

Memorandum of Understanding 19071/05/NL/CP



MELISSA FOOD CHARACTERIZATION: PHASE 1

TECHNICAL NOTE: 98.4.21

PRELIMINARY TRADE-OFF OF CROP CULTIVARS:

TEST PERFORMANCES (BENCH TEST 1)

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List of Abbreviations

AAS:	Atomic Absorption Spectrophotometry
BT1 / BT2:	Bench Test 1 / Bench Test 2
CES:	Controlled Environment Systems
CESRF:	Controlled Environment Systems Research Facility
DAP:	Days After Planting
DI:	Deionised
DM:	Dry Matter
DW:	Dry Weight
EC:	Electrical Conductivity
FID:	Flame Ionization Detector
FW:	Fresh weight
GC:	Gas Chromatograph
HDPE:	High-density polyethylene
HPLC:	High Pressure Liquid Chromatograph
HZPC:	Consultant for hydroponic potato growth
ICP:	Inductive Coupled Plasma
IPL:	Institut Paul Lambein
IRGA:	Infra Red Gas Analyser
LA:	Leaf area
LC-MS/MS:	Liquid chromatography-mass spectrometry
MDL:	Minimum Detection Limit
NCER:	Net Carbon Exchange Rate
NFT:	Nutrient Film Technique
OD:	Optical Density
PAR:	Photosynthetic active radiation



PCA:	Plate Count Agar
PPF:	Photosynthetic Photon Flux
RH:	Relative Humidity
SEC-1 /SEC-2:	Sealed Environment Chambers
T:	Temperature
TDF:	Total Dietary Fibre
TGA:	Total glycoalcaloids
TN:	Technical Note
TVC:	Total Viable Count
UBern:	University of Bern
UCL:	Université Catholique de Louvain
UGent:	Ghent University
UNapoli:	University of Naples
UoGuelph:	University of Guelph
USDA:	United States Department of Agriculture
VOC:	Volatile Organic Compound
VPD:	Vapour Pressure Deficit
YGC:	Yeast extract Glucose Carbonate medium

1 Introduction

This first issue of TN98.4.2 (TN98.4.21) summarizes the results as obtained with the plant bench test measuring plan as defined in TN98.4.11. Timing of the measurements and layout of the cultivars in the bench test setup are included for each setup at the start of the respective sections of the document.

This document has final data for 2 cultivars of durum wheat (as planned in TN98.4.11) (UoGuelph) and final plant growth data and nutritional analysis of the harvest for 4 cultivars (as planned in TN98.4.11) of bread wheat (UBern) and potato (UGent and UCL). For soybean results for 3 cultivars are included, seeds of the 4th cultivar unexpectedly did not germinate.

Durum wheat culture in a sealed growth environment was characterised by harvests with yields well above recorded field data, with a slightly longer culture period due to delayed crop maturation.

Bread wheat culture displayed normal growth and ear formation. Crop maturation and especially kernel ripening also took longer than expected.

Potato culture started from in vitro plants had sufficient tuberisation induction, however shoot and tuber development slowed down followed by dying of the plants. Opportunistic infections were confirmed which are typical for stressed non-optimally growing plants. Non-optimal nutrient availability, especially prolonged nitrogen depletion can have been the cause of low plant performance.

Soybean culture resulted in pod formation. However at this most sensitive developmental stage a phyto-sanitary problem appeared possibly linked to non-optimal nutrient availability as exemplified by visual deficiency symptoms.

The measurement data as reported on a monthly basis in progress files is compiled on a companion CD. Depending on the respective setup hardware, time-lapse logging data is included.

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2 Bread wheat (UBern)

2.1 Experimental Layout

2.1.1 Measuring Plan

Tab. 1 UBern - Timing of the measurements

Measurements	Timing
T, Relative humidity	Automatic
Chamber CO ₂	Once a week
Air temperature at trough level	Weekly min and max
Plant development	Once a week
Temperature of the nutrient solution	Once a week
EC Electrical conductance	Once a week
pH	Once a week
Flow rate	Once a week
Nutrient solution (nutrient content)	Every 4 weeks, before and after exchange of the solution
Biomass	After the harvest
Kernels nutrient content	After the harvest

Plant development

Assessment for one representative plant per Rockwool block of 15 plants (a-d: 4 blocks per gully)

1. height
2. number of tillers
3. number of leaves on the main shoot
4. number of ears
5. number of grains per ear
6. leaf senescence during grain ripening

Recording of time-points of initiation for each the representative plant

- stem elongation
- ear emergence
- anthesis
- ear yellowing

Nutrient solution analysis

K, Ca, Mg, N, P, Fe, Zn, Cu, Mn, Ni

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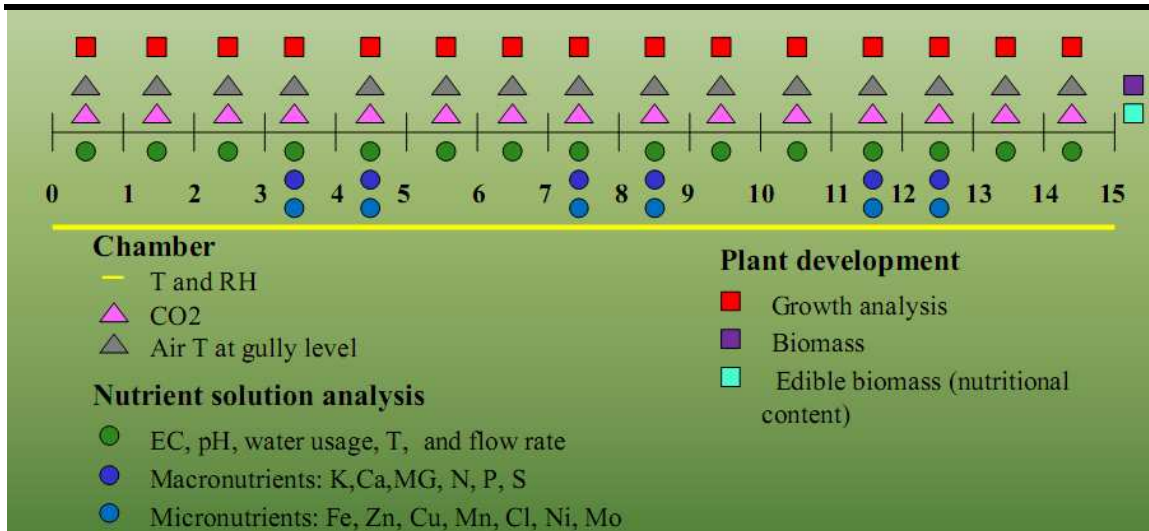


Fig. 1 UBern - Measurement plan

2.1.2 Setup

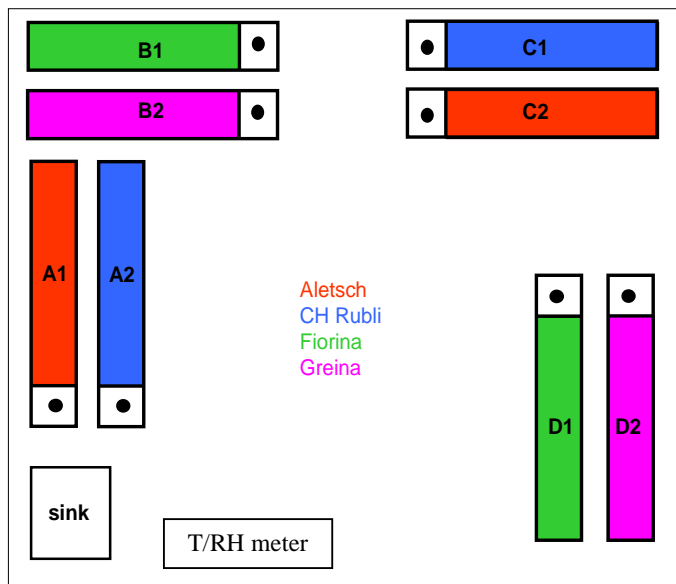


Fig. 2 UBern - Chamber Setup

Plant density was 60 plants per gully of 1m x 19cm width.
Shelf width is 60cm, 2 gullies per shelf makes 60 plants / 0.3m².
Corresponds to 200 plants / m².

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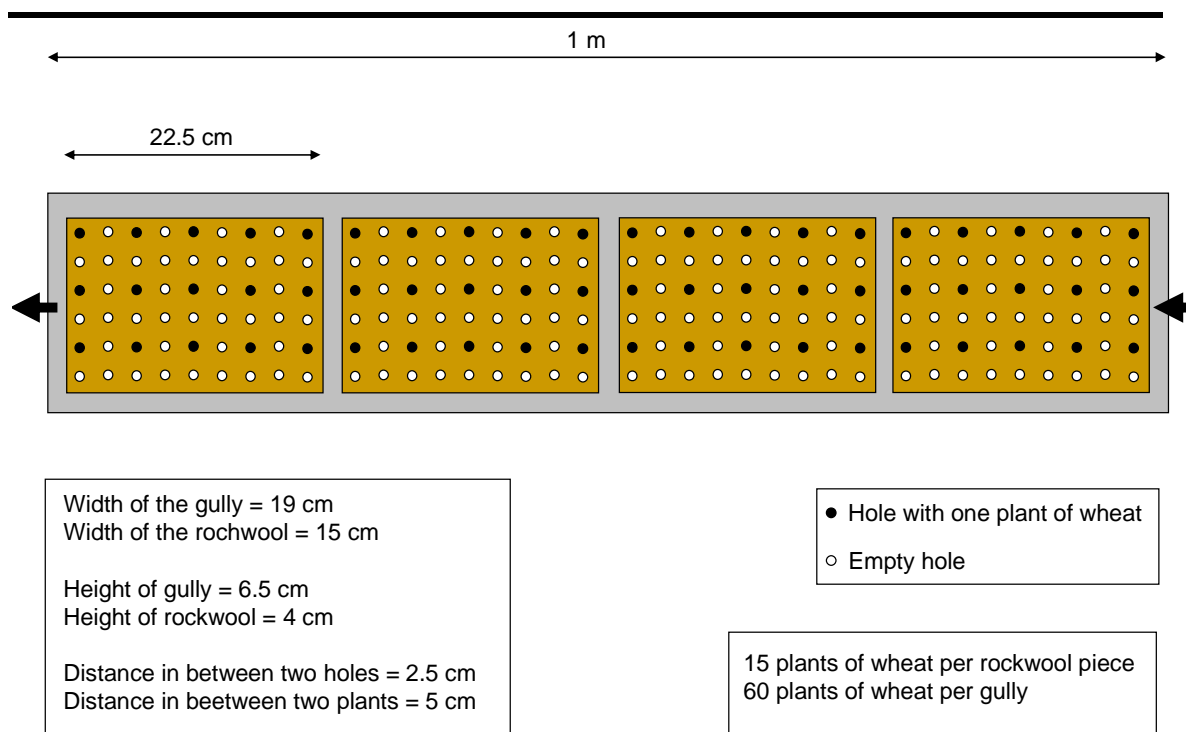


Fig. 3 UBern - Scheme of the gully and the Rockwool

2.2 Growth environment follow-up

2.2.1 Settings

Tab. 2 UBern - Settings

Photoperiod	14h 8:00 – 22:00
Light intensity	200- 450 μ mol/m ² /s
Room temperature	22°C (day), 18°C (night)

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2.2.2 Chamber T/RH evolution



Fig. 4 UBern - Chamber T / RH 28.09.09 – 04.10.09

Humidity and T were measured at the location indicated (Fig. 2 the hygrometer was positioned at the same height as the gullies.

The temperature was stable at 20±1 degree during the day, with a night T at 16±1degree.

Humidity increased during the night, and decreased during the day. The building central air renewal system operates from 06:30 till 22:00.

Humidity was overall higher as the plants developed (Fig. 4/ Fig. 5).

Extra dehumidification needed to be installed to avoid exceeding chamber safety settings.

Tab. 3 shows temperature distribution in the room, according to the setup of thermometers in Fig. 5. Apart from 2 extreme levels at location 3 (see Fig. 6), temperature was within 2,5 degrees (21-23.5) as a function of space and time. A series of measurements at the same timepoint showed values within 1 degree (Tab. 4).

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Fig. 5 UBern - Chamber T / RH 26.10.09 – 01.11.09

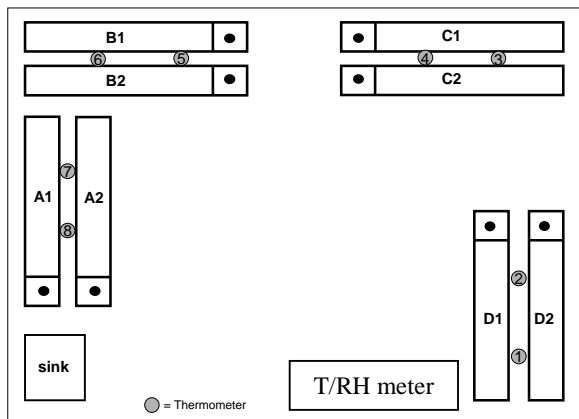


Fig. 6 UBern - Thermometer placement

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Tab. 3 UBern - Temperature at gully level September

Date	Time	Therm. 1	Therm. 2	Therm. 3	Therm. 4	Therm. 5	Therm. 6	Therm. 7	Therm. 8
2/09/2009	14:05	23	23	24,5	23	22	21,5	23	22,5
9/09/2009	10:50	23	23	24,5	23	22	22	23	22
15/09/2009	9:56	22,5	22,5	23,5	23	22	22	22	22
22/09/2009	13:00	22,5	22	22,5	22	21,5	21	21,5	21,5
29/09/2009	10:50	22,5	22	22,5	22	21,5	21	21,5	21,5

Tab. 4 UBern - Temperature at gully level October

Date	Time	Therm. 1	Therm. 2	Therm. 3	Therm. 4	Therm. 5	Therm. 6	Therm. 7	Therm. 8
6/10/2009	11:05	23	22	22,5	22,5	21,5	21,5	22	22
13/10/2009	11:05	23	22,5	23	23	22	22	22,5	22,5
20/10/2009	11:10	23	22,5	23	23	22	22	22,5	22
27/10/2009	11:00	23	22,5	23	23	22,5	22,5	23	22

Tab. 5 UBern - Night T / max. day T

Date	Time		Therm. 1	Therm. 2	Therm. 3	Therm. 4	Therm. 5	Therm. 6	Therm. 7	Therm. 8
1/12/2009	10:40	T max	23,5	23,5	24,5	24,5	23,5	23,5	23	22,5
		T min	14	14	14	14	13	14	14	16
8/12/2009	14:00	T max	23,5	23,5	24,5	24,5	23	24	23,5	22
		T min	14	14	14,5	14	14	13	14,5	15
15/12/2009	13:30	T max	24	23	24,5	25	23,5	23,5	23,5	23
		T min	15	14	15,5	14	13,5	14	14,5	15,5

2.2.3 Chamber CO₂ level

An IRGA system was used to monitor chamber CO₂ level. Ambient air is supplied to the chamber.

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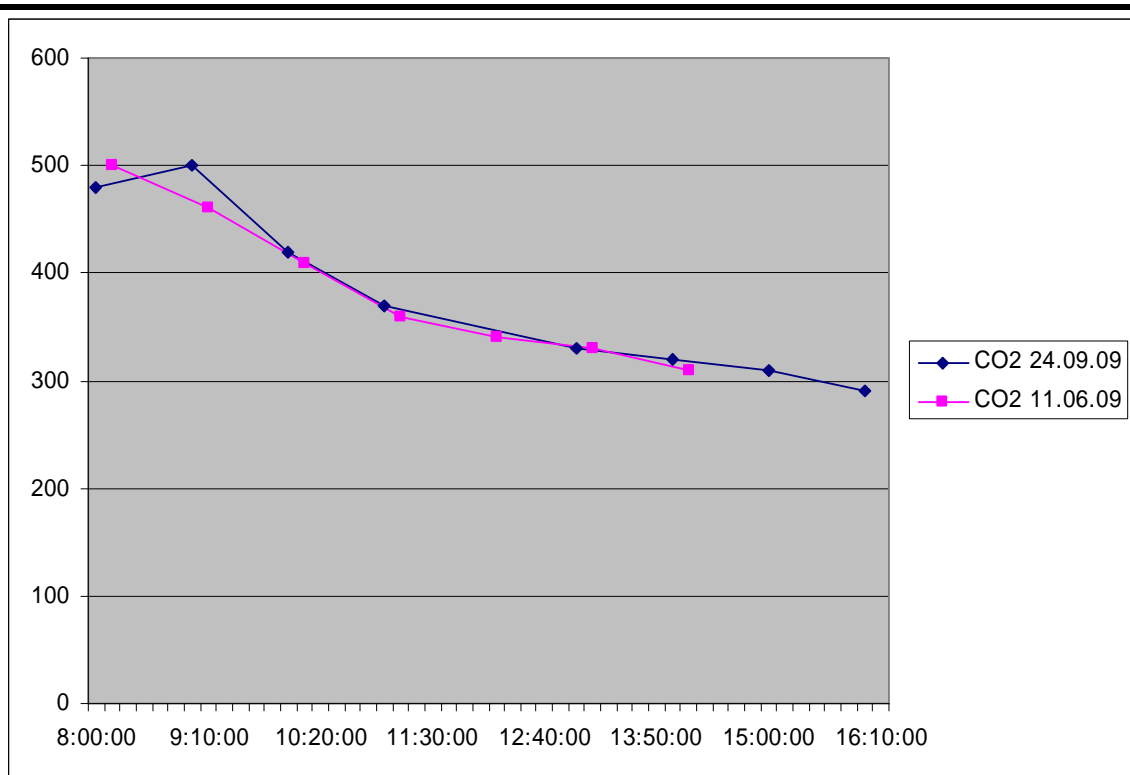


Fig. 7 UBern - Chamber CO₂ level

CO₂ concentration rises during the night, when the conditioned outside air supply system to the chamber is not active (22h-6:30h), and decreases to ambient levels and below during the day, as measured in the middle of the room.

