and outlet of liquid in the liquid loop. Enabling of CO <sub>2</sub> control.	Solution is changed and samples of solution before and after changeover are taken.	11:05 28.05.10  12:30	Base was added in the Base tank till 2,5L.  4L of Base solution was prepared. The solution was changed after 1 week from crop start-up.	C	CH
26	15/6	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	28/05/10	Not performed as it's Saturday. <del>will be done</del> on Monday	C	NT
27	16/6	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	29/05/10	Not performed as it's Saturday. <del>will be done</del> on Monday	C	NT
28	17/6	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	30/05/10 18:40	History graphs from 28.05-31.05 were saved. Photos are taken.	C	NT
29	18/7	Disabling of CO <sub>2</sub> control for taking air sample from the chamber and	Use of peristaltic pump for taking a sample	31/05/10 18:40	Not performed as equipment for analysis is not available	C	NT

Not performed as it's Sunday

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Type	Reference	Chrono :	Page :
MPP-REC	10-4103(0)	01 02	6 / 11

Compartment : CIVb Test Phase : 3 AF 2015/10

		subsequent ethylene analysis. After sample is taken enabling of CO <sub>2</sub> control					
30	18 <sup>7</sup>	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics	31/05/10	Not performed as equipment for analysis is not available	C	NT
31	18 <sup>7</sup>	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	31/05/10 18:40	History graphs from 26.05.10 to 31.05.10 are saved. Pictures are taken.	C	NT
32	18 <sup>4</sup> 19	Taking of 3 samples of nutrient solution from nutrient tank: a. Irrigation system is off. b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this solution but empty it into sewerage. c. After taking 3 samples continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge. d. Enabling of irrigation system in auto mode	Samples of the solution are taken according to the written procedure, nutrient tank is tightly closed	1/06/10 17:22	Samples are taken with 1 day delay as there was confusion with the dates.	C	NT
33	19 <sup>4</sup>	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	1/06/10 17:50 18:05	History graphs are saved. Photos are taken	C	NT
34	20 <sup>15</sup>	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	2/06/10 17:15	Photos are taken. History graphs are not saved as iFix program doesn't work	C	NT
35	21 <sup>30</sup>	Disabling of CO <sub>2</sub> control for taking air sample from the chamber and subsequent ethylene analysis. After sample is	Use of peristaltic pump for taking a sample	3/06/10	Not performed as equipment for ethylene analysis is not available	C	NT

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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10-4103(0)	01 02	7 / 11

Compartment : CIVb Test Phase : 3 AF 20/5/10

		taken enabling of CO <sub>2</sub> control					
36	21%	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics	3/06/10	Not performed, see step 35	C	NT
37	21%	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	3/06/10 11:45 18:45	History graphs for the period 31/05.10-3.06.10 are saved. Photos are taken	C	NT
38	20%	Changeover of the nutrient solution: disabling of irrigation mode, closing with manual valves inlet and outlet of the liquid in the chamber. Opening nutrient tank and taking 3 samples of nutrient solution from the top of the tank (manually) and from the bottom using drain valve (after outpouring first 3 samples into sewerage). Emptying of nutrient tank using drain valve. Rinsing of nutrient tank with distilled water. Preparation of nutrient solution in external tank (see TN 96.3) and transfer into nutrient tank. Taking 6 samples of nutrient solution: 3 from the top and 3 from the bottom. Sealing of nutrient tank. Opening of inlet and outlet of liquid in the liquid loop. Enabling of CO <sub>2</sub> control.	Solution is changed and samples of solution before and after changeover are taken.	4/06/10 15:21 -17:52	Nutrient solution is changed, samples are taken	C	NT
39	22%	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	4/06/10 18:00 18:30	History graphs are saved, increase of pH unexpected from 3.06.10. photos are taken	C	NT

