

Memorandum of Understanding 19071/05/NL/CP



TECHNICAL NOTE: 89.53

TESTS REPORTS, INCLUDING 'AS RUN' ANNOTATED PROCEDURES

prepared by/ <i>préparé par</i>	L. Chaerle, J. Decat, D. Van Der Straeten
reference/ <i>référence</i>	Contract number 19787/06/NL/PA
issue/ <i>édition</i>	1
revision/ <i>révision</i>	0
date of issue/ <i>date d'édition</i>	23oct08
status/ <i>état</i>	Final
Document type/ <i>type de document</i>	Technical Note
Distribution/ <i>distribution</i>	

CONFIDENTIAL DOCUMENT

APPROVAL

Title <i>titre</i>	issue <i>issue</i>	1	revision <i>revision</i>	0
-----------------------	-----------------------	---	-----------------------------	---

author <i>auteur</i>	date <i>date</i>	Error! Reference source not found.
-------------------------	---------------------	--

approved by <i>approuvé by</i>	date <i>date</i>
-----------------------------------	---------------------

CHANGE LOG

reason for change / <i>raison du changement</i>	issue/ <i>issue</i>	revision/ <i>revision</i>	date/ <i>date</i>

CHANGE RECORD

Issue: 1 Revision: 0

reason for change/ <i>raison du changement</i>	page(s)/ <i>page(s)</i>	paragraph(s)/ <i>paragraph(s)</i>

TABLE OF CONTENTS

1	Hardware performance test	1
1.1	Germination / propagation system	1
1.2	Growing system	1
1.2.1	Calibrating control and experimental liquid circuit for equal performance	1
1.2.1.1	Temperature	1
1.2.1.2	Liquid flow	1
1.2.1.3	pH/EC	1
1.2.1.4	Liquid level	2
1.2.1.5	Dissolved O2	2
1.2.1.6	Plant weight.....	2
	The Sartorius load cells, of which 2 were positioned under respectively a control and a treatment gully, were calibrated with calibration weights, and readings proved stable during a 2day test logging period with gullies and nutrient delivery system running.....	2
1.2.2	Air handling system performance	2
1.2.2.1	Chamber T distribution	2
1.2.2.2	Rack T distribution	3
1.2.2.3	Thermal control versus flow	3
1.3	Data management.....	3
1.3.1	Data logging	3
1.3.1.1	DL2 procedure.....	3
1.3.1.2	ACS air handling	3
1.3.2	Image management.....	4
1.3.2.1	Image Capture	4
1.3.2.2	Humicap data coupling	4
2	Plant growth test	5
2.1	Cultivar choice	5
2.2	Germination	5
2.2.1	Petri-dish	5
2.2.2	Capillary material	6
2.3	Propagation	7
2.3.1	Root and shoot assessment.....	7
2.4	Transplant	8
2.5	Growing system performance	8
2.5.1	Robotised imaging system	8
2.5.2	Light.....	9
2.5.3	Air loop.....	9
2.5.3.1	T/RH	9
2.5.3.2	CO2.....	9

2.5.3.3	Ethylene	9
2.5.4	Liquid loop	9
2.5.4.1	pH/EC	9
2.5.4.2	Plant water use.....	10
2.5.4.3	Temperature	11
2.5.4.4	Dissolved O2	11
2.5.4.5	Flow	11
2.5.5	Weight.....	11
2.5.6	Elemental analysis nutrient solution.....	12
3	Plant growth stress assessment experiments – ACC	13
3.1	On-line assessment.....	14
3.1.1	Effects on EC/pH.....	14
3.1.2	Effects on water use.....	14
3.1.3	Ethylene	15
3.1.3.1	Ethylene measurement under cuvette	15
3.1.4	Shoot assessment	20
3.1.4.1	Leaf area imaging	20
3.1.4.2	Visual observation	21
3.1.4.3	Leaf temperature.....	21
3.1.5	Root assessment	21
3.1.5.1	Root response to ethylene treatment.....	21
3.1.6	Weight.....	21
3.2	Destructive.....	22
3.2.1	Microscopic root assessment.....	22
3.2.2	Harvest weight.....	22
3.2.3	Harvest elemental analysis.....	23

L I S T O F T A B L E S

Table 1: Logged parameters nutrient circulation and air plus plant weight and light level	3
Table 2: ACS HVAC logged parameters	4
Table 3: light levels on the 6 racks	9
Table 4: nutrient solution composition and depletion	12
Table 5: elemental analysis of lettuce mg/kg dry weight	23

L I S T O F F I G U R E S

Figure 1: Petri dish germination and NFT propagation setup.....	5
Figure 2: Germination on capillary material, daily images	6
Figure 3: detail of plant germinated on capillary material, and further developed plants.....	7
Figure 4: Iron deficiency symptoms in shoot and root, right root control.....	7
Figure 5: EDDHA Iron chelate	8
Figure 6: water addition log for independent circuits control and treatment during plant growth test	10
Figure 7: continuous weight measurements with load cells during plant growth test.....	11
Figure 8: Experiment 1 after transplanting	13
Figure 9: Experiment 1 before harvest	14
Figure 10: Experiment 2 startup – 18jul08	15
Figure 11: Time lapse growth starting from 14 days old plant till 23 days, 1 picture each day.	16
Figure 12: time course of ethylene accumulation.....	17
Figure 13: Time lapse growth starting from 24 days old plant , 1 picture each day.	18
Figure 14: chamber with NFT lettuce corresponding to last frame of Figure 13.....	19
Figure 15: Side view of rack layout and imaging sequential time-optimized procedure	20
Figure 16: Top view single level imaging sequential procedure.....	21
Figure 17: Root microscopic images, root surface detail, oxygen (air) root, branching, and root tip	22

1 Hardware performance test

1.1 Germination / propagation system

Three setups of 3 Mini-Gullies (1m long x 4cm wide x 2cm high), connected to a 20 l tank (containing 10 l of solution) with a MaxiJet1000 pump were tested for adjustment of liquid flow and nutrient film depth.

1.2 Growing system

1.2.1 *Calibrating control and experimental liquid circuit for equal performance*

1.2.1.1 *Temperature*

The 2 mixing tanks of the independent nutrient delivery circuits (control and treatment) with 30 l solution each were monitored during 2 subsequent days with nutrient recycling system functioning and illumination system on. Isolation needed to be placed between the light system of the rack adjacent to the mixing tank and the nearest liquid mixing tank to obtain equal temperatures ± 0.1 °C.

The nutrient solution quickly warmed under the influence of the illumination system when passing through the gullies to a value of 27 °C, whereas 20 °C is indicated to be optimal. Therefore a recirculating cooling unit with a branched cooling circuit with a plastic (nylon) cooling coil immersed in each of the nutrient solution mixing tanks was installed and confirmed to keep the solution temperature between 19.5 and 20 °C.

1.2.1.2 *Liquid flow*

With 2 NewJet1700 pumps per mixing tank the specification of 200ml/gully⁻¹.min⁻¹ could be obtained per gully by using needle regulator valves on the 2 lower rack levels.

1.2.1.3 *pH/EC*

The pH and EC sensors of both independent circuits were calibrated with reference solutions, and adjusted as to display the same pH/EC value for the prepared nutrient solution. A simple on/off control strategy was chosen and hysteresis was set to a minimum.

The four dosing pumps were calibrated manually to provide a comparable maximal flow of approximately 20ml per minute. The actual volume of pH or EC stock added was calculated by the DeltaT logging software according to a count of the time units (6000 units per minute) the dosing pump was switched on by the controller, divided by the exact calibration flow.

The mixing activity of the circulation pumps proved insufficient to keep the fluctuations of pH below 0.25 units and EC below 100 microS. Therefore a small MaxiJet1000 pump was added to each mixing tank for internal mixing, which provided the abovementioned stability of pH and EC readings.

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

1.2.1.4 Liquid level

The level of the mixing tanks is controlled by a single ‘low level’ signal upon which an exact quantity of 1l of distilled water is added. The low level detection is based on a reed-relais within a gauging rod fitted with a float.

The 2 independent systems were adjusted to have the same level regulation setting and thus liquid volume.

The mixing tanks and the stock solution tanks are fitted with a low level alarm based on the same principle. The mixing tanks also have a high level alarm. Danger of overflow or danger of critically low levels in case of malfunction of the controlling system are thus signalled. Alarm performance was tested for each of these and the level was adjusted for conformity between the tanks of both circuits.

All level measurements and controls are steered by a Mitsubishi Melsec Compact PLC.