2.2.4 Nutrient Solution Environment

Tab. 6 UBern - Nutrient solution environment

Change of nutrient solution	21/10/2009
NFT layer thickness	approximately 0.5 cm
NFT nutrient solution flow	2 l/min Initial setting
Gully inclination	1%

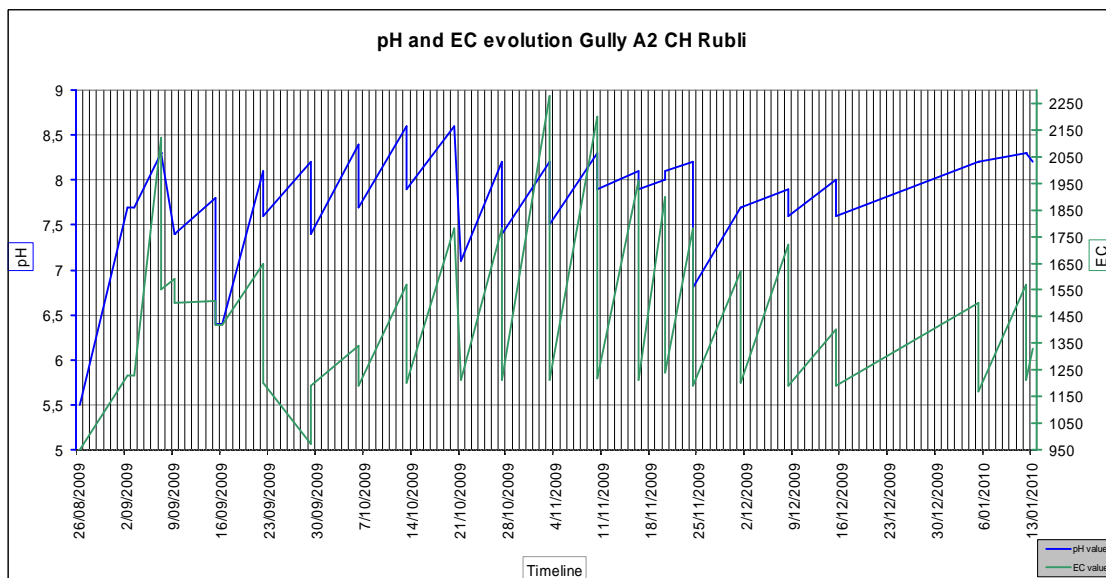
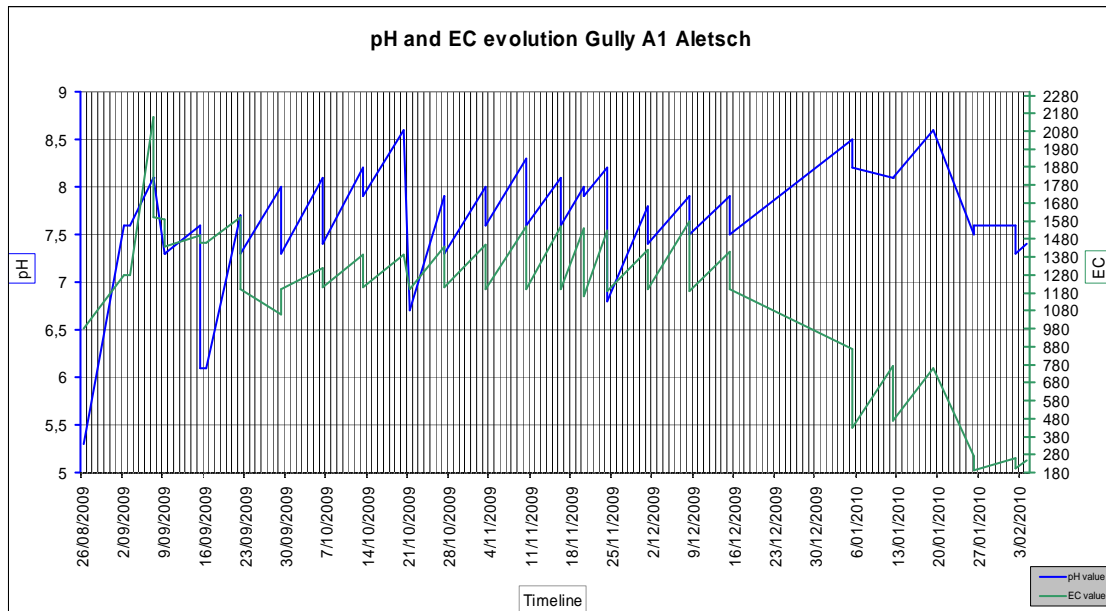
Tab. 7 UBern - NFT nutrient solution flow adjustments

	A1 Aletsch	A2 CH Rubli	B1 Fiorina	B2 Greina	C1 CH Rubli	C2 Aletsch	D1 Fiorina	D2 Greina
Before 24 Nov.	2 l/m	0,52 l/m	1,7 l/m	2 l/m	0,7 l/m	0,9 l/m	2 l/m	1,4 l/m
After 24 Nov.	2 l/m	0,52 l/m	1,7 l/m	1,5 l/m	0,7 l/m	0,9 l/m	1,5 l/m	1,4 l/m

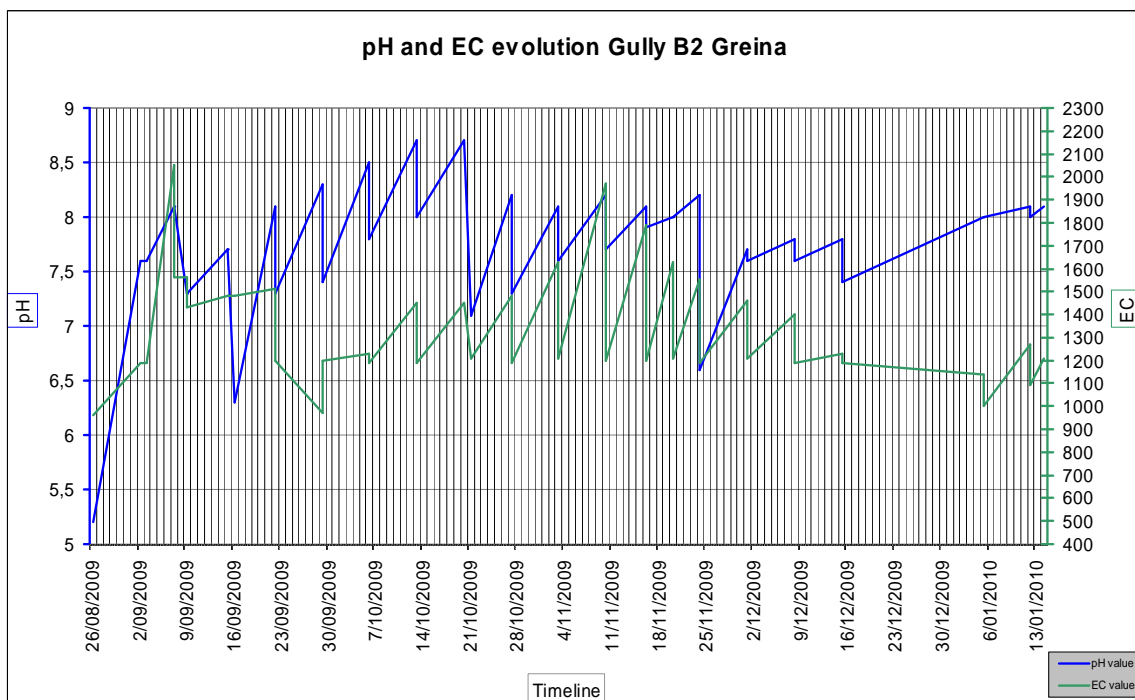
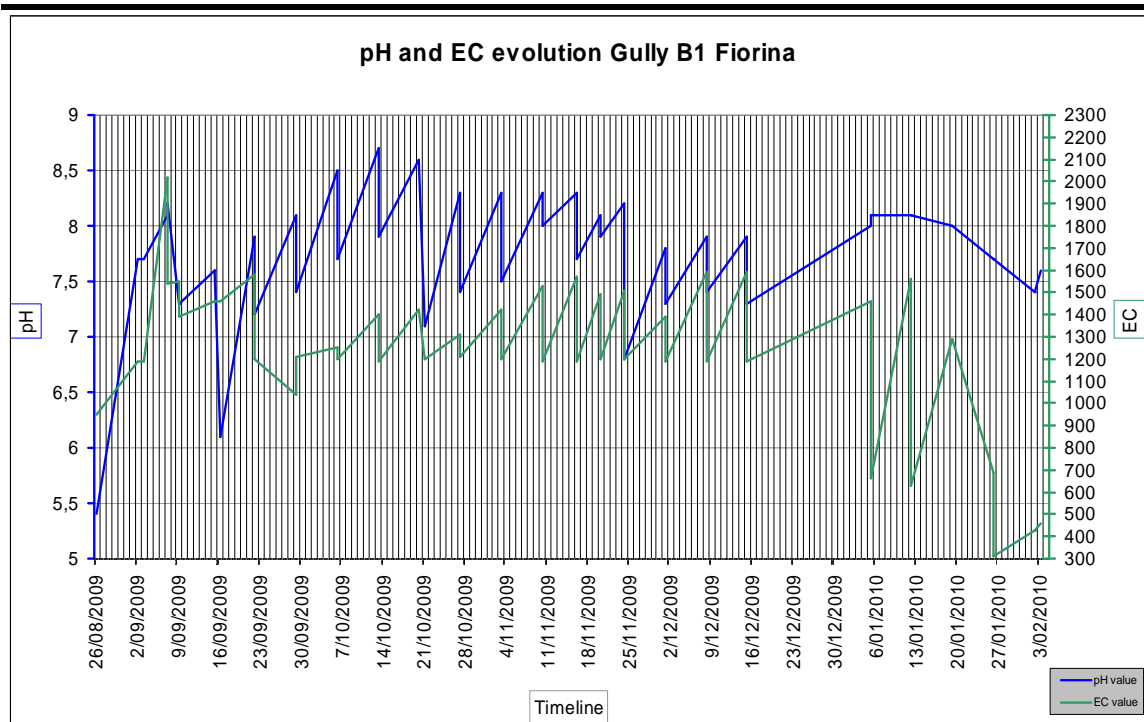
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2.2.5 pH and EC evolution

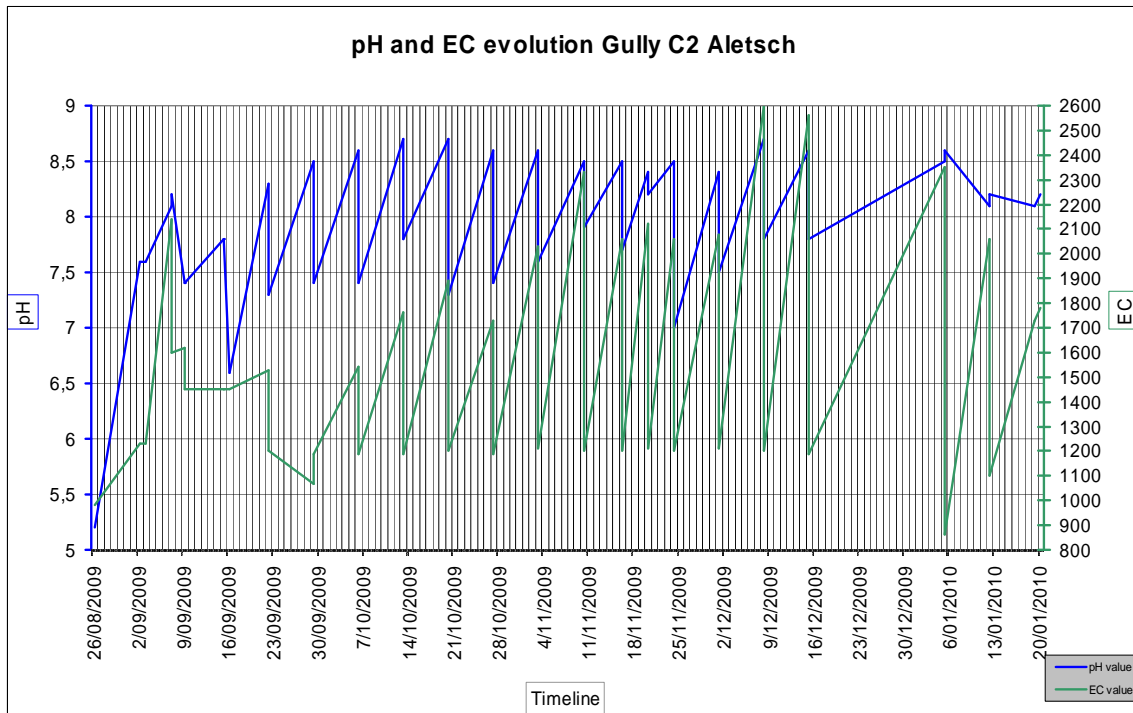
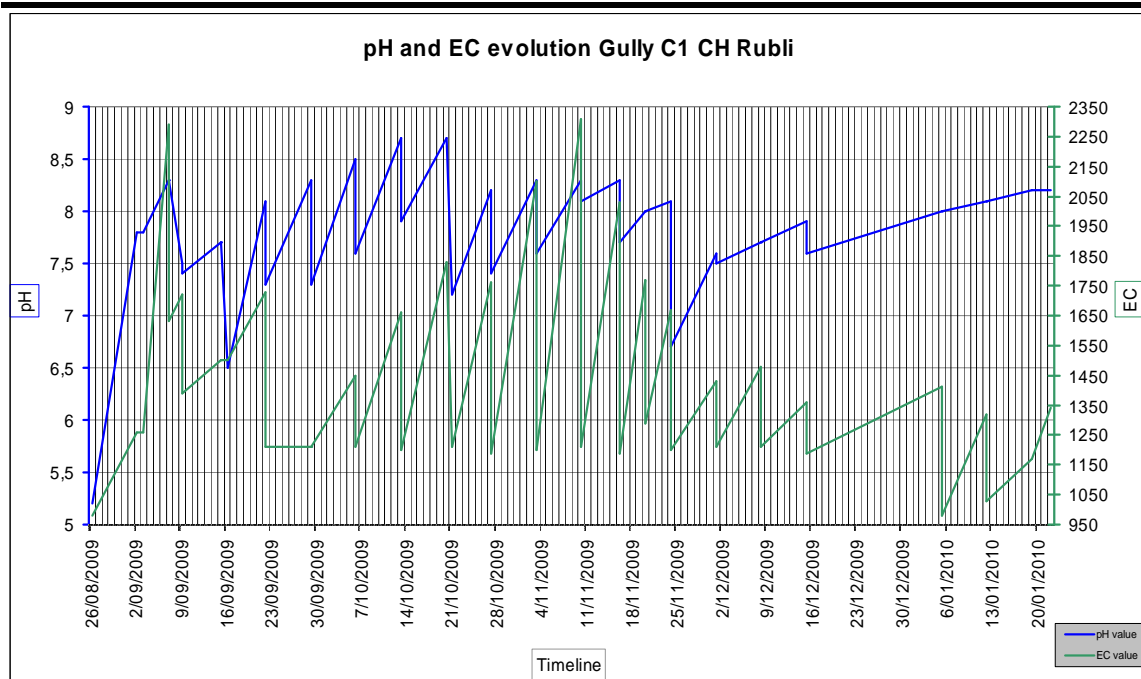
The pH rise of the nutrient solutions was not compensated by acid additions. EC of the nutrient solution was reset to 1200 $\mu\text{S}/\text{cm}$ with stock solution and distilled water, pH fluctuated between 6.5 and 8 between successive reset time points. Nutrient solution changes 16 September, 21 October and 24 November.