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## TEST RECORD SHEET

Type	Reference	Chrono :	Page :
MPP-REC	10-4103(0)	0102	8 / 11

Compartment : CIVb Test Phase : 3 AF 2015/10

40	23 <del>24</del>	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	6/7/06/10	A base solution Kolt was prepared 4L. Before that Base tank was filled with Base solution till 2.5 L	C	OK
41	24 <del>25</del>	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	6/10/06/10	Not performed as it is Sunday, will be done on Monday	C	NT
42	25 <del>26</del>	Disabling of CO <sub>2</sub> control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO <sub>2</sub> control	Use of peristaltic pump for taking a sample	7/06/10	Not performed as equipment analysis is not available. Des. 3	C	NT
43	25 <del>26</del>	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics	7/06/10	See step 42	C	NT
44	25 <del>26</del>	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	7/06/10 15:30-17:00 17:30	History graphs from 4.06.10 to 7.06.10 are saved Photos are taken	C	NT
45	25 <del>26</del>	Taking of 3 samples of nutrient solution from nutrient tank: a. Irrigation system is off. b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this solution but empty it into sewerage. c. After taking 3 samples continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge. d. Enabling of irrigation system in auto mode	Samples of the solution are taken according to the written procedure, nutrient tank is tightly closed	7/06/10 18:00	↓ Samples are taken	C	NT

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		MPP-REC	10-4103(0)	0102	9 / 11		
Compartment : CIVb		Test Phase : 3		AF 20/5/10			
46	26 <del>25</del>	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	8/06/10 17:23 17:50	History graphs are saved. Photos are taken	C	NT
47	27 <del>26</del>	Printing history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied and kept together with test protocol	8/06/10 18:14 18:40	History graphs are saved. Photos are taken	C	NT
48	28 <del>27</del>	Disabling of CO <sub>2</sub> control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO <sub>2</sub> control	Use of peristaltic pump for taking a sample	<del>18:40</del> 18:06/10	Not done	NC	CM
49	28 <del>27</del>	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics		Not done	NC	CM
50	28 <del>27</del>	Disabling of all control loops. Opening the nutrient tank and taking 6 samples of nutrient solution: 3 from the top and 3 from the bottom of the tank. Opening the chamber and taking pictures of the plants. Harvesting of the plants using procedure for rockwool safe manipulation. Taking pictures of plants in each tray. Writing down fresh weight of lettuce shoots (TN 96.4). Putting of lettuce shoots into preliminary weighed labeled paper bags. Weighing of paper bags for roots drying.	Plants are harvested, control system is disabled	18:06/10		C	CM

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0102

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Compartment : CIVb

Test Phase : 3

AF 20/5/10

51	28 <del>28</del>	Delivery of paper bags with lettuce shoots into Veterinary School and plants drying in the oven at 70°C with preliminary fixation at 103°C during 10 minutes. Rockwool cubes are stored in a cold cabinet at 4 °C.	Beginning of lettuce shoots drying	11/06/10		C	CM
52	29 <del>29</del>	Delivery of rockwool cubes with roots inside into Veterinary School and roots separation from rockwool using Procedure for Rockwool safe manipulation	Roots are separated and put into the oven for drying at 70°C	11/06/10	this was done in MPP	NC	CM
53	30 <del>30</del>	Cleaning of the trays with soap and water	Trays are clean and stored in the chamber	X			
54	32 <del>32</del>	Checking of shoots and roots dry weight in Veterinary School	Weight of 3 samples of shoots and 3 samples of roots from each level in the oven, it total 24 samples		Not done	C	CM
55	35 <del>35</del>	Checking of shoots and roots dry weight in Veterinary School	Weight of 3 samples of shoots and 3 samples of roots from each level in the oven, it total 24 samples		Not done	C	CM
56	36 <del>36</del>	Checking of shoots and roots dry weight in Veterinary School. In case weights of the samples don't change, final records of dry biomass should be done next day.	Weight of 3 samples of shoots and 3 samples of roots from each level in the oven, it total 24 samples		Not done	C	CM
57	37 <del>37</del>	Recording of dry weight of lettuce shoots and roots	Dry weights are recorded, samples are taken back to MPP	15/06/10		C	CM
58	38 <del>38</del>	Mixing of dry samples of lettuce shoots in order to have 1 average sample per tray, filling of plastic cups with samples	Samples are prepared for further milling	20.09.10		C	CM