1.2.1.5 Dissolved O₂

On the recollected water line from each circuit (just before re-entry of the solution into the stirred mixed tank), a measuring cuvette was provided to allow measurement of dissolved oxygen, representative of the root conditions at the end of the gullies where oxygen was anticipated to be at its lowest level.

This setup was not functional given the requirement of the Hanna instruments oxygen sensor for a substantial flow at the level of the electrode. Therefore the NewJet circulation pumps in each of the mixing tanks were fitted with a water intake in which the portable DO electrode (not connected to the logging system) can be fitted. Measurements proved stable and within the expected values for the temperature of the solution.

1.2.1.6 Plant weight

The Sartorius load cells, of which 2 were positioned under respectively a control and a treatment gully, were calibrated with calibration weights, and readings proved stable during a 2day test logging period with gullies and nutrient delivery system running.

1.2.2 Air handling system performance

The cross flow system with air entry and exit on the facing walls has an inherent difference in temperature of 1 °C. Fluctuations induced by the T control system were limited to 0.2-0.3 °C.

Due to conditions of high outside temperatures (weather influence) the limits on cooling (difference between air temperature after the cooling unit and at the air exit) had to be enlarged (from 2 to 4 degrees) to allow the cascade regulation to keep the room at the specified temperature. Otherwise the temperature slowly increases till safety light shutoff.

1.2.2.1 Chamber T distribution

Along the rack length air inflow temperature difference was limited to 0.2 °C. Heat load of the TL light modules probably causes this slight difference.

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

Difference between racks was assessed with 5 sensors which were previously adjusted in a comparative experiment. Difference between racks facing the intake respectively outlet wall were limited to 0.2 degrees.

1.2.2.2 Rack T distribution

Heat load of the TL light modules is not homogeneous (in analogy with the light distribution), however the even and high air flow alleviates this largely. Lowering the flow rate will result in a more pronounced temperature gradient from edge till middle of each rack.

1.2.2.3 Thermal control versus flow

Ventilation speed setting can be lowered at the expense of a larger difference in temperature between the air intake and outlet.

1.3 Data management

1.3.1 Data logging

DL2e data logger (Delta-T systems) and ACS (Siemens) system data were converted to (semi) on-line graphical displays.

1.3.1.1 DL2 procedure

Liquid loop parameters pH, EC, T per circuit, light level (middle of rack 6 - mobile), air T/RH, CO2/O2 levels and gully weight per circuit (see Table 1).

Label	Turbine	pmp	pmp	pmp	pmp	pmp	pmp	Vol T	Vol C	pH T	EC T	pH C	EC C	RH air	T Air	PAR	CO2	O2	WT lo	W C lo
Sensor Type	CNT	pH T	EC T	pH C	EC C	CNT	CNT	CNT	CNT	pH	EC	pH	EC	RH	TM2	SKP	CO2	O2	SAR	SAR
Units	l	ml	ml	ml	ml	sec	sec	l	l	/10upH	uS	/10upH	uS	%RH	deg C	uE	ppm	%	gr	gr
29/03/08 6:28	0	0	0	0	0	300	300	0	0	55,87	1790	56,32	1741	59,08	25,32	284,7	370	22,96		
29/03/08 6:33	0	0	0	50,1	0	300	300	0	0	55,87	1790	56,32	1743	62,62	25,07	283,5	371	22,98		
29/03/08 6:38	0	0	0	15	0	300	300	0	0	55,87	1787	52,82	1747	62,36	25,33	284,5	373	22,98		
29/03/08 6:43	0	0	0	0	0	300	300	0	0	55,87	1790	52,42	1747	62,98	25,07	283,3	371	22,99		
29/03/08 6:48	0	0	0	0	0	300	300	0	0	55,87	1790	52,19	1747	62,26	25,29	284,9	372	22,99		
29/03/08 6:53	0	0	0	0	0	300	300	0	0	55,87	1790	52,19	1750	62,16	25,04	284,7	371	22,99	928,6	917,8
29/03/08 6:58	0	0	0	0	0	300	300	0	0	55,87	1790	52,19	1750	62,16	25,32	285,8	373	22,98		
29/03/08 7:03	0	0	0	0	0	300	300	0	0	55,87	1790	52,19	1750	61,8	25,04	283,6	371	22,99		
29/03/08 7:08	0	0	0	0	0	300	300	0	0	55,87	1790	52,19	1750	62,26	25,21	284,4	371	22,98		
29/03/08 7:13	0	0	0	0	0	300	300	0	0	55,87	1790	52,19	1750	58,83	25,07	284,5	371	22,98		
29/03/08 7:18	0	0	0	0	0	300	300	0	0	55,91	1790	52,19	1750	62,41	25,23	284,2	372	22,93		
29/03/08 7:23	0	0	0	0	0	300	300	0	0	55,87	1790	52,19	1747	61,08	25,13	283,6	371	22,99		
29/03/08 7:28	0	0	0	0	0	300	300	0	0	55,87	1790	52,19	1750	62,72	25,15	284,5	371	23,01		
29/03/08 7:33	0	0	0	0	0	300	300	0	0	55,87	1790	52,19	1750	60,88	25,18	284,5	371	22,99		
29/03/08 7:38	0	0	0	0	0	300	300	0	0	55,87	1790	52,19	1753	62,41	25,15	283,6	372	22,99		
29/03/08 7:43	0	0	0	0	0	300	300	0	0	55,87	1790	52,19	1753	60,88	25,25	284,5	370	23,02		
29/03/08 7:48	0	0	0	0	0	300	300	0	0	55,87	1790	52,19	1753	62,05	25,11	284,2	370	23,01		
29/03/08 7:53	0	0	0	0	0	300	300	0	0	55,87	1790	52,19	1753	61,08	25,31	284	370	23,01	928,6	917,8
29/03/08 7:58	0	0	0	0	0	300	300	0	0	55,87	1790	52,19	1753	60,06	25,04	284	371	23,01		

Table 1: Logged parameters nutrient circulation and air plus plant weight and light level

1.3.1.2 ACS air handling

T (chamber-global, air intake, air exit), RH (idem), ventilation speed setting data were established with a frequency of 30seconds and a logging interval of 1 week.

TN 89.53	Tests report, including "as-run" annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization	
Memorandum of Understanding 19071/05/NL/CP	

In Table 1, data are shown as logged each 5 minutes. Weight is logged each hour. Functioning of the circulation pumps is monitored continuously.

Time d/m/y h:m	Room RH (%)	Room T (C)	Pulsion RH (%)	Pulsion T (C)	Heating (%)	Cooling (%)	Humidi- Fication (%)	Chamber Ceiling RH (%)	Chamber Ceiling T (C)	Ventilator frequency (Hz)
11/3/2008 18:44	56	23,4	73	21,0	0	62	12	64	22,6	35
11/3/2008 18:44	55	23,4	73	20,9	0	62	13	63	22,6	35
11/3/2008 18:45	54	23,4	72	20,9	0	63	16	62	22,6	35
11/3/2008 18:45	53	23,4	72	20,7	0	61	19	61	22,6	35
11/3/2008 18:46	53	23,4	72	20,6	0	59	20	61	22,6	35
11/3/2008 18:46	53	23,3	73	20,5	0	57	20	61	22,6	35
11/3/2008 18:47	54	23,3	73	20,4	0	54	20	61	22,6	35
11/3/2008 18:47	54	23,2	74	20,3	0	52	18	62	22,6	35
11/3/2008 18:48	55	23,2	75	20,3	0	50	17	62	22,6	35
11/3/2008 18:48	55	23,2	75	20,3	0	50	15	62	22,6	35
11/3/2008 18:49	55	23,2	75	20,3	0	49	14	63	22,6	35
11/3/2008 18:49	56	23,2	75	20,3	0	49	13	63	22,6	35
11/3/2008 18:50	56	23,2	75	20,4	0	50	13	63	22,6	35
11/3/2008 18:50	55	23,3	74	20,5	0	53	14	63	22,6	35
11/3/2008 18:51	56	23,3	75	20,6	0	55	12	64	22,6	35
11/3/2008 18:51	56	23,4	74	20,7	0	58	12	64	22,6	35
11/3/2008 18:52	56	23,4	74	20,8	0	60	12	64	22,6	35
11/3/2008 18:52	56	23,4	74	20,9	0	62	11	64	22,6	35
11/3/2008 18:53	56	23,5	74	20,9	0	64	10	65	22,6	35

Table 2: ACS HVAC logged parameters

1.3.2 Image management

1.3.2.1 Image Capture

The interfacing of the CMOS video camera's proved problematic. The defect of one of the USB-interfaces, seemingly through a short circuit during connection led to the need to upgrade the camera firmware. This refused to work with the dedicated Labview robotimaging software, and needed extensive searching, modification and testing to obtain a workable platform again.