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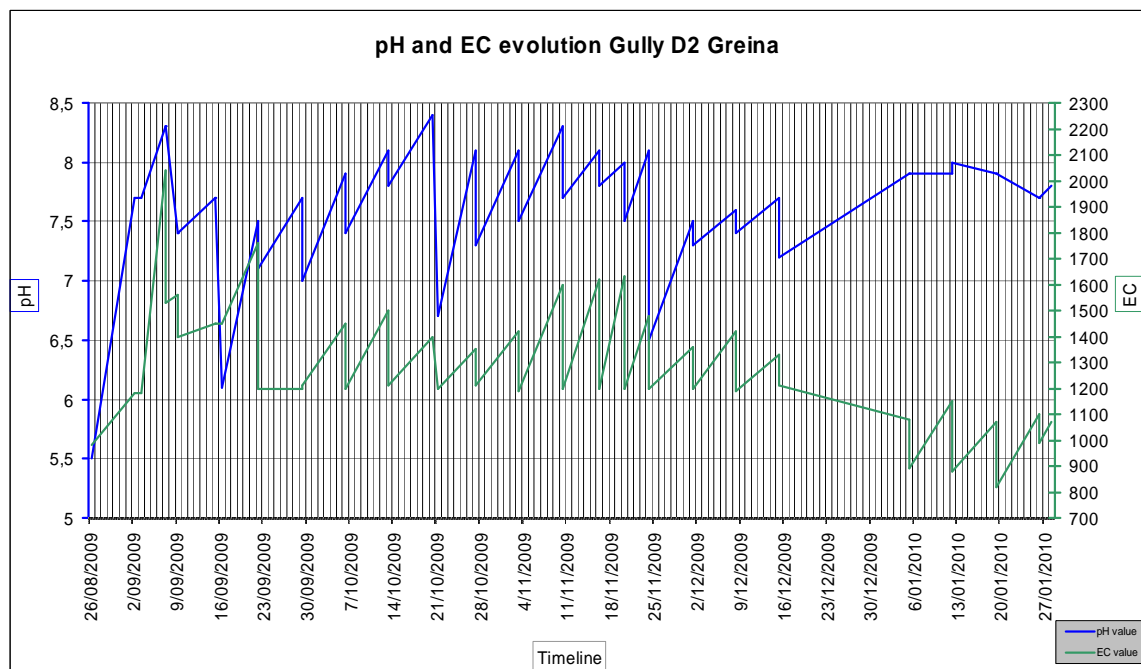
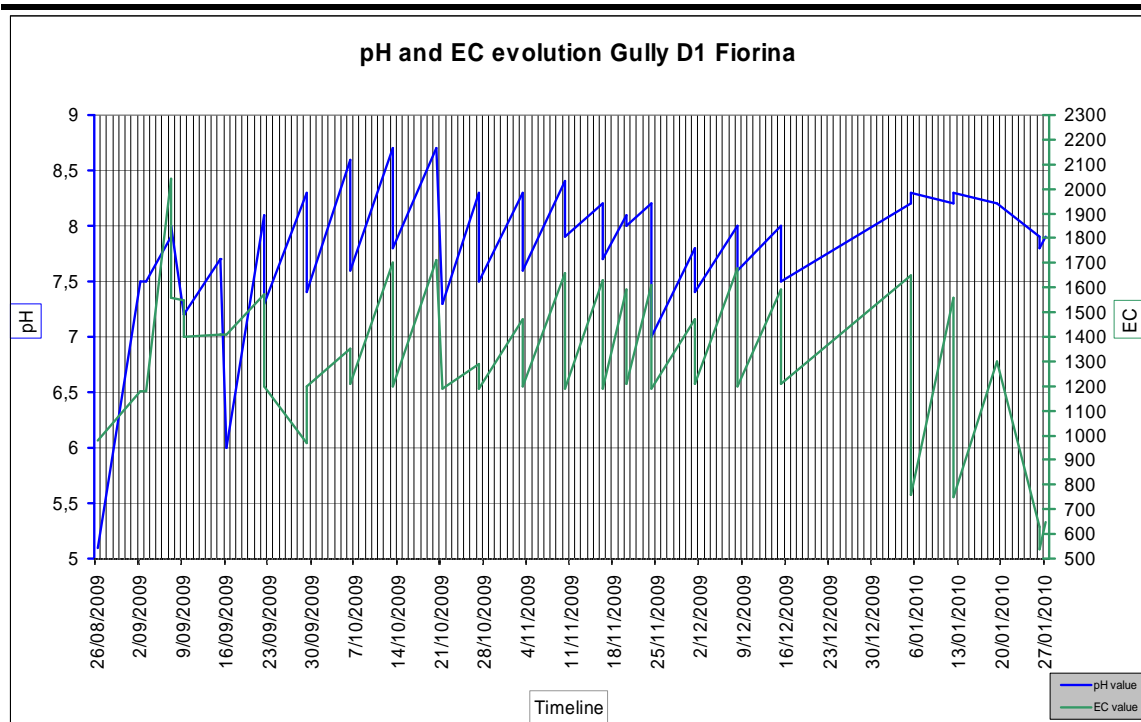


Fig. 8 UBern - pH / EC evolution per gully/cultivar

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2.2.6 Plant water usage

The total amount of liquid added to the 8 individual gully systems during the complete crop developmental period is shown in Fig. 9.

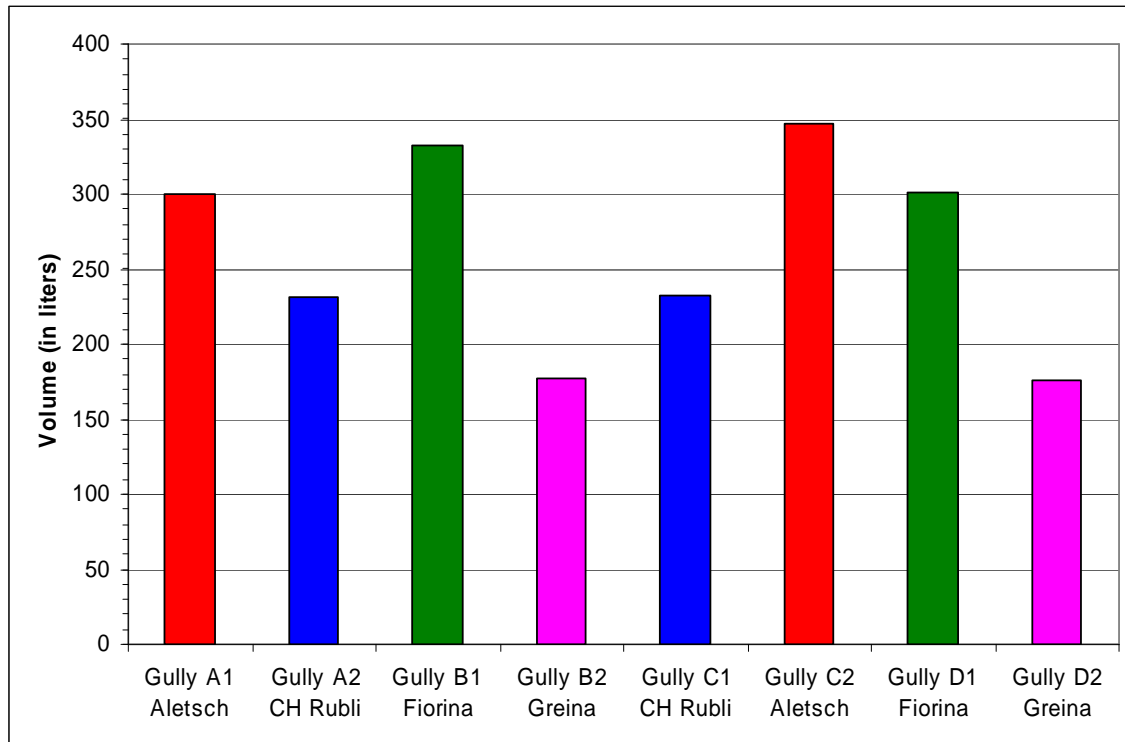


Fig. 9 UBern - Amount of liquid

Plant water usage was determined as starting nutrient solutions (15l) minus the amount left in the system at the time of solution change, plus the water added to adjust the liquid level, plus EC replenishment solution

2.2.7 Nutrient solution T

No nutrient solution cooling was foreseen, Fig. 10 shows temperatures between 25 and 27 degrees, chamber atmosphere T settings being 22 during the day and 18 degrees during the night.

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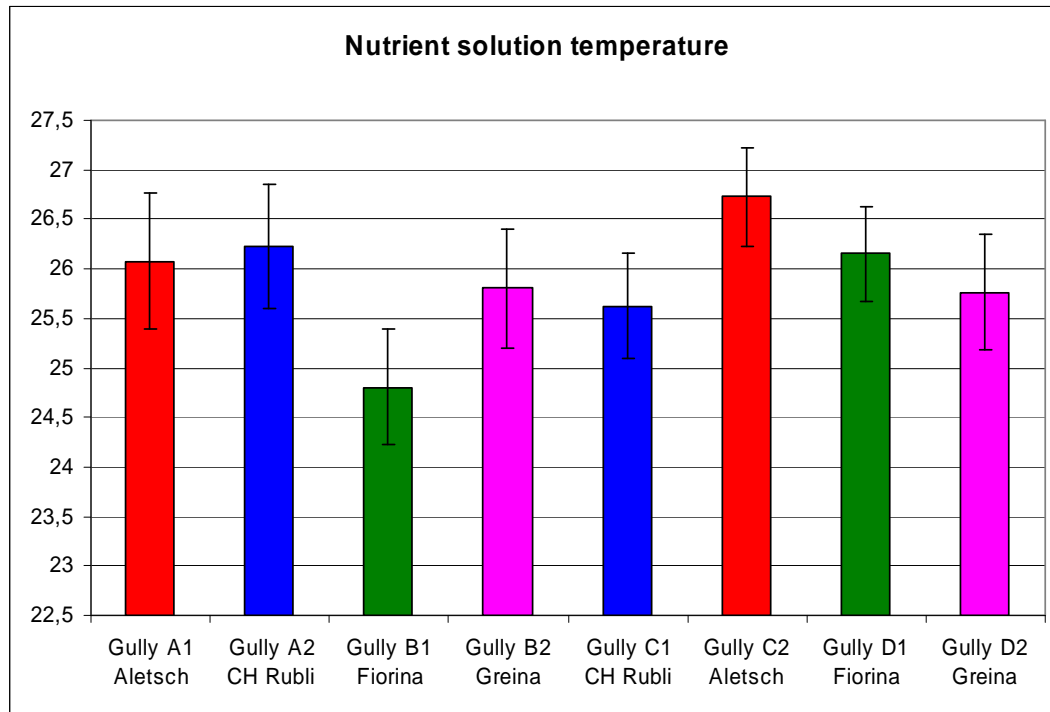
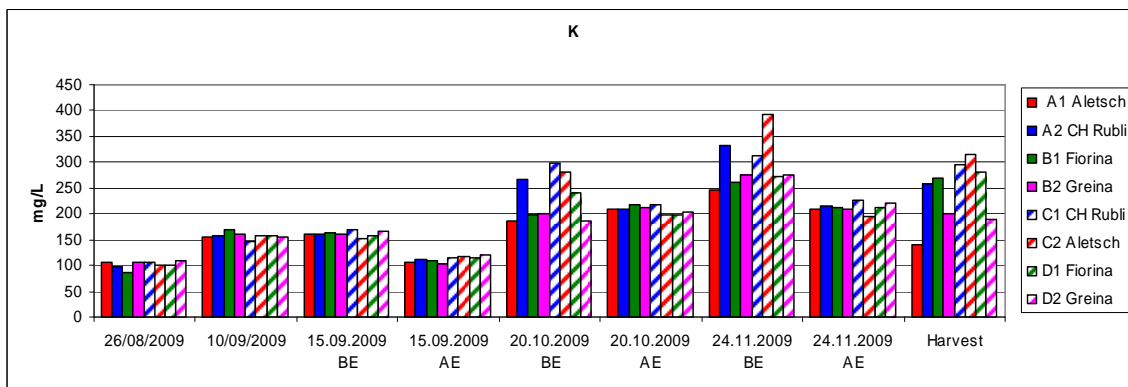
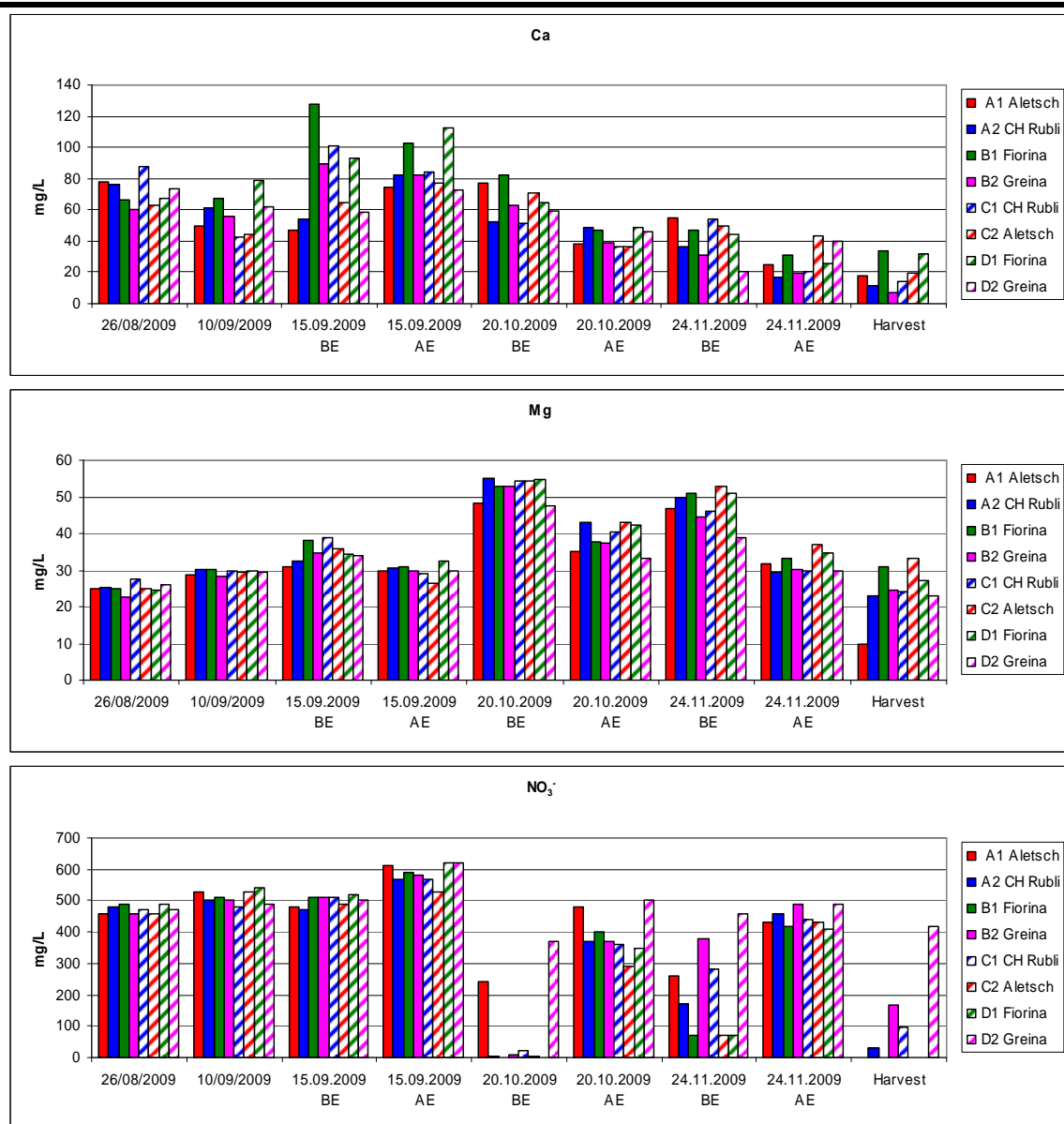


Fig. 10 UBern - Nutrient solution T 24.08.09 – end

2.2.8 Nutrient solution analysis





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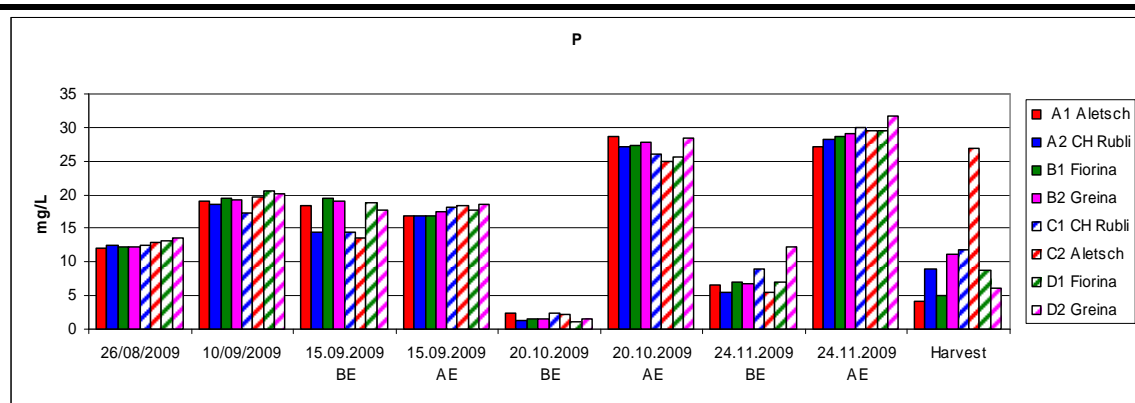
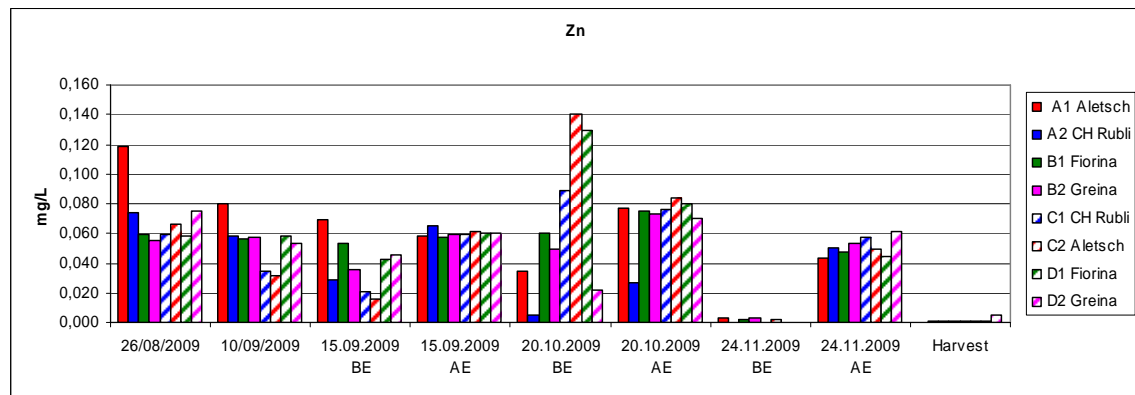
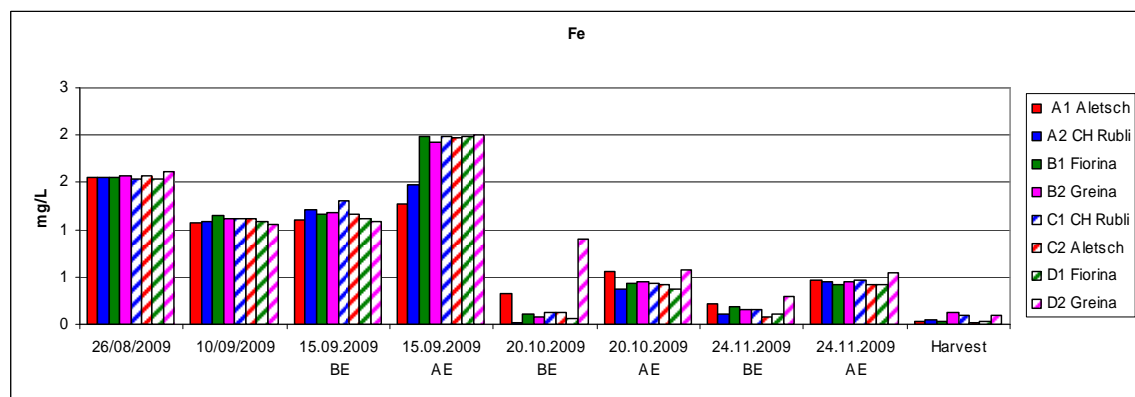