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Compartment : CIVb Test Phase : 3 AF 20/5/10

59	39 <del>38</del>	Mixing of dry samples of lettuce roots in the extraction cabinet of the Department in order to have 1 average sample per tray, filling of plastic cups with samples	Samples are prepared for storage	20-09-10	Done in NPP	C	CM
60	40 <del>39</del>	Delivery of shoots samples to Veterinary School for milling		28/06/10		C	CM

Conclusion for the Test	Name E. PETRO	Signature <i>E. Petro</i>	Date 20.06.10
<input checked="" type="checkbox"/> Passed <input type="checkbox"/> Failed			
- Number of deviations attached to the document : 3 - All deviations have been justified or corrected? YES / (NO)			
Comments			
Checked by <del>TechnoMembranes</del> EP MELISSA Pilot Plant	Name A. FOSSEN	Signature <i>A. Fossen</i>	Date 20/6/10

Appendix 1 for MPP-REC 10-4103(0)-02

Appendix 1 - record of implied personnel

Name	ORGANIZATION	Function	Initials
Natalia Tikhomirova	Visiting Researcher MPP	Plant Scientist	NT
Cynthia Munganga	MPP	Analysis Technician	CM
Enrique Peiro	MPP	Technical manager	EP

Appendix 2 : equal to Appendix 2 for MPP-REC 10-4101(0)-02

EP

Appendix 3 for MPP-REC 10-4103(0)-02

Appendix 3 - deviations list

DEV. FORM # 1	Deviation: Analysis of Ethylene not performed for not available column and not adequate limit of detection of the available method	Criticality Low Medium High
	Corrective action: Search for an alternative method/equipment for ethylene analysis (It could involve a high investment)	Resp. Due date EP
	Corrective action performed and checked: Ref. of retests:	Checked / approved by Closing Date
DEV. FORM # 2	Deviation: Several tasks were not performed as they coincided with week-end: pictures, <del>See dev. 2</del> print history graphs (not EP critical to be performed at that time)	Criticality Low Medium High
	Corrective action: Potential increase the flexibility <del>N.A.</del> of non critical tasks for some EP dates in new version of protocol (end 2009)	Resp. Due date EP End of 2009
	Corrective action performed and checked: Ref. of retests:	Checked / approved by Closing Date
DEV. FORM # 3	Deviation: See Dev. 1.	Criticality Low Medium High
	Corrective action: N.A.	Resp. Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by Closing Date





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<b>TEST RECORD SHEET</b>	Type	Reference	Chrono :	Page :
	MPP-REC	10 -4103(0)	03	1 110

Compartment : CIVb      Test Phase : 3

Test title : Maturity phase

Objectives:

Applicable test plan and test protocols

Hardware:

Person responsible for the test : Natalia Tikhomirova

Test prerequisites :

Step No.	Day No.	Action description	Expected results / Nominal behaviour	Date / Hour	Observed results / calculated / remarks - ref. of Deviation	C/N C	Initials
1	10	Use of procedure for Rockwool safe manipulation: OP-10-4101 (0) in order to prepare rockwool large cubes – Grodan Delta 4G 42/40 for seedlings planting	Rockwool large cubes – Grodan Delta 4G 42/40 are dampened and can be used for the following planting	5/07/10 11:00	OK	C	NT
2	10	Taking trays with seedlings out of the chamber. Taking photos of the seedlings before starting transfer to bigger rockwool cubes.	t <sub>10</sub> of crop test	5/07/10 10:00	OK	C	NT
3	10	Selection of 100 small rockwool cubes with seedlings that look similar (height of the plants, size and quantity of leaves). Taking photos of these plants.	Selected plants are practically the same in their morphology and will be used for transfer to bigger rockwool cubes	5/07/10 10:30	OK	C	NT
4	10	Taking 20 trays out of the chamber	Trays are prepared for planting	5/07/10 8:30	OK	C	NT