1.3.2.2 Humicap data coupling

T and RH are measured with a combined T/RH sensor positioned in the middle of rack 5. Readings are taken synchronised with each image capture, in order to be able to correlate air temperature with thermal imaging results. The readings were written into a tabular form for easy access by software under development.

TN 89.53	Tests report, including "as-run" annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization	
Memorandum of Understanding 19071/05/NL/CP	

2 Plant growth test

2.1 Cultivar choice

Initial tests with the Grand Rapids leaf lettuce cultivar were carried out for germination and propagation.

The Looseleaf lettuce cultivar Lollo Rossa / Bionda (*Lactuca sativa* var. *foliosa*) was chosen based on its reported excellent performance in the installed Hortiplan gullies (Horticultural research centre St-Kaetlijne Waver, B.).

Due to growth space (height) constraints finally a butterhead lettuce heirloom variety (Tom Thumb) was chosen for the two functional tests, after completing one full untreated growth period.

2.2 Germination

Preliminary tests on rockwool cubes with cultivar Grand Rapids proved no vernalisation was needed. Conditions were continuous light, 50 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ PAR, 25 °C and 65% humidity (See figure 1 for both petridish and capillary material setup).



Figure 1: Petri dish germination and NFT propagation setup

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

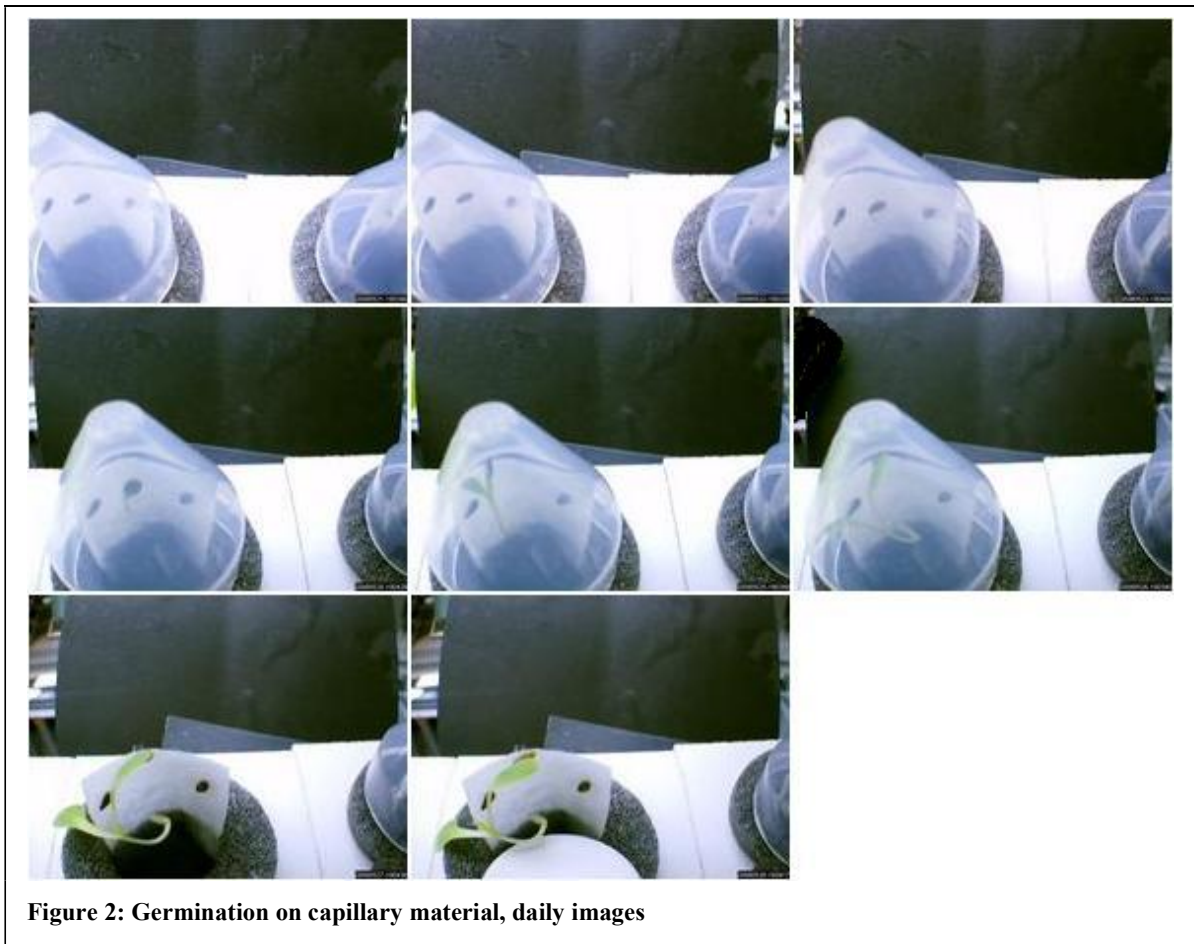
2.2.1 Petri-dish

Germination % of the lettuce cultivar Little thumb was 70%, but when radicle (see root) emergence and development was taken as a criterion, less than 50% of the seeds further developed. The symptom of germination without root development and brown discoloring of the hypocotyl hinted to the possibility of a seed-borne pathogen infection.

Hypocotyl rotting was observed, and the pathogen *Fusarium oxysporum* isolated and diagnosed. An adjusted sterilization procedure (15min 5% hypochlorite instead of 5min 1%) solved this infection problem.

2.2.2 Capillary material

Direct seeding on the capillary material, placed in development gullies proved to be successful and needing the least amount of manual work (See Figure 2).



TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

2.3 Propagation

Three mini gully, tank and pump setups (see 1.1) were used to grow the plants for 2 weeks (See figure 4). The plastic or glass caps maintain a high humidity allowing germination.



Figure 3: detail of plant germinated on capillary material, and further developed plants

2.3.1 Root and shoot assessment

Iron availability proved crucial in the NFT propagation setup, since after prolonged use of Fe-EDTA solutions, clear iron deficiency symptoms appeared (see Figure 5).



Figure 4: Iron deficiency symptoms in shoot and root, right root control

Modification of growing solution was thus needed: EDDHMA Tenso Tenso™ Iron (Yara, Oslo – Norway) was added instead of EDTA, which is less stable.

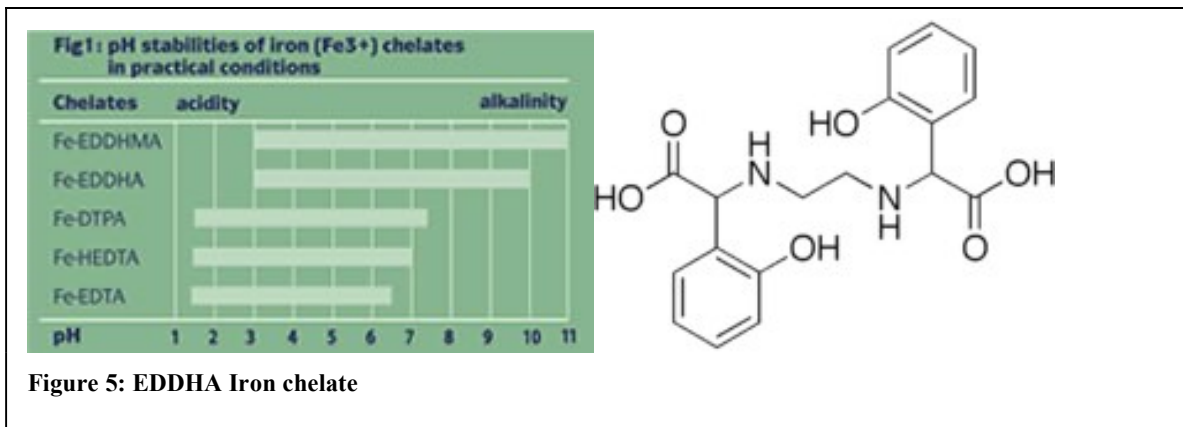
EDDHMA = ethylenediamine-N,N'-bis(2-hydroxy-4methylphenylacetic acid)

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

Composition: Iron (Fe-EDDHMA) 6% Fe of total weight.
Final utilised concentration: 5 microM.

Iron availability is dependent on pH, and different chelating agents are used depending on the pH of the growing medium (see Figure 4).