Fig. 11 UBern - Nutrient solution analysis for macro-nutrients K Ca Mg N P



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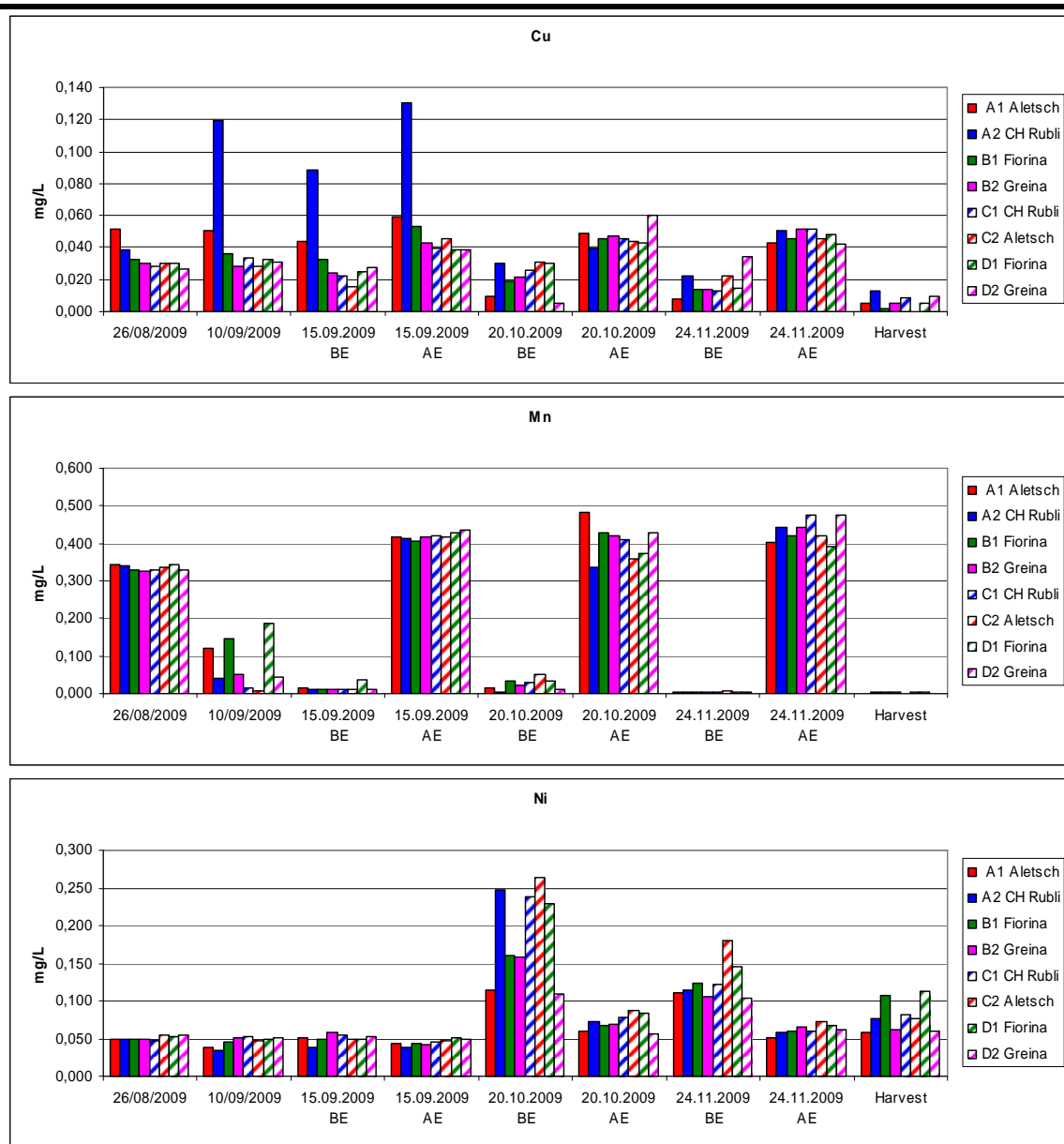


Fig. 12 UBern - Nutrient solution analysis for micro-nutrients Fe Zn Cu Mn Ni

The phosphate analysis (mmol P/liter; P=30.97g/mol) results show a marked depletion after 4 weeks, solution exchange remediated this low level. The higher level at the final measuring point could be explained by the development of a slime layer in the gully, likely of microbial origin.

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2.3 Monitoring of plant development

The growth period varied from 140 to 162 days. This reflects the difference in maturation characteristics between the cultivars (see Tab. 8 and section **Error! Reference source not found.**).

Tab. 8 Growth period and maturation characteristics for bread wheat cultivars

Cultivars	Gully	Germination	Harvest	Number of days	Ripeness	Number of days for ripeness
Aletsch	A1	24.08.2009	04.02.2010	164	not completely mature at harvest	more than 164
CH Rubli	A2	24.08.2009	13.01.2010	142	13.01.2010	142
Fiorina	B1	24.08.2009	03.02.2010	163	not completely mature at harvest	more than 163
Greina	B2	24.08.2009	14.01.2010	143	13.01.2010	142
CH Rubli	C1	24.08.2009	22.01.2010	151	13.01.2010	142
Aletsch	C2	24.08.2009	20.01.2010	149	13.01.2010	142
Fiorina	D1	24.08.2009	27.01.2010	156	not completely mature at harvest	more than 156
Greina	D2	24.08.2009	28.01.2010	157	13.01.2010	142

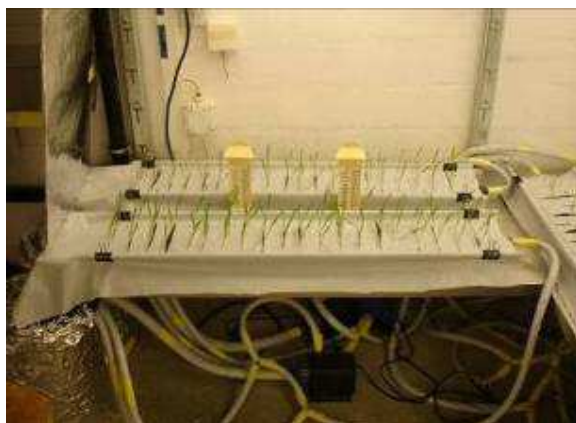
2.3.1 Photographic follow-up - monthly overview

The development of the aerial part (shoot) is shown from the seedling stage to the final development with monthly intervals.

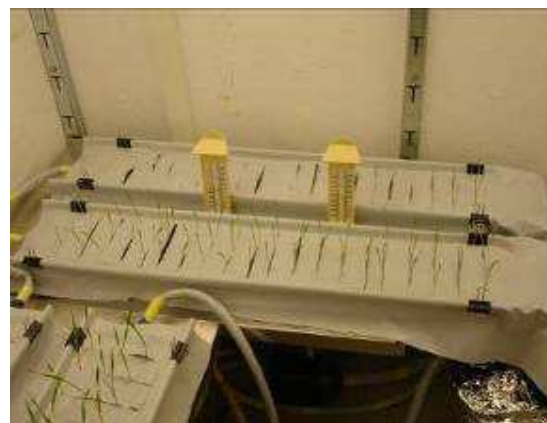
Additional information is available on the companion CD to this TN.

The experiment was started on august 24th.

In the next section 2.3.2, the development of the wheat ears is shown on a monthly basis.

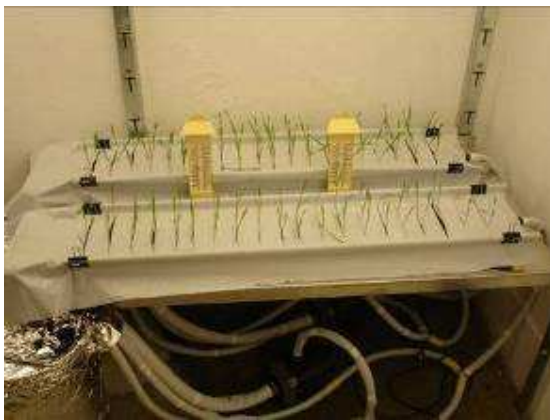


Gullies A1A2, 31 August 2009

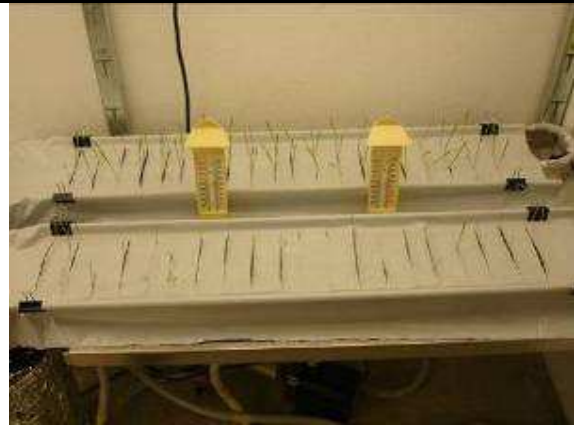


Gullies B1B2, 31 August 2009

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Gullies C1C2, 31 August 2009



Gullies D1D2, 31 August 2009



Gullies A1A2, 29 September 2009



Gullies B1B2, 29 September 2009

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Gullies C1C2, 29 September 2009



Gullies D1D2, 29 September 2009



Gully A1 and A2, 27 October 2009



Gully B1 and B2, 27 October 2009

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Gully C1 and C2, 27 October 2009



Gully D1 and D2, 27 October 2009



Gullies A1 and A2, 24 November 2009



Gullies B1 and B2, 24 November 2009



Gullies C1 and C2, 24 November 2009



Gullies D1 and D2, 24 November 2009

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Gullies A1A2, 15 December 2009



Gullies B1B2, 15 December 2009



Gullies C1C2, 15 December 2009



Gullies D1D2, 15 December 2009

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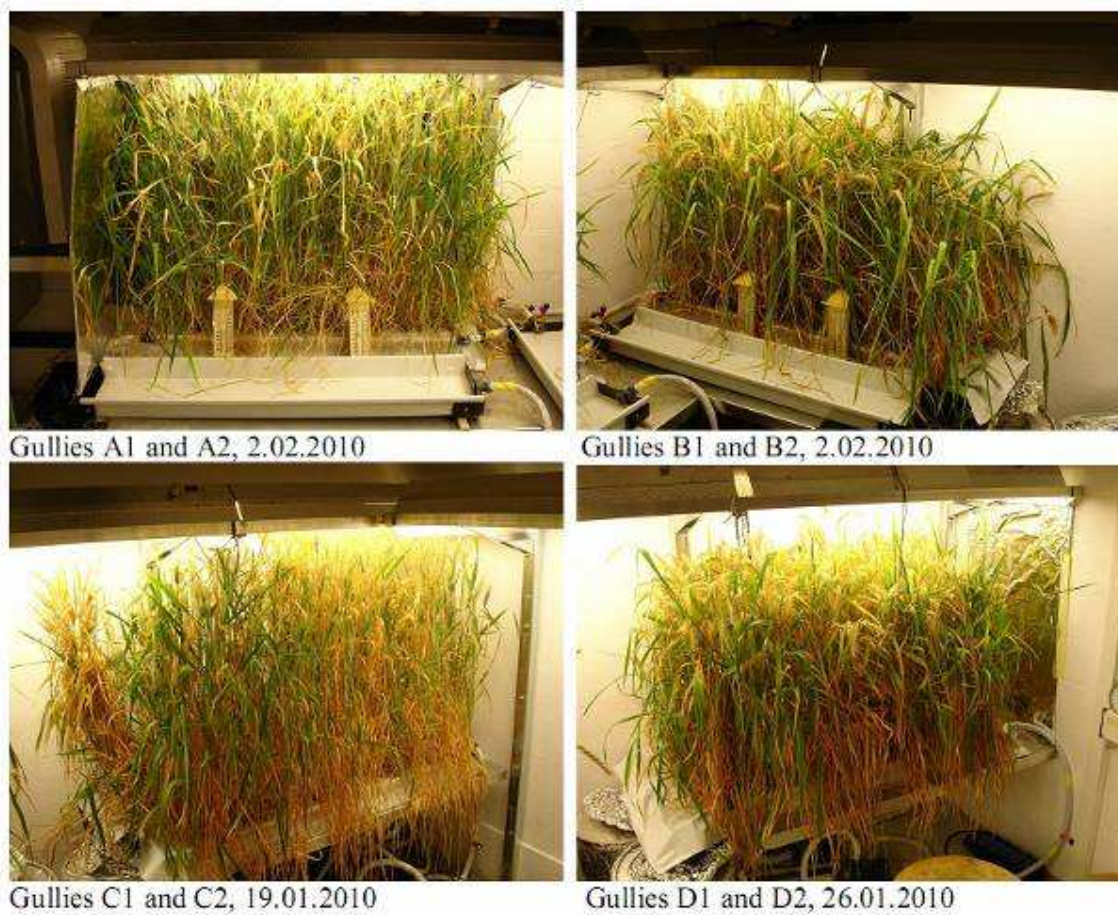
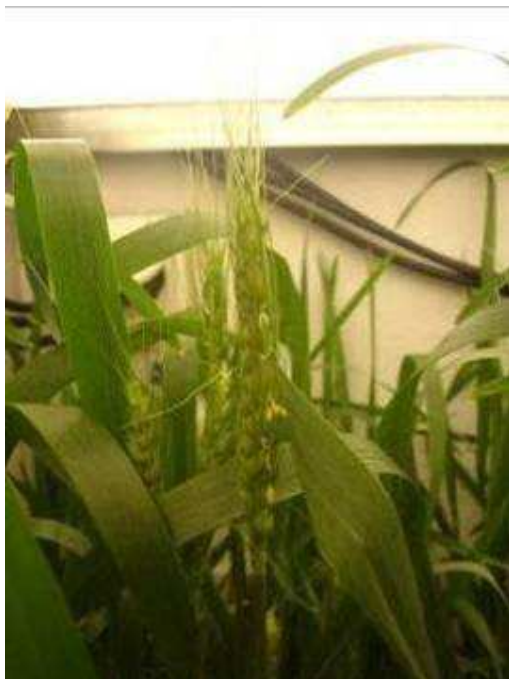


Fig. 13 UBern - Photographic follow up

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2.3.2 Detailed photographic observations



Ear of CH Rubli flowering, A2, 26 Oct.'09
 Ear of CH Rubli flowering, A2, 26 Oct.'09



Ear of Greina flowering, B2, 26 Oct.'09
 Ear of Greina flowering, B2, 26 Oct.'09



Yellowing ears: CH Rubli (A2), 24Nov.'09
 Yellowing ears: CH Rubli (A2), 24Nov.'09



Yellowing ears: Greina (B2), 24 Nov.'09
 Yellowing ears: Greina (B2), 24 Nov.'09

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Ears of CH Rubli, gully A2, 15 Dec.'09



Ears of Greina, gully B2, 15 Dec.'09



Ears of Fiorina, gully B1, 15 Dec.'09



Ears of Aletsch, gully A1, 15 Dec.'09

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Ears of CH Rubli, gully C1, 15 December 2009.



Ears of Greina, gully D2, 15 December 2009.



Ears of Fiorina, gully D1, 15 December 2009.



Ears of Aletsch, gully C2, 15 December 2009.