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Compartment : CIVb Test Phase : 3

5	10	Placement of 5 big Rockwool cubes into each tray, dampen with distilled water if necessary	Cubes are prepared for planting	5/07/10 12:00	OK	c	NT
6	10	Covering of each tray with polypropylene nutrient film. Placement of small rockwool cubes with seedlings into each hole of big rockwool cubes	Attention should be paid during transfer process. Seedlings are not to be under the nutrient film. Size of the holes in the nutrient film can be increased if necessary.	5/07/10 12:00 - 13:00	OK	c	NT
7	10	Taking of photos of several trays with transferred seedlings. Placement of 20 trays with transferred seedlings into HPC1	Beginning of maturity phase of crop test	5/07/10 12:00 - 13:00	OK	c	NT
8	10	Activation of control system: Light Mode: auto, fan mode: auto, all lamps on, day/night=16hours/8hours Temp. and Hum.mode: auto, air blower mode: auto, day average temperature =26°C, night average temperature=20°C, day average humidity=50%, night average humidity=70%. Irrigation mode: auto, pH mode: auto, EC mode: auto, pH set point=5.9, EC set point=1.9 mS/cm, Condensate level: auto.	All activated loops are running	5/07/10 14:00	OK	c	NT
9	10	When all lamps are on taking photos of all trays with seedlings inside the chamber before closing the chamber	View of the plants inside the chamber before tightening of the system	5/07/10 13:55	OK	c	NT

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<b>TEST RECORD SHEET</b>	Type	Reference	Chrono :	Page :
	MPP-REC	10 -4103(0)	03	3 / 10

Compartment : CIVb      Test Phase : 3

10	10	Closing of the inner airlock doors (curtains) and latching of the outer doors. Activating of CO <sub>2</sub> mode: auto, CO <sub>2</sub> setpoint= 1000 ppm	Chamber is tightly closed and crop test is started	5/07/10 13:57	OK	C	NT
11	11	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants from 3 windows	Results are studied	6/07/10 15:30	Results are ok. Photos are taken	C	NT
12	12	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants from 3 windows	Results are studied	7/07/10 16:55	Results are ok. Photos are taken	C	NT
13	13	Disabling of CO <sub>2</sub> control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO <sub>2</sub> control	Use of peristaltic pump for taking a sample	8/07/10	Not performed as not possible to detect low ethylene concentrations with the equipment that is available	NC	NT Dev. 1 EP
14	13	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants from 3 windows	Results are studied NT	8/07/10			
15	13	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics	8/07/10	see step 13	NC	NT Dev. 2 EP
16	13	Taking of 3 samples of nutrient solution from nutrient tank: a. Irrigation system is off. b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this solution but empty it into sewerage. c. After taking 3 samples continuously into 3	Samples of the solution are taken according to the written procedure, nutrient tank is tightly closed	8/07/10 13:30	The samples were taken	C	NT

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		plastic cups (preliminary labelled) and placement them into the fridge. d. Enabling of irrigation system in auto mode		8/7/10 17:30	photos taken		
17	14	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied	8/7/10 (09/07/10)	photos taken		
18	15	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied				
19	16	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied				
20	17	Changeover of the nutrient solution: disabling of irrigation mode, closing with manual valves inlet and outlet of the liquid in the chamber. Disabling of CO <sub>2</sub> mode. Taking 3 samples of nutrient solution from the bottom of the tank using drain valve (after outpouring first 3 samples into sewerage). Emptying of nutrient tank using drain valve. Preparation of nutrient solution in external tank (see TN 96.3) . Opening inlet of the liquid in the chamber by the manual valves and transfer of nutrient solution through drain valve into nutrient tank. Taking 3 samples of nutrient solution from the bottom. Opening of inlet and outlet of liquid in the liquid loop. Enabling of CO <sub>2</sub> control.	Solution is changed and samples of solution before and after changeover are taken.	12/7/10 9:10	OK		W