<http://www.micronutrients.info/Applications/StabilityandpH/>



2.4 Transplant

The procedure involved removing the 2 week old plants in their 4 x 4cm plastic support plates from the propagation gullies, carry them in a flat tray with a layer of nutrient solution (avoiding root drought stress) to the PSDU setup and insert the plants with support plate in the gullies, paying attention that the whole root system is in contact with the recirculating nutrient solution film of the NFT setup.

2.5 Growing system performance

2.5.1 Robotised imaging system

A custom-made NI-Labview program was used for capturing thermal and video images. Layout of the capture sequence per rack and gully needed a redefinition of settings, to optimize time-use (see 3.4.1 for a visualisation).

Images were converted into time-lapse sequences and organized using an adapted freeware javascript user-configurable image cataloguing application that provides a web-interface to the collected data on a per-experiment basis, allowing easy data viewing and retrieval.

Offline Analysis of the organized thermal image data was performed with ImageJ (<http://rsb.info.nih.gov/ij/>), and thermal information was extracted with a preliminary version of a developed Matlab program.

Growth environment measurements T/RH were coupled to thermal image data capture time point by carrying out a modification of the Labview program.

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

2.5.2 Light

The updated measurement Table 1 indicates a maximum light level of 200 micromoles $m^{-2} s^{-1}$ in the middle of the rack. For rack 1 to 3, levels at the far end of the rack were 65% of maximum. Extra side illumination increased that value for rack 5 and 6 to just over 70%. Light photoperiod was 20h light and 4h darkness.

	begin	middle	end
rack 1	155	199	126.7
rack 2	138	199	126.9
rack 3	146.5	200	126.7
rack 4	Not meas.	200	Not meas.
rack 5	143.7	199	150
rack 6	147.1	201	146.7

Table 3: light levels on the 6 racks

2.5.3 Air loop

2.5.3.1 T/RH

Performance was similar as during the hardware test. The combined T/RH sensor located on rack 2 was positioned above the plant height level and was modified to avoid influence of the heat radiation of the TL lamps.

2.5.3.2 CO₂

No depletion was measured by using a single input line located above rack 1.

2.5.3.3 Ethylene

The photoacoustic system was setup outside of the measuring room, using the same sampling line as for the PPSystems CO₂ analyser, of which it needed the flow to work. A gas bottle reference hydrocarbon free air was used to keep the system calibrated for long time measurement. The procedure involved 110 minutes of sampling line measurement followed by 10 minutes of reference air.

Preliminary measurements on sampled air indicated no detectable in the measuring room and in outside air, indicating levels below 0.5 ppb.

2.5.4 Liquid loop

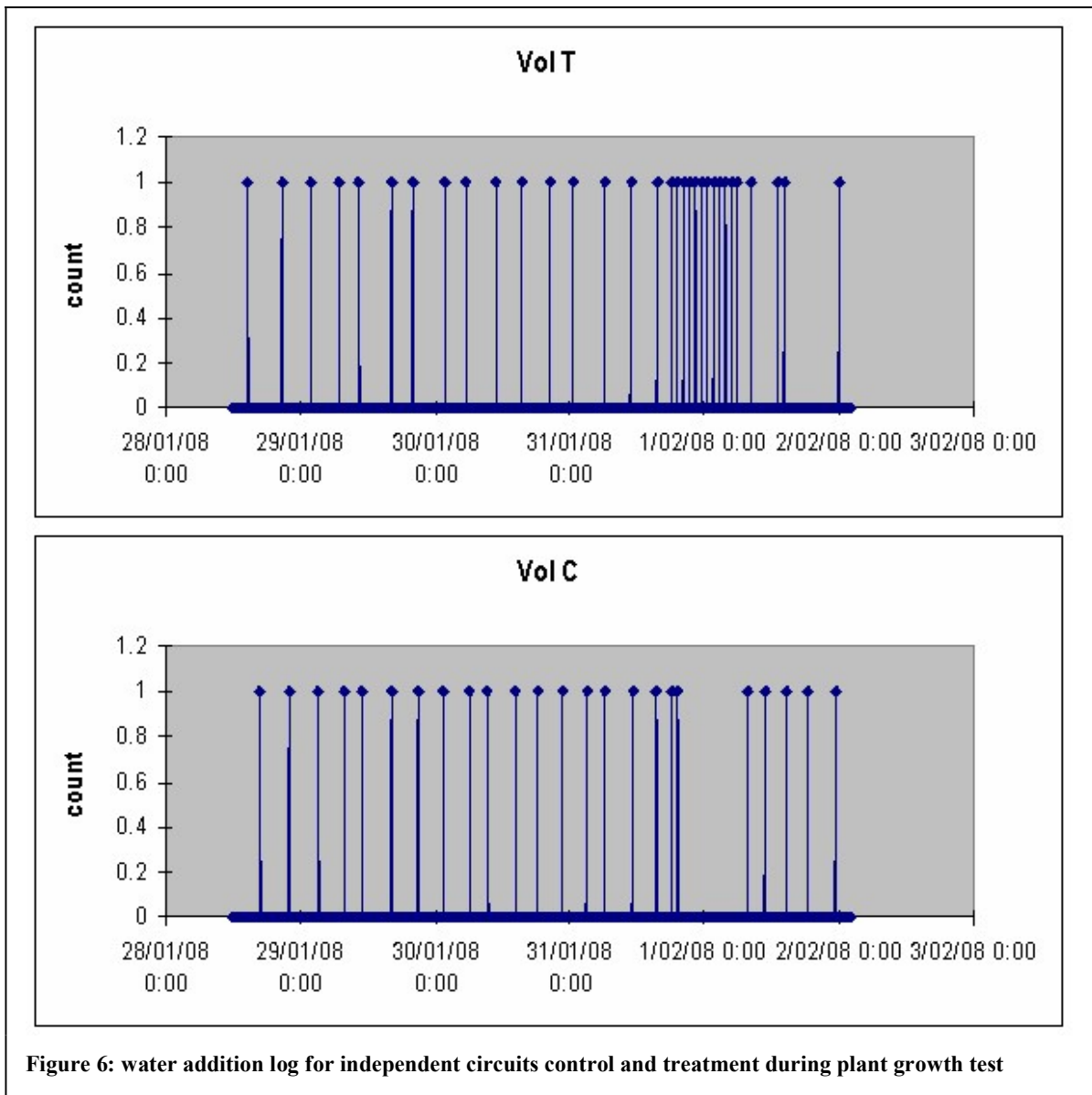
2.5.4.1 pH/EC

Plant growth caused the pH of the solution to increase (since NO₃⁻ was used as the sole N source). Nutrient uptake caused the conductivity to drop, additions were however mostly triggered by the addition of dd water due to liquid level drop caused by plant evapotranspiration.

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

2.5.4.2 Plant water use

The collected condensate water from the HVAC chilled water/air heat exchanger was fed into the replenishment tank for the hydroponics system.



The amount of external dd water (specs conductivity lower than 1 microS) added was measured by a turbine (see Table 2). Figure 6 indicates equal performance of T and C circuits. Water addition logs provided a quick way of checking differences in water consumption between treatments.

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization	
Memorandum of Understanding 19071/05/NL/CP	

2.5.4.3 Temperature

Measurements proved equal performance of the cooling system for both tanks.

2.5.4.4 Dissolved O₂

Point measurements (not logged) in both tanks did not show any difference.

2.5.4.5 Flow

The lower rack flows proved most difficult to control since the needle valves logically needed to be most closed and consequently most prone to clogging.