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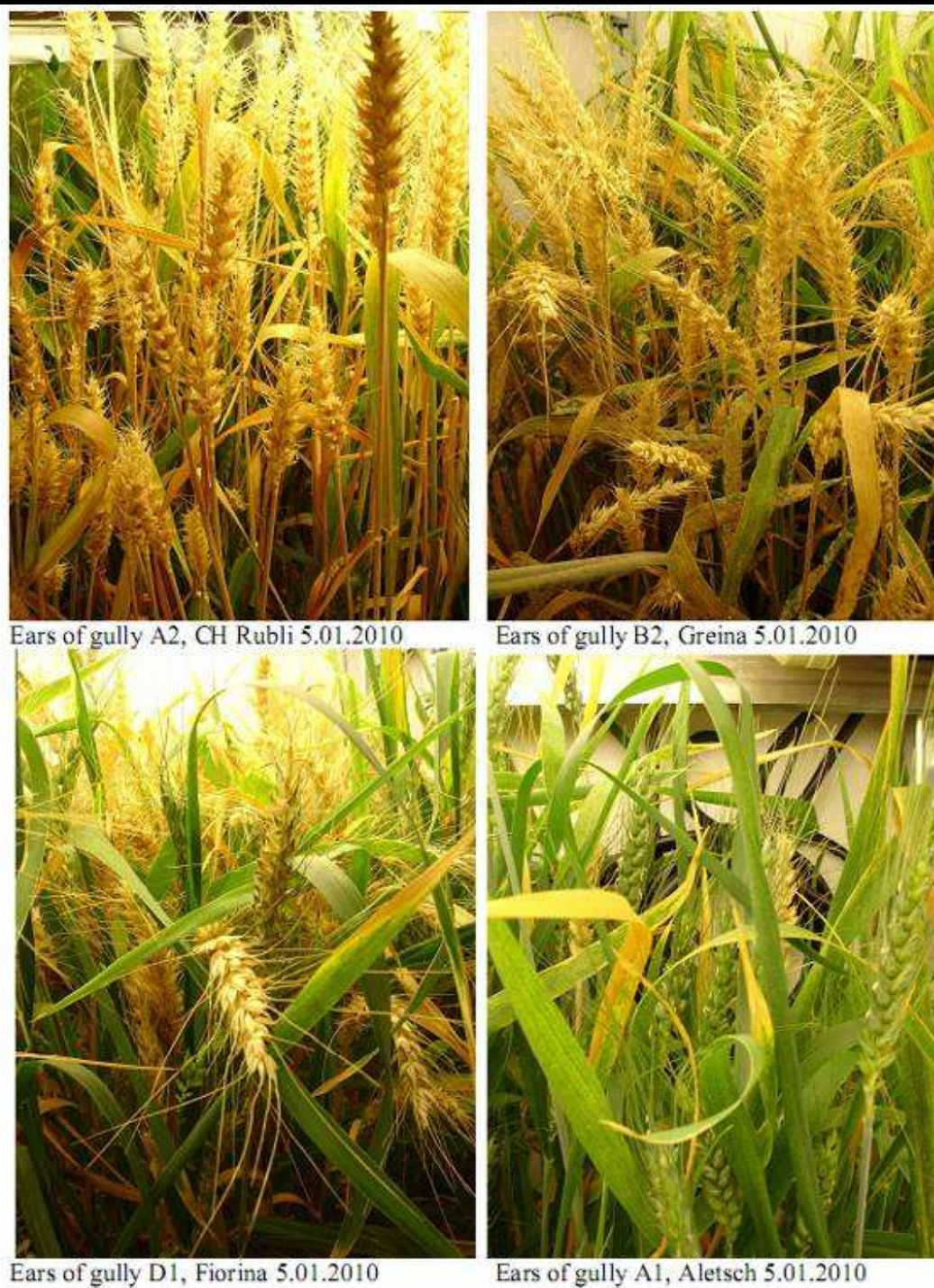


Fig. 14 UBern - Ears of the flowering bread wheat

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2.3.3 Growth assessment

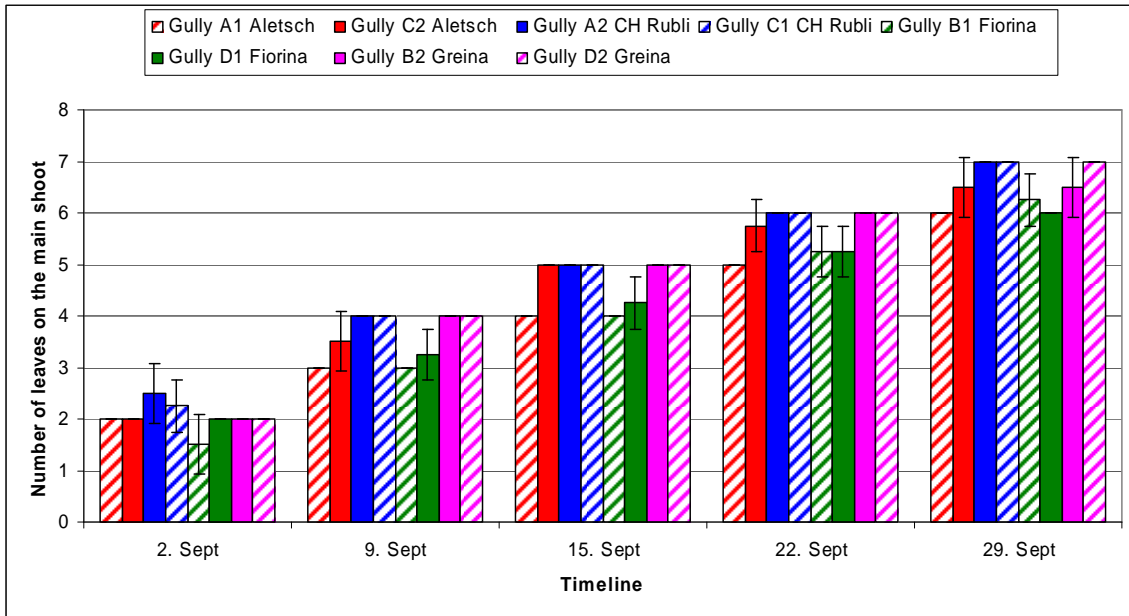


Fig. 15 UBern - Number of Leaves on the main shoot
Count was limited to the 6th leaf.

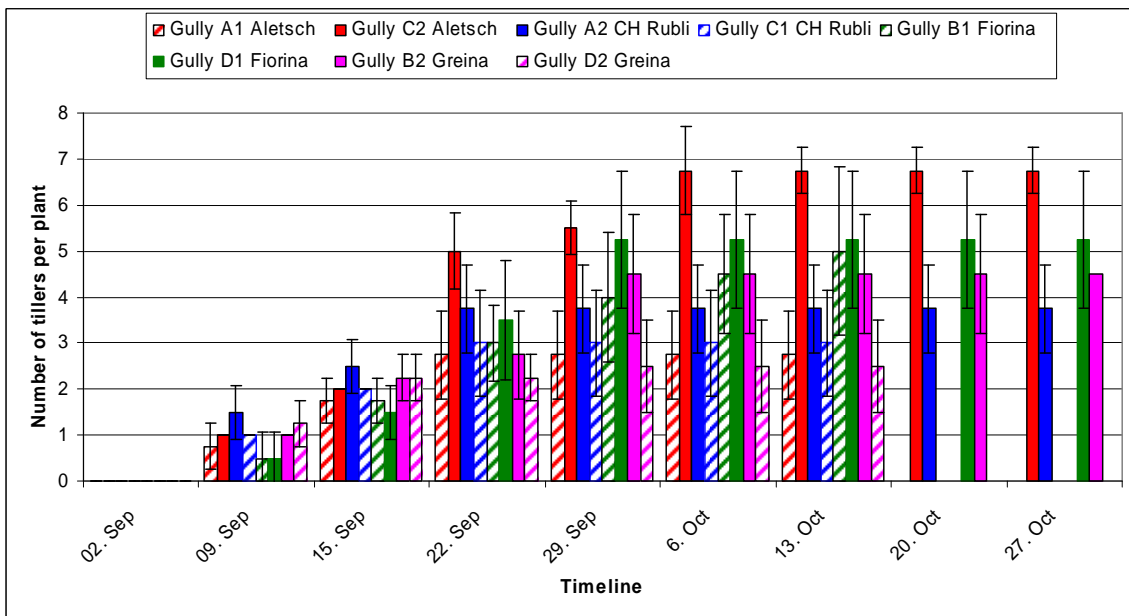


Fig. 16 UBern - Number of tillers per plant

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In Fig. 15 and Fig. 16 only the gullies from each shelf facing the centre of the room were accessible for measurements (A2 B2 C2 D1).

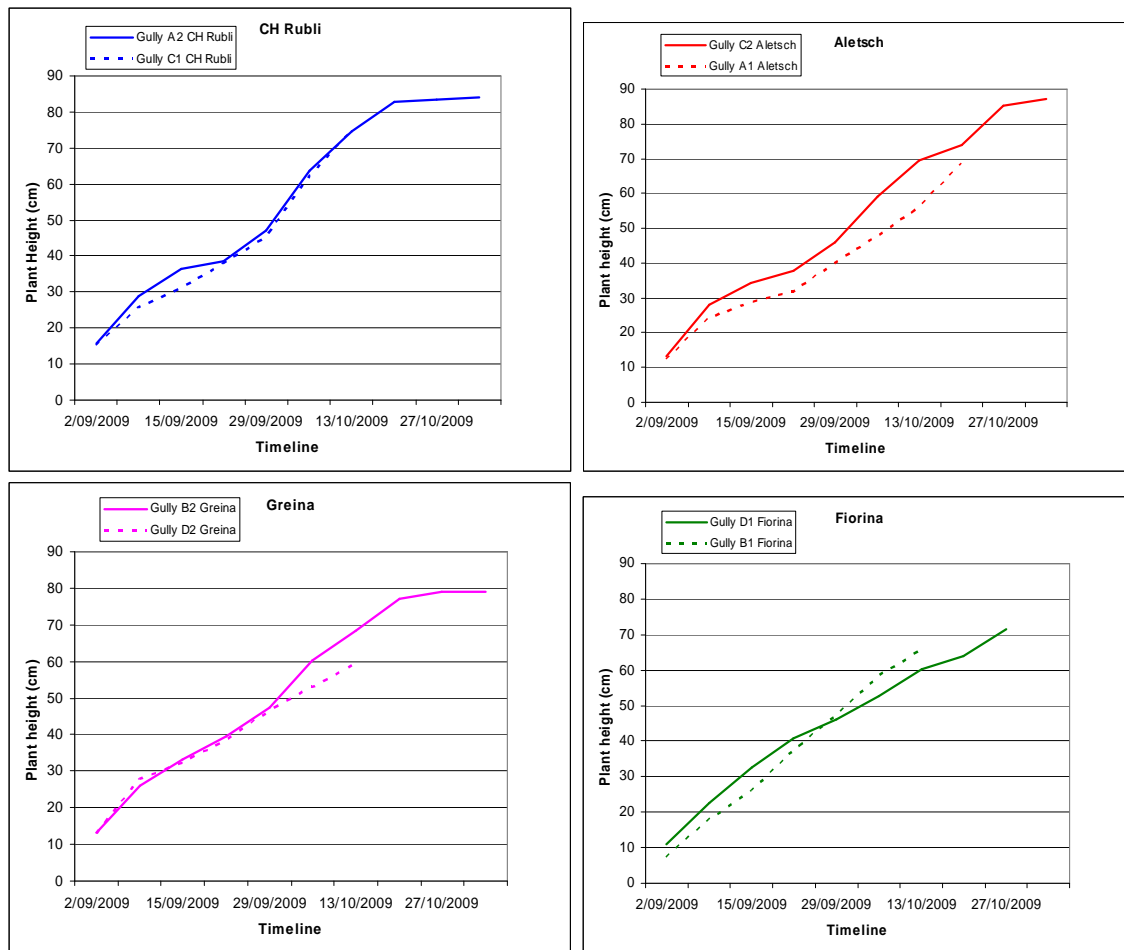


Fig. 17 UBern - Plant height

2.3.4 Gas exchange data

No plant level gas exchange measurements were carried out. See Fig. 7 and Fig. 9 on chamber level CO₂ and plant evaporation.

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2.4 Harvest results

The two gullies per cultivar taken together (0.6m² growth area) produced 180.35 g (Aletsch), 239.95 g (Fiorina), 247.47 g (CH Rubli) and 248.05 g (Greina) of kernels, which correspond to an average yield of 300 g/m² for Aletsch, 400 g/m² for Fiorina, 412 g/m² for CH Rubli and 413 g/m² for Greina.

The yield expected in the field was reported to be good for CH Rubli and Fiorina, middle for Aletsch and middle to weak for Greina (see Table 2, TN98.3.1). The field catalogue and BT1 values are summarized in the table below (Tab. 9).

The difference in yield may be explained by the unequal growth period lengths. CH Rubli and Greina were mature at harvest.

The **maturation** of Fiorina and Aletsch took a longer time, certainly related to the nutrient solution not being well adapted to these cultivars needs. After five and a half month of growth, Fiorina and Aletsch were finally harvested without being completely mature.

Moreover, Aletsch (Gully A1) suffered more severely from the problem of **chlorosis** that occurred at the beginning of the growth period, and this cultivar took more time than the others to recover (see TN98.4.21, 2.4 for detailed harvest info). Together with the delayed maturation this could explain the approximately 25% lower yield compared to the other cultivars.

The number of **green ears** (not mature) was high for Aletsch and Fiorina. The number of green ears was also high for CH Rubli, but for this cultivar, new ears appeared after the maturation of the previous ears, likely induced by a too high N level in the nutrient solution.

Only few green ears were found at the harvest of Greina.

The cultivar Greina appears to cope best with the non-optimal constant nutrient solution composition.

The full harvest amounts are reported in Tab. 9 below.

Harvest index (Tab. 11), based on analysis of the dry weight of the different parts (root, shoot, kernels, debris) of the two gullies together of each cultivar.

Water content of kernels (Tab. 10), determined on 1 representative plant per rockwool piece (pad) containing 15 plants. After harvest, plants were stored a few days at room temperature before analysis.

Micronutrient analysis (Tab. 13), also based on 1 plant per rockwool block, 4 plants per cultivar per gully.

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Tab. 9 UBern - Yield of all cultivars

Variety	Fiorina	Aletsch	Greina	CH Rubli
Field Yield (g/m ²)	445	382	371	464
BT1 Yield (g/m ²)	400	300	413	412
BT1 growth period (days)	154	147	141	140

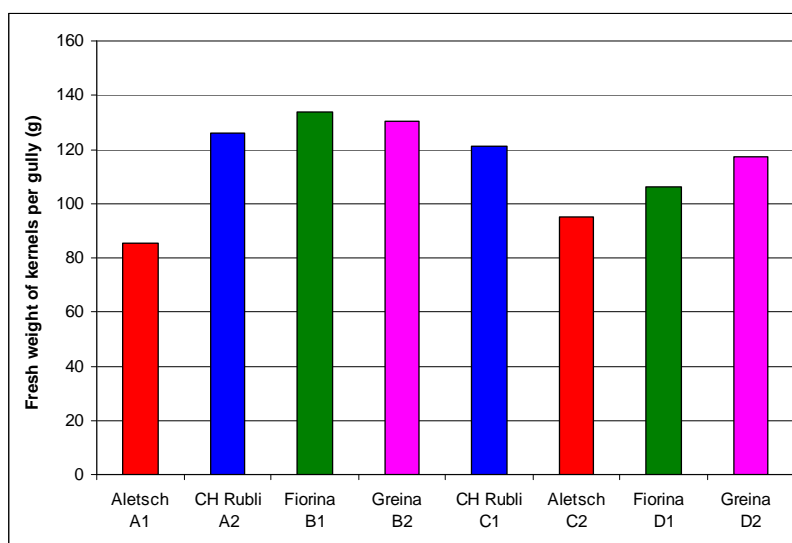


Fig. 18 UBern - Fresh weight of kernels per gully

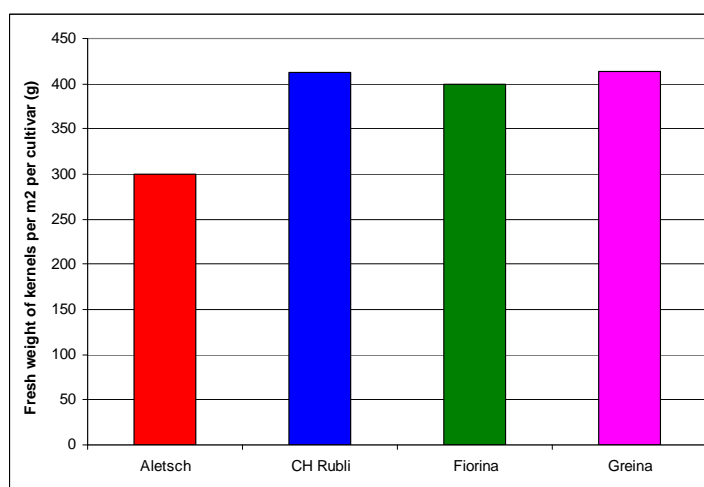


Fig. 19 UBern - Fresh weight of kernels per m² per cultivar

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Tab. 10 UBern - Kernels water content

	Room temperate stored kernels (g)	Water content %	Estimated DW kernels
Aletsch	180.35	6.39	168.82
CH Rubli	247.47	6.39	231.65
Fiorina	239.944	6.58	224.15
Greina	248.052	6.28	232.49

Tab. 11 UBern - Harvest index

	DW Kernels * (g)	DW straw (g)	DW roots (g)	DW threshing debris** (g)	Harvest index
Aletsch	168.82	699.78	222.73	126.93	0.14
CH Rubli	231.65	491.45	119.96	109.26	0.24
Fiorina	224.15	733.83	194.52	207.72	0.16
Greina	232.49	337.34	91.76	78.57	0.31