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Compartment : CIVb      Test Phase : 3

21	17	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied				
22	18	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied	13/07/10 16:00	The photos are taken. The results are ok	c	NT
23	19	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied	14/07/10 18:35	The photos are taken. The results are ok	c	NT
24	20	Disabling of CO <sub>2</sub> control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO <sub>2</sub> control	Use of peristaltic pump for taking a sample	15/07/10	Not performed as not possible to detect low ethylene concentrations with the present instrument		NT Dev3
25	20	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics		See step 24	NC	NT
26	20	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied	15/07/10 17:00	The photos are taken. The results are ok	c	CM
27	20	Taking of 3 samples of nutrient solution from nutrient tank: a. Irrigation system is off. b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this solution but empty it into sewerage. c. After taking 3 samples continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge. d. Enabling of irrigation system in auto mode	Samples of the solution are taken according to the written procedure, nutrient tank is tightly closed	15/07/10 16:45 17:10	Irrigation made 1044 in order to take samples The samples are taken.	c	NT



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Compartment : CIVb

Test Phase : 3

28	21	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied	16.07.10 16:50	Base solution prepared and filled in the Beck bank photos taken	C	CM
29	22	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied		Week end		
30	23	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied		Week end		
31	24	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics		See step 24	NE	CM
					Dev. 4	EP	
32	24	Changeover of the nutrient solution: disabling of irrigation mode, closing with manual valves inlet and outlet of the liquid in the chamber. Disabling of CO <sub>2</sub> mode. Taking 3 samples of nutrient solution from the bottom of the tank using drain valve (after outpouring first 3 samples into sewerage). Emptying of nutrient tank using drain valve. Preparation of nutrient solution in external tank (see TN 96.3) . Opening inlet of the liquid in the chamber by the manual valves and transfer of nutrient solution through drain valve into nutrient tank. Taking 3 samples of nutrient solution from the bottom. Opening of inlet and outlet of liquid in the liquid loop. Enabling of CO <sub>2</sub> control.	Solution is changed and samples of solution before and after changeover are taken.	19.07.10 9:00 15:15	OK photos taken	C	CM
					Pb with Blower it stopped! AV Hum : 97% AV Temp: 35°C and power cut during 17 <sup>h</sup> starting to 18.07.15 at 14:15. CO <sub>2</sub> = 2120-1000		
					Dev. 5	EP	

take out the tub from the stock A, B Acid and Base



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Compartment : CIVb

Test Phase : 3

33	24	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied	19.07.10	<del>See step 24</del> EP	NC	CM
34	25	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied	20.07.10	<del>See step 24</del> EP photos taken	NC	CM
35	25	Labeling of 100 paper bags for drying lettuce leaves, 100 paper bags for drying lettuce roots, 100 plastic bags for storing rockwool roots with roots inside in a fridge	Done	21.07.10	Addition of Base and Stock A & B → refill Stock B OK  photos taken paper bags for Rockwool have not been done	NC	CM
36	26	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied	22.07.10	See step 24	NC	CM
37	26	Weighing of each paper bag for leaves and roots drying and recording its weight (special template is ready for that)	Done	22.07.10 11:00  16:30	OK  photos taken	NC	CM
38	26	Labeling 3 plastic cups for taking samples of nutrient solution in the end of the test.	Done	22.07.10  15:45	OK	NC	CM
39	27	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied	22.07.10	<del>See step 24</del> EP	NC	CM
40	27	Placing of 2 scales to HPC room from the department and their adjustment.	Done	22.07.10  15:30	OK (only one scale is placed)	NC	CM
40	28	Taking of 3 samples of nutrient solution from nutrient tank:	Samples of the solution are taken according to the written procedure, nutrient tank is tightly				