2.5.5 Weight

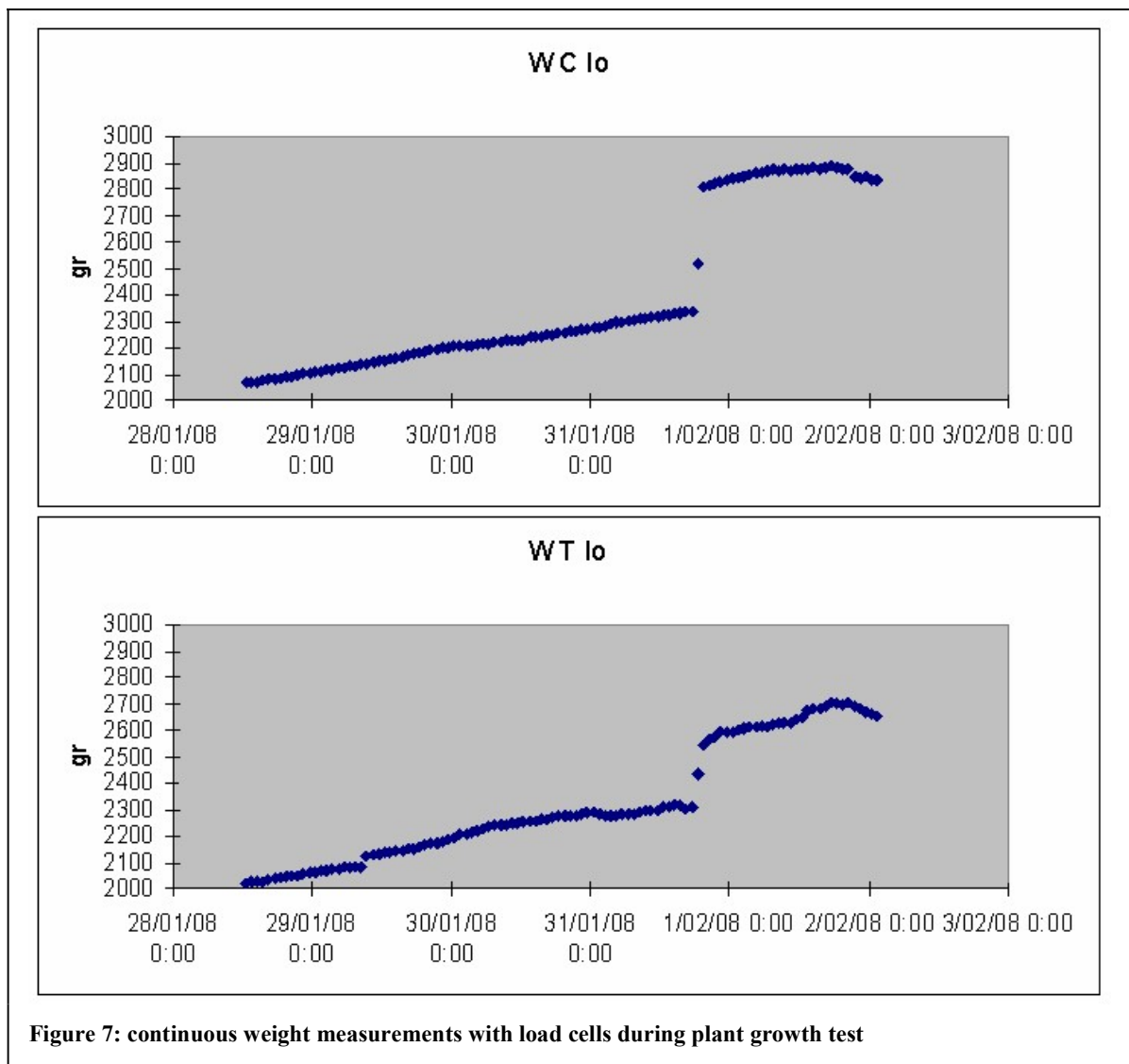


Figure 7: continuous weight measurements with load cells during plant growth test

Interference from liquid flow fluctuations was obvious in the logged data (Figure 7.)

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization	
Memorandum of Understanding 19071/05/NL/CP	

2.5.6 Elemental analysis nutrient solution

Nutrient solution sampling at start and end culture revealed the following:

- depletion of K (271 to 6.4 mg/l)
- depletion of Cl⁻
- depletion of Mn⁻

The high levels of Zn, Cu, Ni are due to the use of messing-containing junctions in the liquid loop, which were subsequently replaced by acetate plastic compounds.

	1xCT1-S	1xCT1-E
Ca	170	228
Mg	31	37.2
K	271	6.4
Na	0.07	0.46
P	54.7	62.8
N	10.8	< 7
SO ₄ ²⁻	129	178
Cl ⁻	25.4	< 1
NO ₃ ⁻	784	538
Fe	0.099	0.118
Mn	0.25	0.02
Zn	0.46	3.15
Cu	0.34	3.81
Pb	0.007	0.05
Al	< 0.05	< 0.05
Mo	0.053	0.023
Ni	0.023	0.097
B	0.26	0.53

Table 4: nutrient solution composition and depletion mg/l

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

3 Plant growth stress assessment experiments – ACC



Figure 8: Experiment 1 after transplanting

After transplanting the plants were grown for 2 more weeks before treatment. In Figure 8 the upper and middle (partial) level racks are visible. Lower level (left with load cells) cannot be seen. Yellow and Red markings indicate the circuit (treatment and control) to which the gully is connected.

The positioning system (Cartesian robot) is visible in the front. When the system is in its 'home' or 'reference' position, the operator can access the space between the 2 racks for observation (shoot and root).

For transplanting or harvest, the positioning system is moved to the position defined at the back of the room 'space-saving position', which implies stopping the image capturing software routine.

TN 89.53	Tests report, including "as-run" annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	



Figure 9: Experiment 1 before harvest

3.1 On-line assessment

3.1.1 Effects on EC/pH

The effect of 10 micromoles ACC treatment on EC stock solution addition was inconsistent. Experiment 1 indicated a higher consumption of nitric acid in the ACC treated circuit compared to the control circuit, whereas the amount of concentrated stock consumption was lower. In experiment 2 this trend was reversed.

3.1.2 Effects on water use

The ACC treated circuit showed both in experiment 1 and 2 a higher water addition rate than the control circuit (10 versus 6, and 9 versus 6 liters) indicating a higher transpiration of the plants growing on the solution with ACC.

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

3.1.3 Ethylene

Whole chamber measurement with the photoacoustic ethylene detection system after ACC addition to the nutrient solution did not reveal any increase in ethylene levels above the detection limit of 0.5 ppb.

Next, a single lettuce head was enclosed in a glass bulb (cuvette) and connected to the sampling line with a 1 l/min flow rate. The air sampled was drawn in through the bottom of the cuvette, and was a mix of chamber air and root-zone air. The volume of the cuvette was roughly 5 liters.

3.1.3.1 Ethylene measurement under cuvette

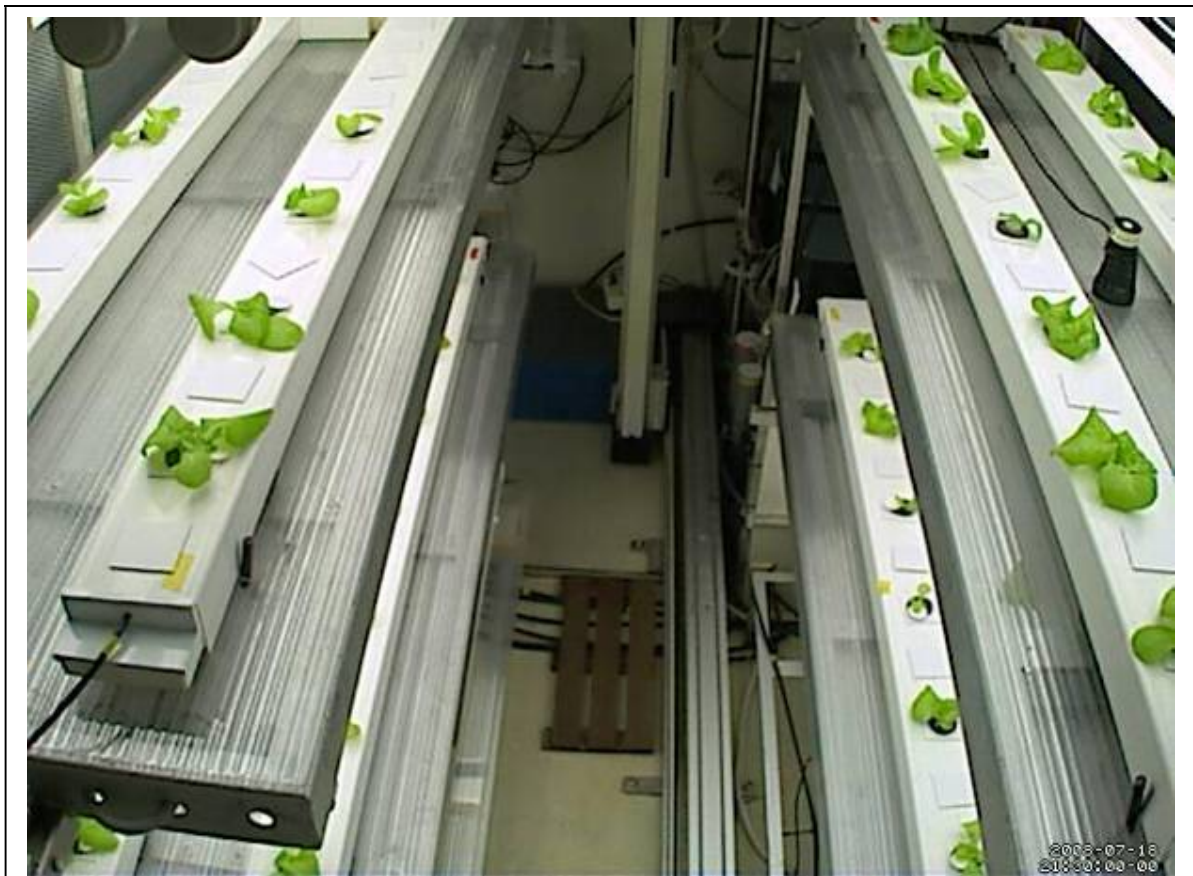


Figure 10: Experiment 2 startup – 18jul08

History of the experiment 2 plants:

- sowing date: 02/07/2008
- seeds sown on Klaver® HC-80 capillary matting strip in mini gullies
 - After 14 days, plants were transferred to HPC- room (16/07/2008 – Figure 10)

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

Figure 11 shows the growth of the selected lettuce plant enclosed in the glass single plant bulb before treatment.