Tab. 12 UBern - Ears maturity and amount of debris per cultivar

**	Average % debris per ear	Yellow ears (g)	Green ears (g)	Total ears (g)	Estimation debris
Aletsch	30.63	210.67	203.74	414.41	126.93
CH Rubli	24.06	322.81	131.24	454.05	109.26
Fiorina	34.73	293.24	304.95	598.19	207.72
Greina	22.56	343.67	4.65	348.32	78.57

Tab. 13 UBern - Micronutrient analysis of kernels of all cultivars

				K	Ca	Mg	P	Fe	Zn	Cu	Mn	Ni
				mg K per g DW kernels	mg Ca per g DW kernels	mg Mg per g DW kernels	mg P per g DW kernels	µg Fe per g DW kernels	µg Zn per g DW kernels	µg Cu per g DW kernels	µg Mn per g DW kernels	µg Ni per g DW kernels
Aletsch	Gully A1	Rockwool a	Kernels plant 1	4.676	0.264	2.002	5.714	40.26	65.93	14.70	36.91	2.38
Aletsch	Gully A1	Rockwool b	Kernels plant 2	5.772	0.219	1.774	4.924	24.02	50.43	13.77	29.13	2.67
Aletsch	Gully A1	Rockwool c	Kernels plant 3	4.054	0.291	1.827	5.218	27.99	44.82	12.82	21.59	2.77
Aletsch	Gully A1	Rockwool d	Kernels plant 4	4.010	0.277	1.685	5.613	27.64	49.38	12.39	23.74	2.65
CH Rubli	Gully A2	Rockwool a	Kernels plant 1	4.201	0.170	1.886	4.962	20.08	32.82	8.14	49.31	1.84
CH Rubli	Gully A2	Rockwool b	Kernels plant 2	3.737	0.132	1.834	5.246	24.71	44.88	8.16	69.27	2.52
CH Rubli	Gully A2	Rockwool c	Kernels plant 3	4.155	0.174	3.625	5.185	25.05	38.37	8.48	57.22	2.18
CH Rubli	Gully A2	Rockwool d	Kernels plant 4	5.470	0.183	1.978	5.437	24.42	34.56	9.14	46.84	2.37
Fiorina	Gully B1	Rockwool a	Kernels plant 1	5.699	0.217	1.775	5.355	12.44	27.69	12.75	24.64	2.79
Fiorina	Gully B1	Rockwool b	Kernels plant 2	6.094	0.182	2.165	5.387	13.73	31.33	12.73	26.14	2.76
Fiorina	Gully B1	Rockwool c	Kernels plant 3	4.576	0.159	1.952	6.317	23.66	43.77	16.81	44.88	4.44
Fiorina	Gully B1	Rockwool d	Kernels plant 4	5.790	0.160	1.833	5.561	29.39	32.33	9.00	30.93	1.65
Greina	Gully B2	Rockwool a	Kernels plant 1	4.183	0.206	1.692	4.802	26.97	39.99	7.04	42.35	1.17
Greina	Gully B2	Rockwool b	Kernels plant 2	3.646	0.162	1.761	4.675	17.45	31.48	7.75	36.56	1.72
Greina	Gully B2	Rockwool c	Kernels plant 3	4.180	0.173	1.819	4.878	20.48	31.33	8.01	31.91	1.75
Greina	Gully B2	Rockwool d	Kernels plant 4	3.424	0.215	1.576	3.821	27.15	38.38	5.58	52.76	0.95
CH Rubli	Gully C1	Rockwool a	Kernels plant 1	4.479	0.127	2.038	5.020	16.87	44.46	7.69	73.22	1.99
CH Rubli	Gully C1	Rockwool b	Kernels plant 2	5.003	0.176	2.069	5.791	33.99	42.02	7.80	57.64	2.57
CH Rubli	Gully C1	Rockwool c	Kernels plant 3	5.275	0.073	1.774	6.151	32.18	47.73	8.09	59.52	2.51
CH Rubli	Gully C1	Rockwool d	Kernels plant 4	4.789	0.133	1.897	5.071	23.53	44.00	9.27	64.00	2.34
Aletsch	Gully C2	Rockwool a	Kernels plant 1	3.526	0.244	2.113	5.290	19.60	46.76	11.33	49.28	1.69
Aletsch	Gully C2	Rockwool b	Kernels plant 2	3.238	0.268	1.900	5.457	17.67	40.12	11.47	53.18	1.80
Aletsch	Gully C2	Rockwool c	Kernels plant 3	4.477	0.215	1.780	5.673	22.70	45.27	11.36	50.25	2.39
Aletsch	Gully C2	Rockwool d	Kernels plant 4	4.417	0.220	1.843	5.522	27.98	42.20	11.04	54.26	3.02
Fiorina	Gully D1	Rockwool a	Kernels plant 1	5.947	0.231	1.981	5.492	15.88	36.91	13.54	27.99	3.08
Fiorina	Gully D1	Rockwool b	Kernels plant 2	6.142	0.178	1.835	6.745	13.09	33.49	13.88	36.38	3.58
Fiorina	Gully D1	Rockwool c	Kernels plant 3	6.090	0.201	1.840	5.281	13.81	34.26	13.34	33.51	2.36
Fiorina	Gully D1	Rockwool d	Kernels plant 4	6.152	0.158	1.960	6.050	17.42	42.86	15.66	35.45	3.01
Greina	Gully D2	Rockwool a	Kernels plant 1	3.526	0.135	1.790	5.523	19.83	54.45	9.39	51.18	2.09
Greina	Gully D2	Rockwool b	Kernels plant 2	3.584	0.185	1.851	5.390	30.71	57.76	9.21	62.39	2.43
Greina	Gully D2	Rockwool c	Kernels plant 3	3.893	0.542	1.880	5.148	20.71	49.19	8.68	46.69	1.92
Greina	Gully D2	Rockwool d	Kernels plant 4	4.158	0.184	1.946	5.337	17.96	47.53	9.56	39.79	2.16
Fiorina	market samples			3.772	0.309	1.128	4.282	28.17	24.36	5.92	30.06	0.44
Greina	market samples			2.872	0.330	1.101	3.913	37.92	28.38	4.04	19.06	0.33
CH Rubli	market samples			3.570	0.253	1.181	4.081	37.71	38.45	4.25	41.46	0.24
Aletsch	market samples			3.152	0.262	1.247	4.021	38.17	18.14	5.48	39.97	0.57

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3 Durum wheat (UoGuelph)

3.1 Experimental Layout

3.1.1 Measuring Plan

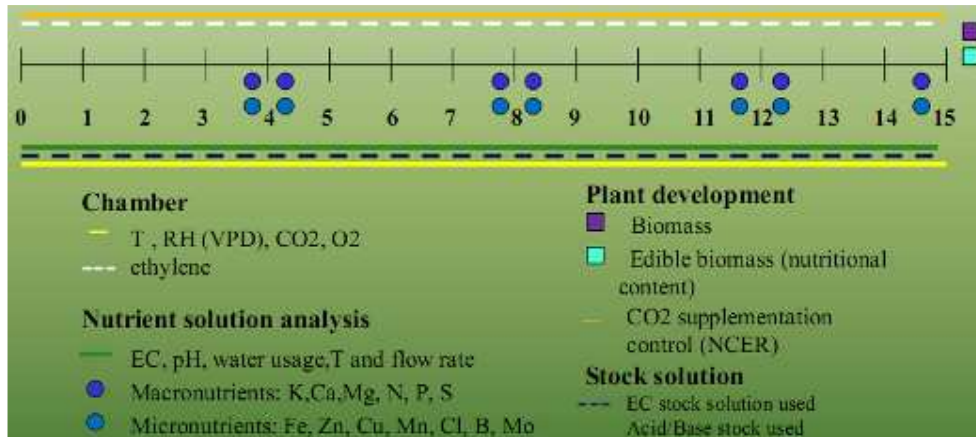


Fig. 20 UoGuelph - Measuring plan

3.1.2 Setup

Plant density: the plant growth area corresponds to 2.5m length (gully length 2.45m) x 2m width. Gully width is 0.17m. Crops of each gully have an area of 2.5x0.4m (1 m²) to develop. Planting density: 3 times 45 plants per gully = 135 plants, density = 135 plants / m², 675 total.

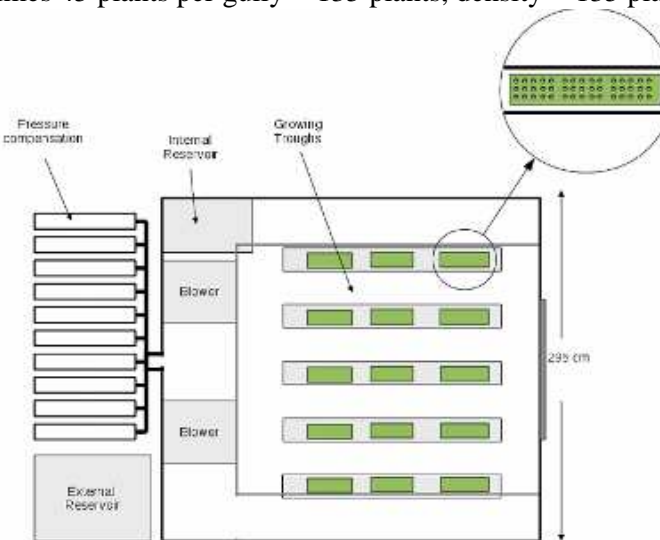


Fig. 21 UoGuelph - Setup

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3.2 Growth environment follow-up

3.2.1 Settings

Set point was 23 day and night for temperature. RH set point was 60%.

3.2.2 Chamber T/RH evolution

Profiles of chamber atmospheric temperature, humidity were recorded at six minute intervals for the duration of this experiment. Figures indicate standard deviation for each days data. Temperature control was good throughout the experiment with the only perturbations during chamber access for flooding remediation and during a University wide steam system failure.

Temperature was kept at an isothermal 23°C during the majority of growth, but was raised to 26°C after approximately 12 weeks in order to improve seed filling.

Relative humidity was set to 60% until 15 weeks after planting, at which point it was set to 0% to facilitate crop drying prior to harvest.

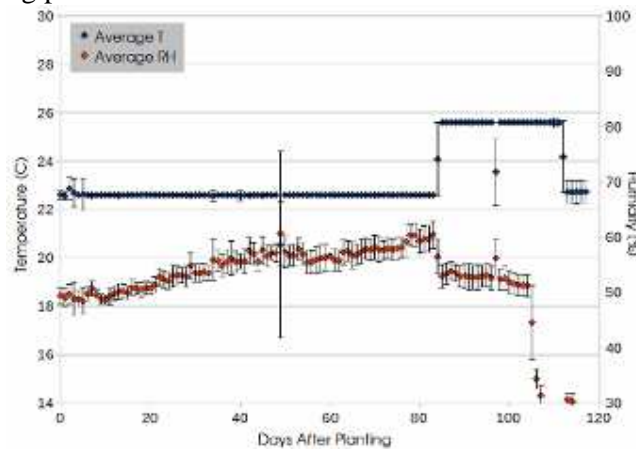


Fig. 22 UoGuelph - T/RH control (Avonlea).

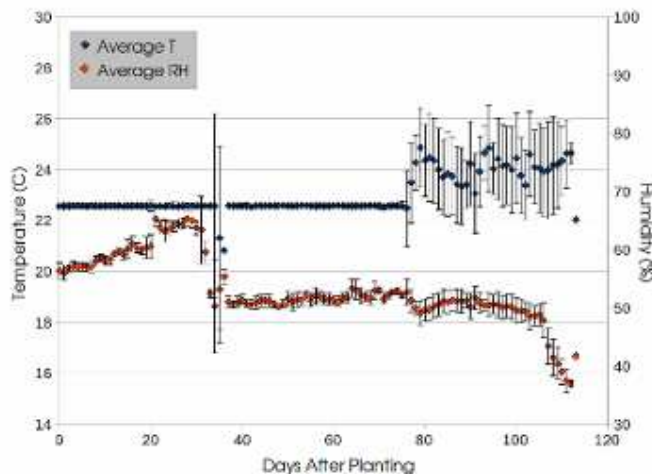


Fig. 23 UoGuelph - T/RH control (Strongfield).

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3.2.3 Chamber NCER and evapotranspiration

Daily carbon assimilation calculated based on CO₂ additions to the chamber atmosphere (set point 1200ppm) and condensate production increased steadily till about 80-90 days of growth. Chamber opening is indicated by yellow triangles, nutrient solution exchange by green triangles.

NCER and transpiration followed typical profiles found in plant growth and development (Fig. 224 and Fig. 25). Both cultivars had similar peak productivity, however Avonlea productivity dropped off rapidly at approximately 80 days whereas Strongfield productivity dropped at a slower rate. As this is during the seed filling stage, higher productivity by Strongfield at this time may be the reason for its higher overall kernel production.

A reduction in NCER was observed in both treatments immediately after the first solution change, however the reason for this is currently unknown. Nutrient solution analysis did not show any discrepancy nor did environment control.

Avonlea evapotranspiration peaked at approximately 60 litres per day whereas Strongfield had daily water production of over 90 litres per day.

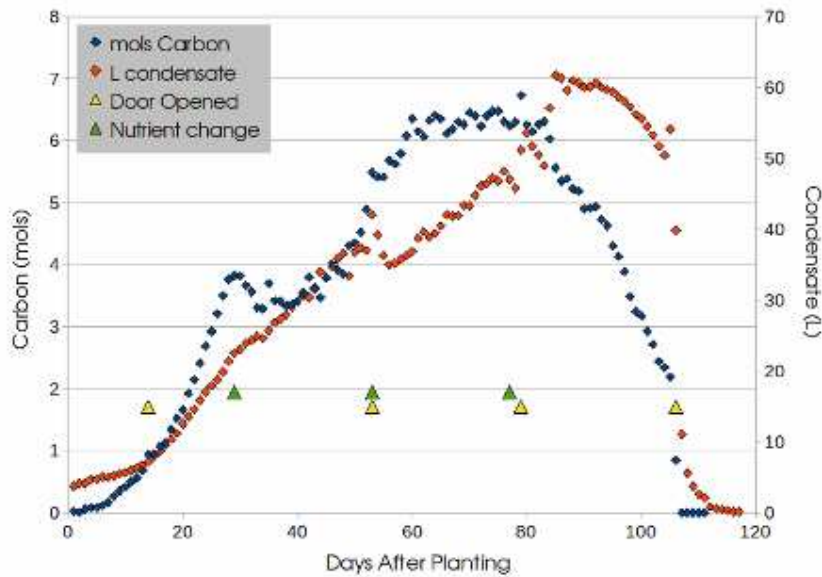


Fig. 24 UoGuelph - NCER/evapotranspiration Avonlea

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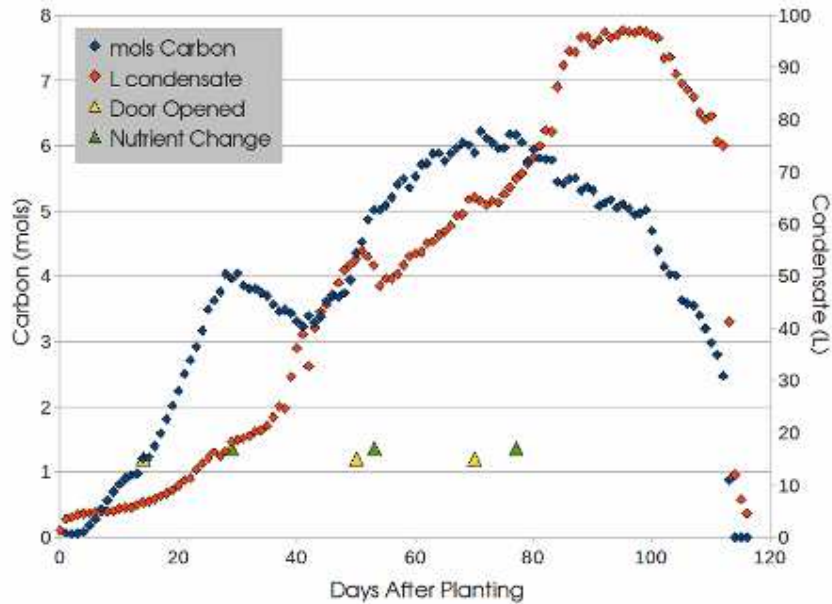


Fig. 25 UoGuelph - NCER/evapotranspiration Strongfield

3.2.4 Ethylene production

Ethylene levels increased rapidly in the case of Avonlea, several times exceeding the 50ppb level. Yellow triangles indicate chamber opening as was first needed for root mass removal.