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Test Phase : 3

		a. Irrigation system is off. b. Opening of drain valve and filling a plastic cup with nutrient solution 3 times without closing the valve. Don't keep this solution but empty it into sewerage. c. After taking 3 samples continuously into 3 plastic cups (preliminary labelled) and placement them into the fridge.	closed	23.07.10 8:15	OK	NC	CM
41	28	Saving history graphs for all loops for the period of 24 hours. Taking photos of the plants	Results are studied	23.07.10 8:25	See Step 24 <u>Dev.8</u>	NC	CM
42	28	Disabling of CO <sub>2</sub> control for taking air sample from the chamber and subsequent ethylene analysis. After sample is taken enabling of CO <sub>2</sub> control	Use of peristaltic pump for taking a sample	22.07.10 10:30	CO <sub>2</sub> control was not disabled <u>Dev.9</u> Analysis ethylene OK	NC	CM
43	28	Analysis of ethylene concentration in the air sample with GC of the department at least with 5 replications	Results of analyses are presented as graphics	22.07.10 11:00	OK but only 3 replications (time of planning → Dpt)	NC	CM
44	28	Disabling of all control loops. Opening the chamber and taking pictures of the plants. Harvesting of the plants using procedure for rockwool safe manipulation. Taking pictures of plants in each tray. Writing down fresh weight of lettuce shoots (TN 96.4). Putting of lettuce shoots into preliminary weighed labeled paper bags. Weighing of paper bags	Plants are harvested, control system is disabled	23.07.10 8:30			

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Compartment : CIVb Test Phase : 3

		for roots drying.					
45	28	Delivery of paper bags with lettuce shoots into Veterinary School and plants drying in the oven at 70°C with preliminary fixation at 103°C during 10 minutes. Rockwool cubes are stored in a cold cabinet at 4 °C.	Beginning of lettuce shoots drying	13:00 23.07.10			NC CM
46	28	Cleaning of the trays with soap and water	Trays are clean and stored in the chamber	11:00 23.07.10	OK		NC CM
47	29	Delivery of rockwool cubes with roots inside into Veterinary School and roots separation from rockwool using Procedure for Rockwool safe manipulation.	Roots are separated and put into the oven for drying at 70°C	02/08/10 08:00 04/08/10 13:30	it was done into USIB laboratory		NC CM
48	32	Checking of shoots and roots dry weight in Veterinary School	Weight of 3 samples of shoots and 3 samples of roots from each level in the oven, it total 24 samples	28/07/10 9:00	only for shoots was done → weight		NC CM
49	35	Checking of shoots and roots dry weight in Veterinary School	Weight of 3 samples of shoots and 3 samples of roots from each level in the oven, it total 24 samples		not done		NC CM
50	36	Checking of shoots and roots dry weight in Veterinary School. In case weights of the samples don't change, final records of dry biomass should be done next day.	Weight of 3 samples of shoots and 3 samples of roots from each level in the oven, it total 24 samples		Not done		NC CM
51	37	Recording of dry weight of lettuce shoots and roots	Dry weights are recorded, samples are taken back to MPP	28/07/10 9:00	OK		NC CM
52	38	Mixing of dry samples of lettuce shoots in order to have 1 average sample per tray, filling of plastic	Samples are prepared for further milling	20/09/10			C CM

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Compartment : CIVb      Test Phase : 3

		cups with samples					
53	39	Mixing of dry samples of lettuce roots in the extraction cabinet of the Department in order to have 1 average sample per tray, filling of plastic cups with samples	Samples are prepared for storage	21.09.10			C CM
54	40	Delivery of shoots samples to Veterinary School for milling		20.09.10			C CM

Conclusion for the Test	Name E. PEIRS	Signature Enrique Peirs	Date 30.07.10
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Passed    Failed

- Number of deviations attached to the document : 10
- All deviations have been justified or corrected? YES / NO

Comments

Checked by TechnoMembranes MELISSA Pilot Plant	Name A. FOSSEN	Signature 	Date 30/7/10
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Appendix 1 for MPP-REC 10-4103(0)-03