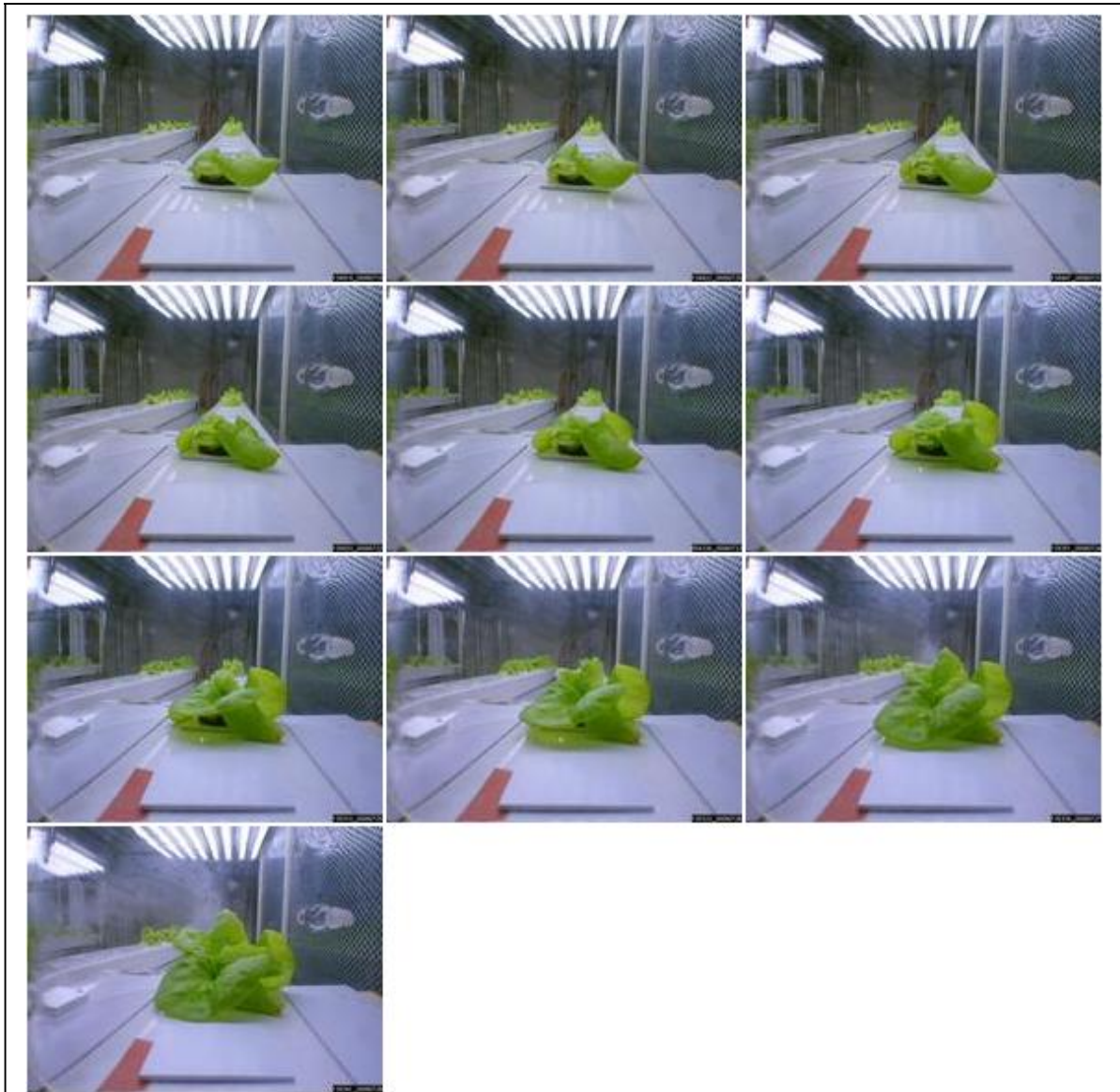


Figure 11: Time lapse growth starting from 14 days old plant till 23 days, 1 picture each day.
Date pictures: 16/07/08 till 25/07/08

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

Figure 12 shows time-course analysis of ethylene levels in both treated and control lettuce plants enclosed in a single plant cuvette.

Day 0-6 (plant age: 24-30): - alternating ethylene production measurement from 2 plants under cuvette (1 from control tank (yellow), 1 from treatment tank (blue))
 - day 2 (plant age: 25): addition of 10 μ M ACC to the treatment tank: thereafter increase of C₂H₄ production for the treated plant
 - day 6 (plant age:30): ethylene production from treated plant returned to background level

Day 6-13 (plant age: 30-37): - only ethylene production measurement from 1 new plant from treatment tank (green)
 - day 7 (plant age: 31): adding 5 μ M ACC to treatment tank: increase of C₂H₄ production of treated plant. Less production was seen than with 10 μ M ACC.