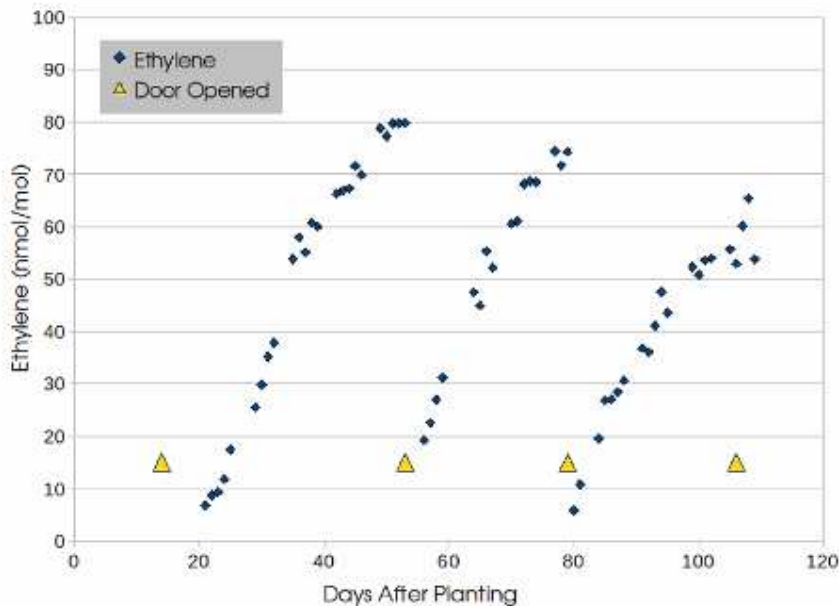


Fig. 26 UoGuelph - Ethylene production Avonlea

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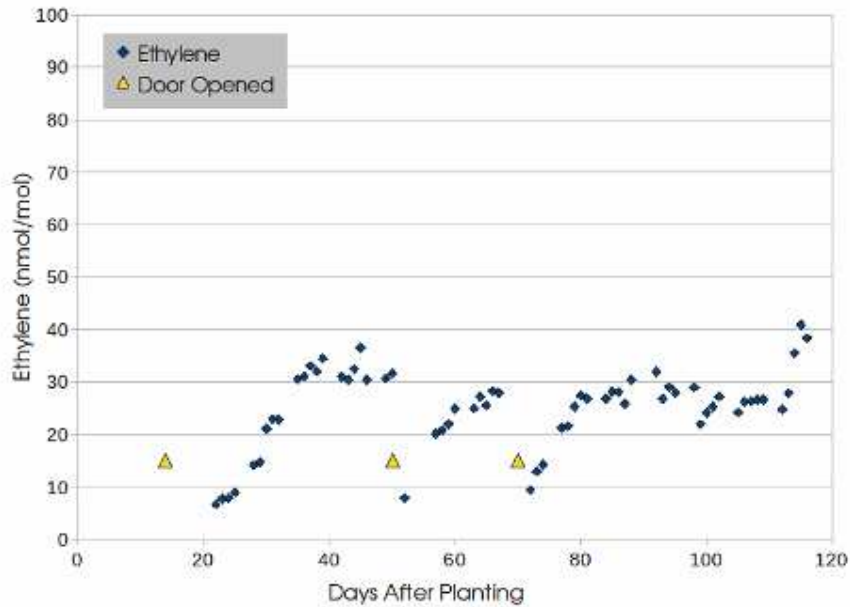


Fig. 27 UoGuelph - Ethylene production Strongfield

Leakage rates of the chambers were 6,59% (Strongfield) versus 0,51% (Avonlea)

3.2.5 Oxygen production

In the Avonlea culture O₂ levels rose till 28%, the available data for Strongfield indicate a slower initial rise to 23%. A mechanical error caused the oxygen measurements to fail for the rest of the measuring period.

The high oxygen immediately prior to the observed decrease in NCER may have been a contributing factor as high oxygen reduces the efficiency of photosynthesis by competing with CO₂ for the acceptor 1,5-bisphosphate (Warburg effect).

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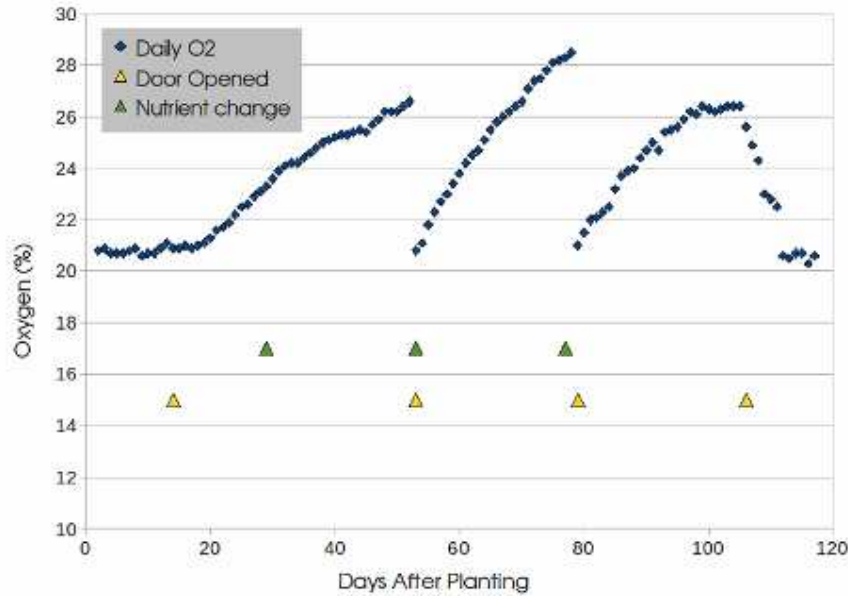


Fig. 28 UoGuelph - Oxygen production Avonlea

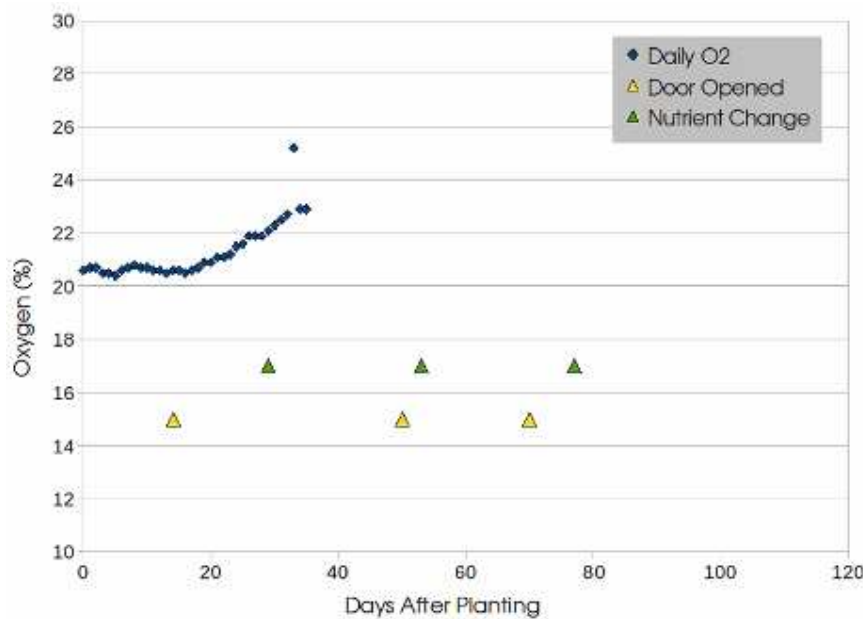


Fig. 29 UoGuelph - Oxygen production Strongfield

3.2.6 Nutrient Solution Environment

NFT flow was intermittent with a 2min pump on, 8min pump off cycle. The period was adjusted to 3min on / 7min off to increase nutrient availability when the plants were 1 month old, and returned to 2min on / 8min off at the 2 month time point.

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3.2.7 pH and EC evolution

pH and EC were automatically measured and adjusted on a daily basis by the control system. Control was excellent with deviations from setpoint only during initial operation (Avonlea - injection pump failure) and during solution changes or flooding events.

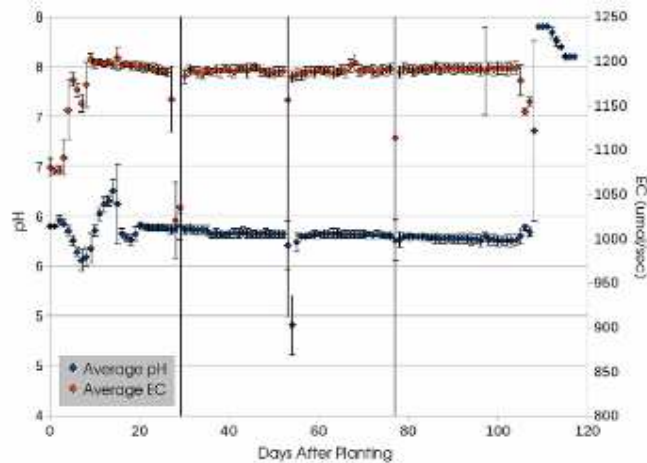


Fig. 30 UoGuelph - pH/EC control (Avonlea)

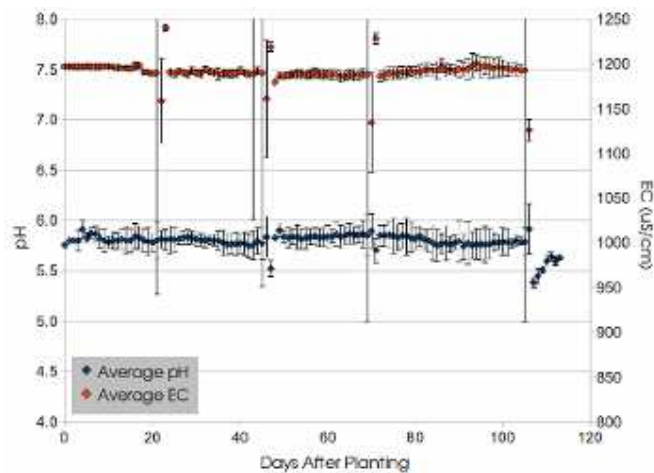


Fig. 31 UoGuelph - pH/EC control (Strongfield)

Set points were 5.8 for pH and 1200 microS/cm (1.2 mS/cm) for EC.
 pH adjustment with 0.5 M HNO₃ needed 11.5 l for Avonlea and 12.5 l for Strongfield.

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3.2.8 Nutrient solution T

A cooling system was not used; temperature values were recorded at the moment of nutrient solution exchange.

For Avonlea T values ranged from 23.6 to 24.1 degrees C.

Values for Strongfield were between 22.2 and 24 degrees C.

Temperature of the starting solution was 27degrees, corresponding to the T of the building distilled water delivery system.

3.2.9 Nutrient solution analysis

Sampling of hydroponics solution was performed at the beginning and end of each 4 week nutrient solution interval (Tab. 1414)

Depletion in P, K and the micronutrient Mn were apparent for both cultivars

Tab. 14 UoGuelph - nutrient solution analysis

Sample number	Cultivar name	Sample date	Sample date	NO3-N ppm	P ppm	K ppm	Ca ppm	Mg ppm	Cl ppm	S ppm	NH4-N ppm	Na ppm	Zn ppm	Mn ppm	Cu ppm	Fe ppm	B ppm	Mo ppm	Si ppm
1	Avonlea	July06/09	Start	120.00	16.13	128.23	111.50	24.38	4.00	92.04	6.63	<1.0	0.02	0.46	0.04	2.88	0.14	<0.01	<0.1
2	Avonlea	Aug04/09	End	83.00	<1.0	4.11	126.79	41.97	<1.0	154.85	<0.5	1.35	0.02	0.01	0.04	2.89	0.25	<0.01	<0.1
3	Avonlea	Aug04/09	Start	120.00	15.27	135.50	107.79	24.19	4.00	89.78	6.24	1.26	0.02	0.43	0.05	2.80	0.13	<0.01	<0.1
4	Avonlea	Aug28/09	End	95.00	6.71	22.06	145.45	59.42	<1.0	250.52	<0.5	<1.0	0.02	0.06	0.11	4.07	0.35	<0.01	<0.1
5	Avonlea	Aug28/09	Start	118.00	15.76	128.98	109.38	24.07	3.00	94.27	6.31	<1.0	0.01	0.45	0.04	2.97	0.14	<0.01	<0.1
6	Avonlea	Sep21/09	End	96.00	<1.0	24.63	135.18	63.06	<1.0	221.78	<0.5	1.41	0.02	0.01	0.03	3.87	0.28	<0.01	<0.1
7	Avonlea	Sep21/09	Start	114.00	15.06	124.80	114.64	25.50	4.00	98.76	6.91	<1.0	0.01	0.47	0.04	2.84	0.14	<0.01	<0.1
8	Avonlea	Oct19/09	End	80.00	<1.0	90.70	77.60	29.83	<1.0	110.30	<0.5	1.33	0.02	0.01	0.04	2.08	0.11	<0.01	<0.1
9	Strongfield	July13/09	Start	113.00	15.81	124.99	108.95	23.97	4.00	94.30	6.32	<1.0	0.01	0.45	0.04	2.89	0.13	<0.01	<0.1
10	Strongfield	Aug11/09	End	100.00	1.82	53.72	121.52	37.55	<1.0	142.60	<0.5	1.8	0.06	0.02	0.14	2.65	0.24	<0.01	<0.1
11	Strongfield	Aug11/09	Start	116.00	16.01	126.75	107.64	23.93	4.00	93.67	6.54	<1.0	0.02	0.44	0.04	3.05	0.13	<0.01	<0.1
12	Strongfield	Sept04/09	End	86.00	<1.0	6.91	143.57	49.36	<1.0	196.88	<0.5	<1.0	0.02	0.02	0.11	4.04	0.29	<0.01	<0.1
13	Strongfield	Sept04/09	Start	123.00	16.16	129.02	116.46	25.39	4.00	98.8	7.13	<1.0	0.01	0.47	0.04	2.96	0.14	<0.01	<0.1
14	Strongfield	Sept28/09	End	88.00	<1.0	9.26	140.22	54.36	<1.0	210.14	<0.5	<1.0	0.01	0.01	0.08	3.85	0.27	<0.01	<0.1
15	Strongfield	Sept28/09	Start	122.00	16.44	129.28	118.65	25.52	4.00	96.32	6.91	<1.0	0.02	0.47	0.05	2.87	0.13	<0.01	<0.1
16	Strongfield	Nov02/09	End	84.00	<1.0	77.46	83.16	32.38	<1.0	112.24	<0.5	1.39	0.01	0.01	0.03	2.10	0.11	<0.01	<0.1

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3.3 Monitoring of plant development

The 2 durum wheat cultivars were grown for nearly 4 months

112 days for Avonlea

119 days for Strongfield

3.3.1 *Photographic follow-up*



Durum wheat (cv. Strongfield) 17 days after planting in SEC2-2



Durum wheat (cv. Avonlea) 24 days after planting in SEC2-1

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Fig. 32 UoGuelph - durum wheat photographs

3.3.2 *Growth assessment*

Given the usage of a sealed chamber, only carried out at harvest, see 3.4.

3.3.3 *Gas exchange data*

Carried out at chamber level, see 3.2.3, 3.2.4, 3.2.5

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3.4 Harvest results

Avonlea produced over 2.1 kg of wheat kernels while Strongfield produced over 3.7 kg Germination was 69% of seeds for both cultivars (on a total of 675 seeds)

Plant growth parameters measured at the end of the growth period were dry weight of roots, kernels, and straw. Data was collected on a per pad basis for the entire chamber (Tab. 1515, Tab. 166). Results of proximate analysis are shown in Tab. 177 and Tab. 188.

Tissue analysis results are presented in Tab. 199 and Tab. 20, and fibre/lignin analysis is shown in Tab. 2121 and Tab. 22.