Appendix 1 - record of implied personnel

Name	ORGANIZATION	Function	Initials
Natalia Tikhomirova	Visiting Researcher MPP	Plant Scientist	NT
Cynthia Munganga	MPP	Analysis Technician	CM
Enrique Peiro	MPP	Technical manager	EP

Appendix 2: equal to Appendix 2 for MPP-REC 10-4102(0)-03

EP

Appendix 3 to MPP-REC 10-4103(0) - 03

Appendix 3 - deviations list

<b>DEV. FORM #</b> 1	<b>Deviation:</b> Ethylene measurement not performed	<b>Criticality</b> Low Medium High	
	<b>Corrective action:</b> Search for alternative method/equipment (It could require a big investment)	<b>Resp.</b> EP	<b>Due date</b>
	<b>Corrective action performed and checked:</b> <b>Ref. of retests:</b>	<b>Checked / approved by</b>	<b>Closing Date</b>
<b>DEV. FORM #</b> 2	<b>Deviation:</b> See Dev. 1	<b>Criticality</b> Low Medium High	
	<b>Corrective action:</b> N.A.	<b>Resp.</b>	<b>Due date</b>
	<b>Corrective action performed and checked:</b> <b>Ref. of retests:</b>	<b>Checked / approved by</b>	<b>Closing Date</b>
<b>DEV. FORM #</b> 3	<b>Deviation:</b> See Dev. 1	<b>Criticality</b> Low Medium High	
	<b>Corrective action:</b> N.A.	<b>Resp.</b>	<b>Due date</b>
	<b>Corrective action performed and checked:</b> <b>Ref. of retests:</b>	<b>Checked / approved by</b>	<b>Closing Date</b>

# Appendix 3 to MPP-REC 10-4103(0)-03

## Appendix 3 - deviations list

DEV. FORM # 4	Deviation:  See dev. 1	Criticality Low Medium High	
	Corrective action:  N.A.	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date
DEV. FORM # 5	Deviation: Environmental conditions in the chamber failed: blower OFF; humidity 97%; temp. 35°C since 18.07 at 14:15 until 19.07 in the morning (17h)	Criticality Low Medium High	
	Corrective action: The reason was probably a power cut during the week-end, so an alarm remote system is needed for the HPC, and to consider professional supervision every day as alternative	Resp. EP	Due date End of COO9
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date
DEV. FORM # 6	Deviation: Some non critical tasks were not performed, like taking pictures or printing graphs. They didn't affect the process neither the objective of the test.	Criticality Low Medium High	
	Corrective action:  N.A.	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date

Appendix 3 to MPP-REC 10-4603 (0)-03

Appendix 3 - deviations list

DEV. FORM # 7	Deviation: Placing only one scale for weighing (not critical for the process)	Criticality Low Medium High	
	Corrective action: To flexibilise the protocol in the new version if <del>these</del> two scales are not mandatory for the test (end of COO)	Resp. EP	Due date End of COO9
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date
DEV. FORM # 8	Deviation: See Dev. 6	Criticality Low Medium High	
	Corrective action: N.A.	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date
DEV. FORM # 9	Deviation: CO <sub>2</sub> control not disabled for ethylene determination.	Criticality Low Medium High	
	Corrective action: Not big impact on the experiment, especially for communication purposes. N.A.	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date

Appendix 3 to MPP-REC 10-4103(0) -03

Appendix 3 - deviations list

DEV. FORM # 10	Deviation:  Weighing for dry weight determination not performed sequentially, only at the end of the drying phase	Criticality Low Medium High	
	Corrective action:  N.A. (not relevant, as final weight doesn't change)	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date
DEV. FORM #	Deviation:	Criticality Low Medium High	
	Corrective action:	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date
DEV. FORM #	Deviation:	Criticality Low Medium High	
	Corrective action:	Resp.	Due date
	Corrective action performed and checked: Ref. of retests:	Checked / approved by	Closing Date