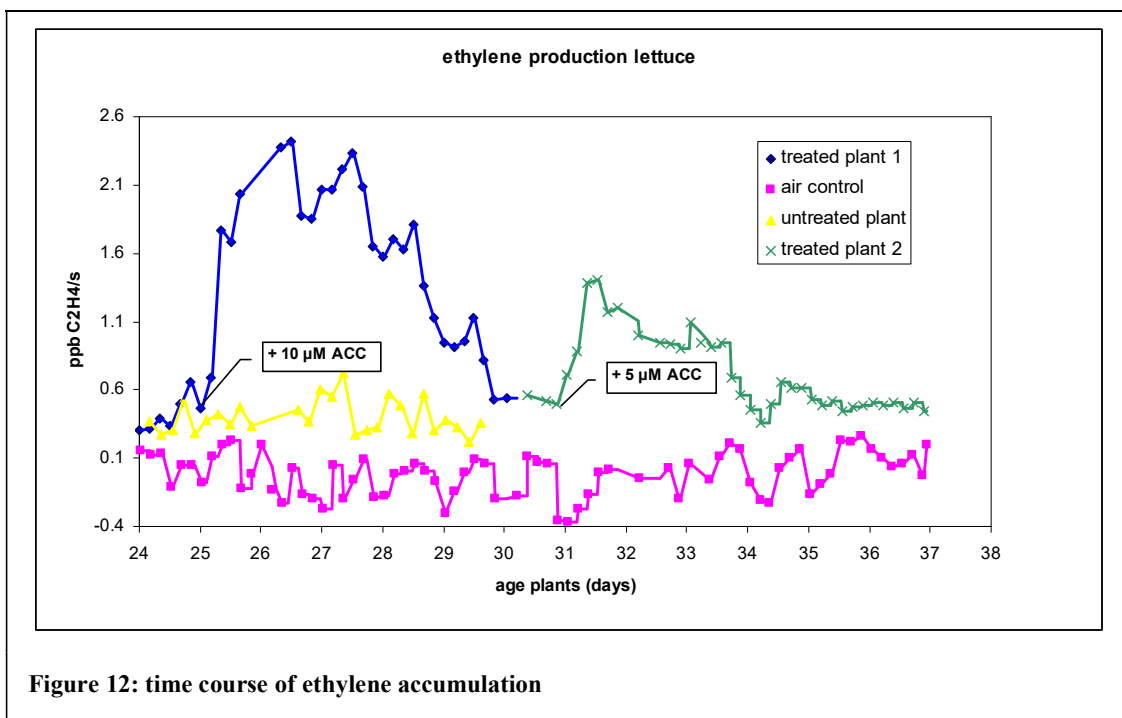
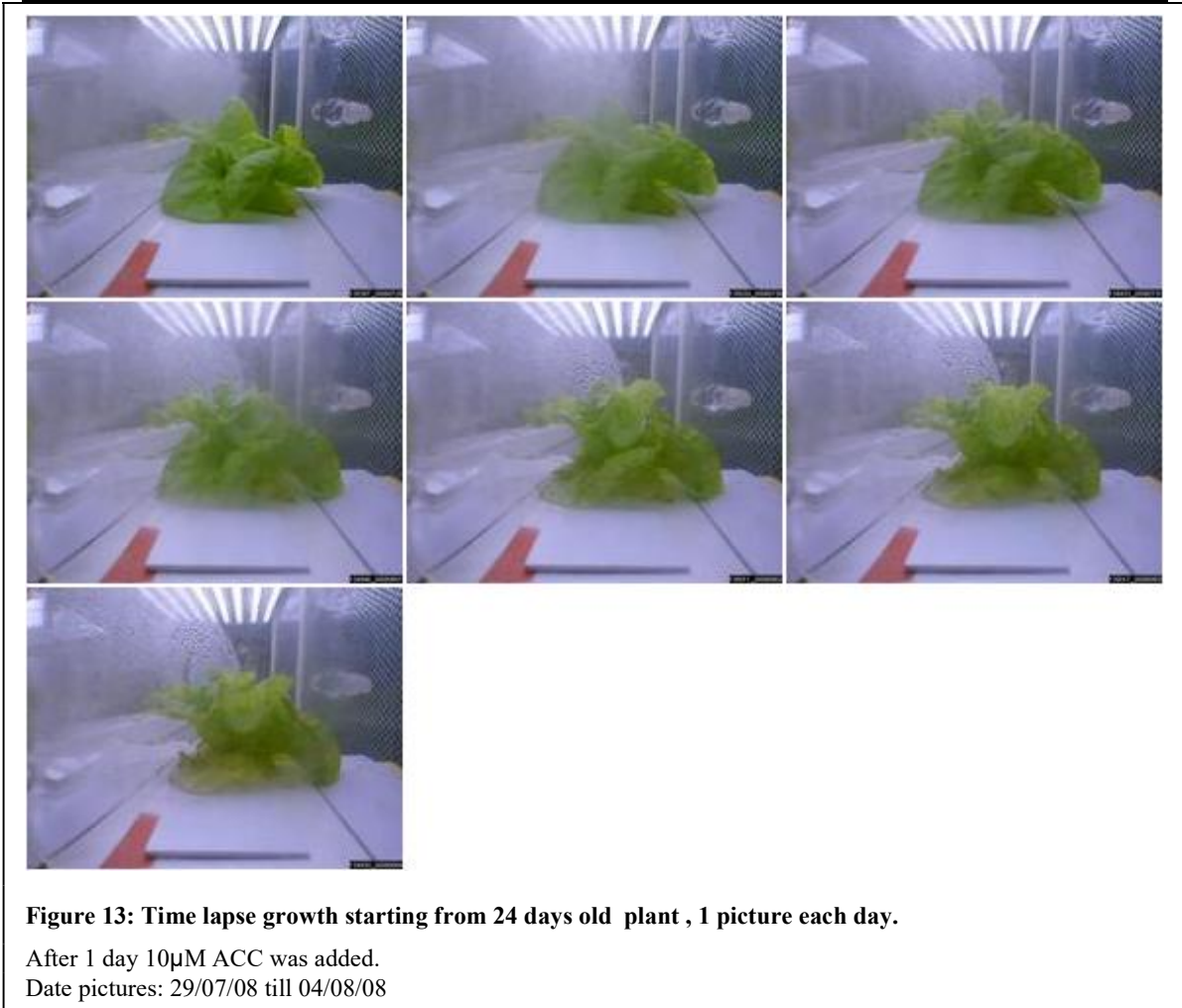


Figure 13 shows time lapse growth pictures taken during the experiment (treated plant 1, blue line in graph).

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization	
Memorandum of Understanding 19071/05/NL/CP	



CO₂ depletion was observed when the lettuce head grew mature – lowest measured level was 250ppm. Levels returned to ambient over night. Also moisture accumulated on the walls during the day period.

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	



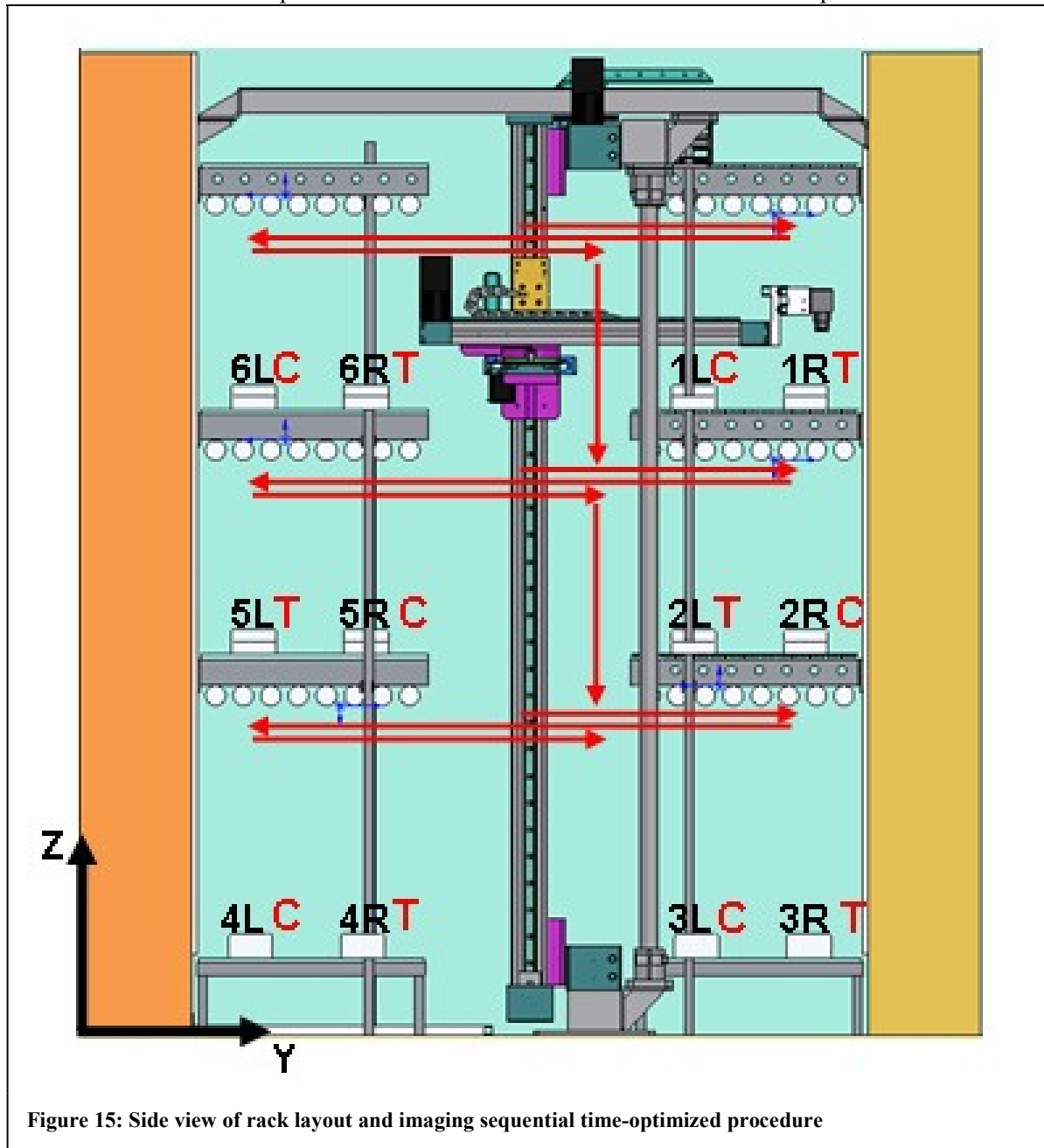
Figure 14: chamber with NFT lettuce corresponding to last frame of Figure 13

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

3.1.4 Shoot assessment

3.1.4.1 Leaf area imaging

The robotimaging Labview program procedure captured time-lapse sequences of each lettuce plant. Automatic leaf area selection is under development.



TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

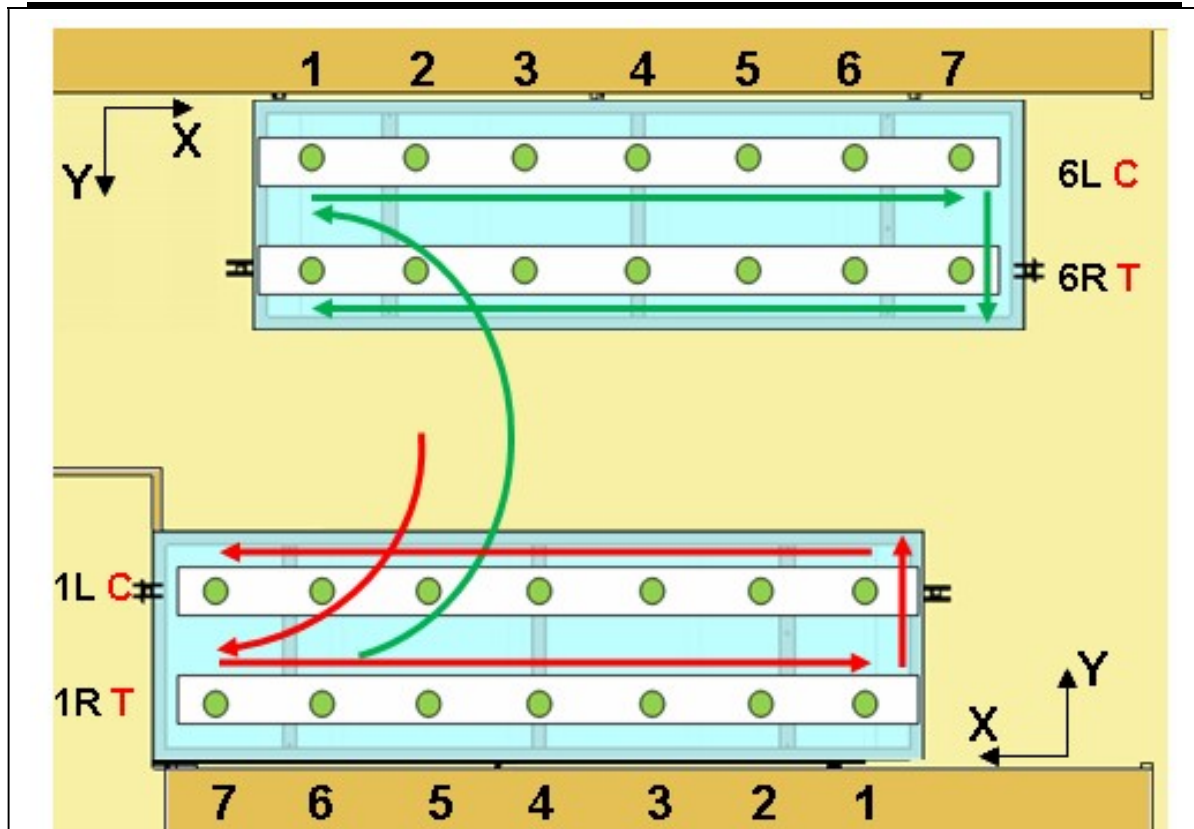


Figure 16: Top view single level imaging sequential procedure.

3.1.4.2 Visual observation

Plants were assessed daily, both shoot and root (through removable covers on the gully). Most noticeably, necrosis was seen at the leaf margins.

3.1.4.3 Leaf temperature

The thermal camera was calibrated before starting each experiment, by using its proprietary software. A miniature wheel is turned between the lens and the detector and the 2 amplifying circuits of the camera are equilibrated. If not properly calibrated a distinctive vertical banding pattern is visible..

3.1.5 Root assessment

3.1.5.1 Root response to ethylene treatment

Root curling was observed in roots developing after ACC addition. This loss of orientation is a typical ethylene effect.

3.1.6 Weight

Load cell results confirmed the larger biomass accumulation over time in ACC treated plants.

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

3.2 Destructive

3.2.1 *Microscopic root assessment*

Root morphology was observed at different levels of detail. The roots growing in contact with the air had a coverage of very fine air roots. Root caps, being composed of dead cells were typically brownish coloured.

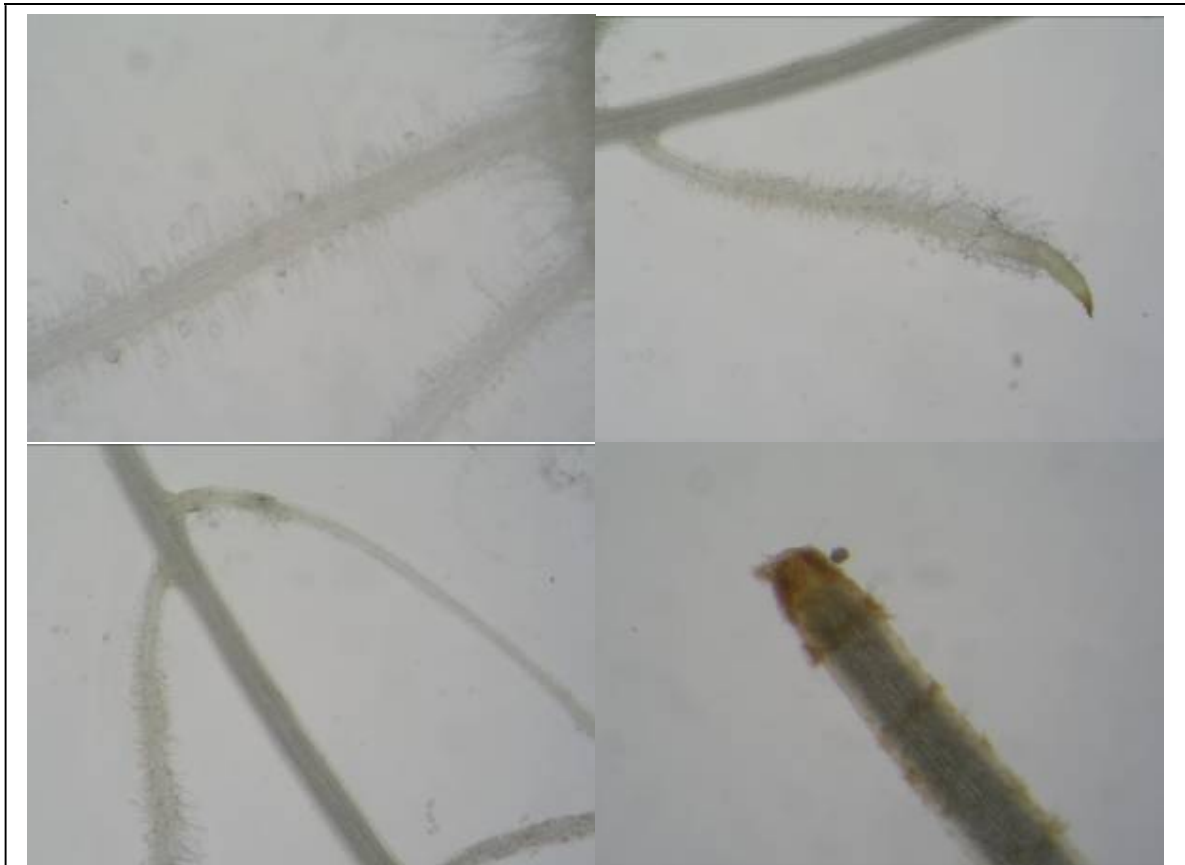


Figure 17: Root microscopic images, root surface detail, oxygen (air) root, branching, and root tip

3.2.2 *Harvest weight*

Experiment 1: ACC treated plants have a root weight comparable to the control plants, but foliar part was 135% of the control value (80 plants).

Individual crop weight (shoot part only) was 50g for treatment plants, with 7% dry weight.

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	

3.2.3 Harvest elemental analysis

The aluminium contamination was due to aluminium foil used in the drying process. Subsequently a stainless steel recipient was used.

Organan. mat.	84.54 %	Fe	32.3
Ca	15749	Mn	56.3
Mg	4998	Zn	70.2
K	33320	Cu	9.15
Na	80	Pb	0.72
P	5730	Al	281
N	27125	Mo	2.09
		Ni	1.46
Cl ⁻	133	B	18.8

Table 5: elemental analysis of lettuce mg/kg dry weight

TN 89.53	Tests report, including “as-run” annotated procedures
UGent	
This document is confidential property of the MELISSA partners and shall not be used, duplicated, modified or transmitted without their authorization Memorandum of Understanding 19071/05/NL/CP	