Tab. 15 UoGuelph - Dry mass analysis Avonlea

Trough number	Plot number	Number of heads			Plant height avg (cm)	Dry Weight(g)					Total DW above ground	Cultivar % lodged	Number of Plants	Rockwool DW(g)
		Yellow heads	Green heads	Total # heads		Heads straw seeds	Seeds only	Straw only	Roots with rockwool	Roots only				
1	1	320	134	454	87	245.0	122.4	575.4	252.7	106.3	820.4	90	31	146.4
1	2	305	150	455	88	278.0	145.5	543.8	250.8	98.7	821.8	85	30	152.1
1	3	296	172	468	86	319.4	178.4	632.5	275.5	123.6	951.9	90	32	151.9
Total		921	456	1377		842.4	446.3	1751.7	779	328.6	2594.1		93	450.4
2	4	232	96	328	86	235.3	123.8	456.2	229.4	75.4	691.5	90	26	154.0
2	5	185	104	289	88	174.7	100.9	436.0	196.3	52.5	610.7	20	30	143.8
2	6	301	76	377	82	277.5	164.6	506.2	241.7	101.0	783.7	95	32	140.7
Total		718	276	994		687.5	389.3	1398.4	667.4	228.9	2085.9		88	438.5
3	7	202	97	299	85	182.5	101.7	420.7	213.0	72.6	603.2	55	32	140.4
3	8	280	101	381	86	292.6	171.6	541.7	231.9	99.3	834.3	80	36	132.6
3	9	317	142	459	87	295.0	179.7	547.0	232.1	89.5	842.0	90	28	142.6
Total		799	340	1139		770.1	453.0	1509.4	677.0	261.4	2279.5		96	415.6
4	10	178	136	314	85	140.9	75.3	335.9	194.1	59.1	476.8	50	36	135.0
4	11	235	95	330	87	290.2	183.4	413.5	225.1	79.8	703.7	90	34	145.3
4	12	313	73	386	87	314.0	201.3	478.7	226.4	100.0	792.7	90	27	126.4
Total		726	304	1030		745.1	460.0	1228.1	645.6	238.9	1973.2		97	406.7
5	13	289	61	350	86	214.6	127.6	393.9	218.1	90.5	608.5	70	31	127.6
5	14	200	55	255	85	223.2	144.2	362.4	196.7	61.9	585.6	90	28	134.8
5	15	262	63	325	82	201.2	112.6	434.6	227.5	81.1	635.8	90	36	146.4
Total		751	179	930		639.0	384.4	1190.9	642.3	233.5	1829.9		95	408.8
Total in CH-1		3915	1555	5470		3684.1	2133.0	7078.5	3411.3	1291.3	10762.6		469	2120.0

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Tab. 16 UoGuelph - Dry mass analysis Strongfield

Trough number	Plot number	Number of heads			Plant height avg (cm)	Dry Weight(g)					Total DW above ground	% lodged	Number of Plants	Rockwool DW(g)
		Yellow heads	Green heads	Total # heads		Heads straw seeds	Seeds only	Straw only	Roots with rockwool	Roots only				
1	1	190	80	270	76	291.3	189.5	372.2	220.3	75.0	663.5	95	35	145.3
1	2	162	94	256	79	296.0	194.0	364.7	209.0	68.7	660.7	95	34	140.3
1	3	163	111	274	79	290.3	183.2	356.0	218.6	85.2	646.3	95	31	133.4
Total		515	285	800		877.6	566.7	1092.9	647.9	228.9	1970.5		100	419.0
2	4	218	86	304	87	416.6	291.6	442.8	226.9	83.0	859.4	90	34	143.9
2	5	177	69	246	86	323.6	225.2	442.0	264.6	115.0	765.6	90	25	149.6
2	6	202	53	255	86	318.7	208.9	388.3	233.2	76.8	707.0	90	27	156.4
Total		597	208	805		1058.9	725.7	1273.1	724.7	274.8	2332.0		86	449.9
3	7	251	68	319	85	480.8	350.3	514.6	238.5	89.6	995.4	90	29	148.9
3	8	210	72	282	87	515.1	334.7	548.5	306.0	186.0	1063.6	95	32	120.0
3	9	199	80	279	84	399.8	285.2	474.5	232.0	99.6	874.3	90	27	132.4
Total		660	220	880		1395.7	970.2	1537.6	776.5	375.2	2933.3		88	401.3
4	10	270	100	370	85	545.8	358.7	505.2	252.4	119.1	1051.0	75	36	133.3
4	11	239	94	333	88	519	361.5	547.8	276.6	154.4	1066.8	85	31	122.2
4	12	184	69	253	85	288.6	180.5	349.8	196.6	52.7	638.4	75	32	143.9
Total		693	263	956		1353.4	900.7	1402.8	725.6	326.2	2756.2		99	399.4
5	13	148	90	238	84	284.4	200.1	360.7	203.3	70.5	645.1	90	29	132.8
5	14	158	111	269	86	296.6	200.7	390.0	235.8	86.7	686.6	90	29	149.1
5	15	200	32	232	83	333.9	207.3	439.0	204.7	73.2	772.9	90	35	131.5
Total		506	233	739		914.9	608.1	1189.7	643.8	230.4	2104.6		93	413.4
Total in CH-2		2971	1209	4180	84	5600.5	3771.4	6496.1	3518.8	1435.8	12096.6		466	2083.0

The samples mentioned in tables 16 through 21 are a mix of all harvests from all plots from the 5 gullies (throughs).

Tab. 17 UoGuelph - Results of proximate analysis Avonlea

Sample number	Material	Fat %	Protein %	Moisture %	Ash %	Carboh. %
1	seeds mix	0.98	17.16	8.21	2.35	71.30
2	seeds mix	1.56	17.15	8.20	2.54	70.55
3	seeds mix	1.11	16.96	8.09	2.28	71.55

Tab. 18 UoGuelph - Results of proximate analysis Strongfield

Sample number	Material	Fat %	Protein %	Moisture %	Ash %	Carboh. %
1	seeds mix	1.62	15.78	7.70	2.23	72.68
2	seeds mix	1.52	16.55	7.93	2.16	71.83
3	seeds mix	1.49	16.58	7.87	2.05	72.02

Tab. 19 UoGuelph - Results of tissue analysis for Avonlea expressed as percentage of dry mass

Sample name	Material	Trough number	Plant number	Total C %	N %	P %	K %	Mg %	Ca %
1	seeds	mix	mix	41.60	2.67	0.49	0.50	0.16	0.06
2	straw	mix	mix	39.40	2.52	0.62	4.34	0.29	1.00
3	roots	mix	mix	36.30	5.14	0.45	6.69	0.15	0.48

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Tab. 20 UoGuelph - Results of tissue analysis for Strongfield, expressed as percentage of dry mass

Sample name	Material	Trough number	Plant number	Total C %	N %	P %	K %	Mg %	Ca %
1	seeds	mix	mix	41.50	2.63	0.45	0.52	0.14	0.05
2	straw	mix	mix	41.40	2.10	0.47	3.57	0.31	1.04
3	roots	mix	mix	36.00	5.62	0.6	6.91	0.17	0.55

Tab. 21 UoGuelph - Results of fibre/lignin analysis for Avonlea, expressed as percentage of dry mass

Sample number	Material	Cultivar	NDF %	ADF %	Lignin %
1	mix seeds	Avonlea	24.68	4.44	1.04
2	mix seeds	Avonlea	23.59	4.84	0.75
3	mix seeds	Avonlea	28.09	4.24	0.63
4	mix straw	Avonlea	49.80	31.70	1.94
5	mix straw	Avonlea	49.08	31.23	1.78
6	mix straw	Avonlea	49.80	31.31	2.99
7	mix roots	Avonlea	54.40	21.50	6.20
8	mix roots	Avonlea	57.28	21.51	5.03
9	mix roots	Avonlea	54.83	21.34	7.18

Tab. 22 UoGuelph - Results of fibre/lignin analysis for Strongfield, expressed as percentage of dry mass

Sample number	Material	Cultivar	NDF %	ADF %	Lignin %
1	mix seeds	<u>Strongf.</u>	20.01	4.68	0.73
2	mix seeds	<u>Strongf.</u>	21.55	4.32	0.65
3	mix seeds	<u>Strongf.</u>	25.15	5.02	0.62
4	mix straw	<u>Strongf.</u>	50.61	32.84	4.47
5	mix straw	<u>Strongf.</u>	52.25	31.96	4.16
6	mix straw	<u>Strongf.</u>	50.98	32.41	4.17
7	mix roots	<u>Strongf.</u>	53.33	21.02	7.44
8	mix roots	<u>Strongf.</u>	52.12	20.39	7.24
9	mix roots	<u>Strongf.</u>	51.34	21.56	7.51

A kernel quality analysis was performed at the Canadian Cereal Research Centre (Tab. 2323)

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Tab. 23 UoGuelph - Kernel quality analysis

ID	CHK	Whole-Meal Protein (%)	Falling Number	Gluten Index (%)	Semolina Protein (%)	Oven Moisture (%)	Semolina Ash (%)	Semolina Yield (%)	Semolina b*	Alveograph			
										W	L	P	P/L
		15.5	589	33.5	13.2	15.1	0.72	67.4	24.1	49	25	46	1.98
Avonlea	Y	14.7	642	23	12.7	14.8	0.76	68.2	23.4	24	15	34	2.27
Strongfield	Y	16.3	536	44	13.8	15.4	0.68	66.6	24.8	74	35	58	1.69
Avonlea	N	15.7	231	26	13.4	14.9	0.81	63.5	26	12	10	23	2.32
Strongfield	N	15.1	222	46	12.2	14.8	0.71	60.9	26.3	47	37	38	1.02

The CHK 'Y' refers to data from field trials that was analyzed at the same time.

The protein levels were quite good as compared with the field samples.

When comparing this trial data to data from the field (N vs. Y), the biggest change was in the falling number. The falling number measures starch degradation (due to alpha-amylase activity).

The gluten index correlates with the diversity between the cultivars (see TN 98.3.1).

The alveograph W and P strength parameters are also lower than in the field samples.

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4 Potato (UGent)

Potato in vitro plants were obtained from UGent consultant HZPC.

A pre-test was carried out at the HZPC greenhouse (see TN98.3.1, 4.2.4), followed by a trial at UGent in a similar type of gully-setup in a test-room (see TN98.4.12, 4.3.12).

For the first bench test a batch of in-vitro plants of the selected cultivars was distributed for culture at UGent and UCL and for greenhouse culture at HZPC.

The results from bench test 1 are reported in this document (this section for UGent; subsection 4.5 for HZPC greenhouse test). See section 0 for UCL results.

The in vitro plants obtained from HZPC were grown for 3 weeks in-vitro at HZPC, subsequently acclimatised for 1 week in an open gully at HZPC, transported to UGent and UCL and then temporary put on deep-water hydroculture for one day.

The Innovator cultivar in vitro plants were clearly smaller as compared with the other 3 cultivars (Annabelle, Bintje and Desiree). These 4 cultivars were chosen based on a preliminary listing derived in TN98.3.1.

At UGent the plants of the 4 cultivars were grown in propagation gullies in the propagation room for 4 more weeks (see TN98.4.11 section 4.3.2), before transplanting to the production gullies in the bench test room.

See section 5 for UCL: the plants were transplanted to bench test gullies after 5 days of deep water culture.

4.1 Experimental Layout

4.1.1 Measuring Plan

As an overview, the list of parameters to be measured from TN 98.4.11 is repeated below, and a measuring timeline plan is added.

Tab. 24 UGent - Parameters and frequency of logging

		Frequency logging	Online/ Manual
Fixed	airflow		
	Solution flow	Weakly check	Manual
Daily measurements	Light quantity	5 min	Online
	Air temperature	30sec and 5 min	Online
	Humidity	30sec and 5 min	Online
	CO₂ in air	5 min	Online
	O₂ in air	5 min	Online
	Ethylene	1 min	Online

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	Oxygen in solution	weekly	Manual
	pH	5 min	Online
	EC	5 min	Online
	Solution temperature	5 min	Online
	Weight gully 4	1 h	Online
	EC stock solution used	5 min	Online
	Water stock used	5 min	Online
	Acid/Base stock used	5 min	Online
	Video imaging	1 h	Online
	Thermal imaging	1 h	Online
Weekly measurements	projected leaf area 1-->16: results from images captured by robot video cam		Online
	leaf area temperature 1-->16: results from images captured by robot thermal cam		Online
	individual tuber area measurement – manual / image analysis of manually captured images		Manual
	CO2 assimilation ADC2250 IRGA system with small leaf cuvette or whole plant cuvette		Online / 1day period
	Ethylene emanation measurement Sensorsense system small leaf cuvette or whole plant cuvette		Online / 1day period
	O2 level measurement leaf cuvette or whole plant cuvette		Online/ 1day period
	Plant height		Manual
	Number of stolons		Manual
	Number of tubers		Manual
	Date of stolon appearance		Manual
	Date of tuber appearance		Manual
	Date of flowering		Manual
Week 3, 8 and harvest	Complete nutrient solution composition control		Manual
Harvest	Foliage fresh weight		Manual
	Stem fresh weight		Manual
	Root fresh weight		Manual
	Tuber fresh weight		Manual
	Foliage dry weight		Manual
	Stem dry weight		Manual
	Root dry weight		Manual
	Nutritional analysis by IPL, average per category		Manual
	plant 1-4 suboptimal light		Manual
	plant 5-12 optimal light		Manual
	plant 13-16 suboptimal light		Manual

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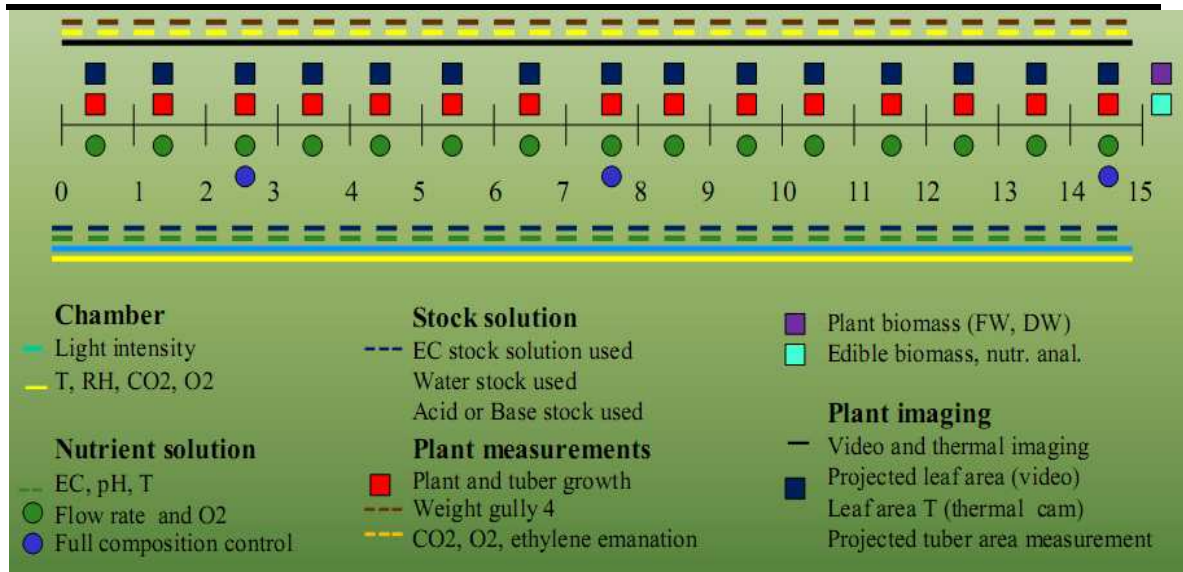


Fig. 33 UGent - Measuring schedule

4.1.2 Setup bench test UGent growth chamber

The Setup with the 4 gullies is shown below, air enters from the left perforated wall and exits through the right one. For more details see TN 98.4.11. See 4.3.1 for overviews of the plant growth shown as overviews in the configuration of the left panel of Fig. 344.

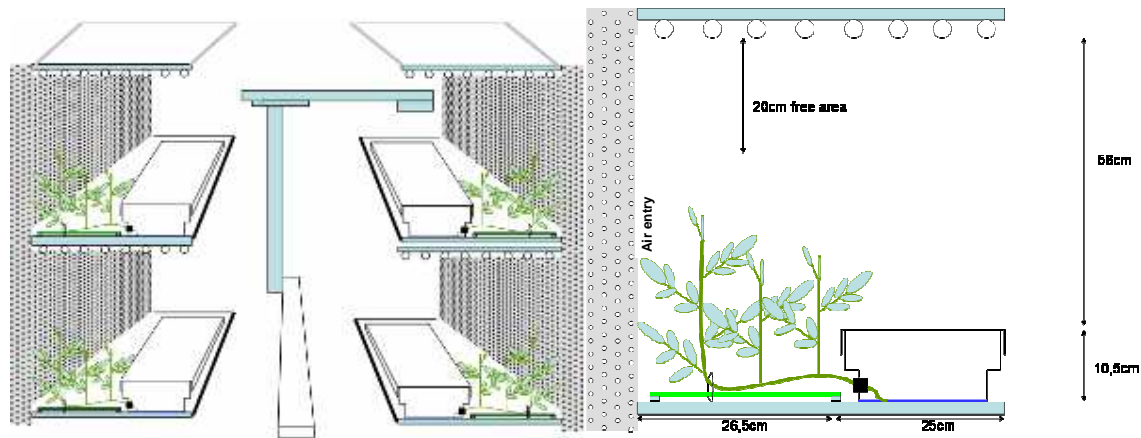


Fig. 34 UGent - Setup

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4.2 Growth environment follow-up

4.2.1 Settings

Tab. 25 UGent - Settings

Room	Nutritive solution
RH 70%	pH 5.5
T 20°C	EC 1800
	T 18.5 °C

4.2.2 Chamber T/RH evolution

Chamber level T and RH remained stable at the setpoints 20.3 degrees and 70% humidity.

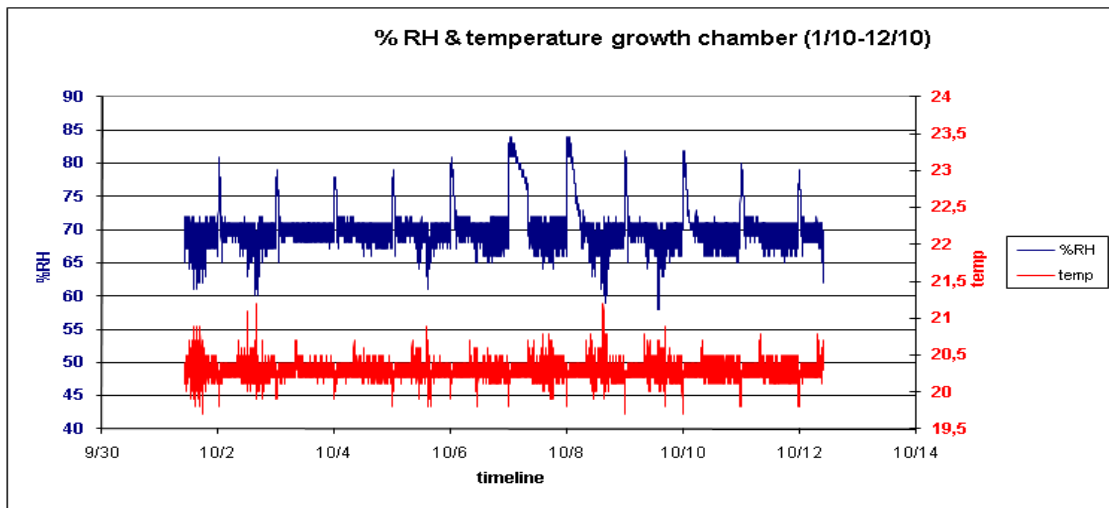


Fig. 35 UGent - RH/ T growth room

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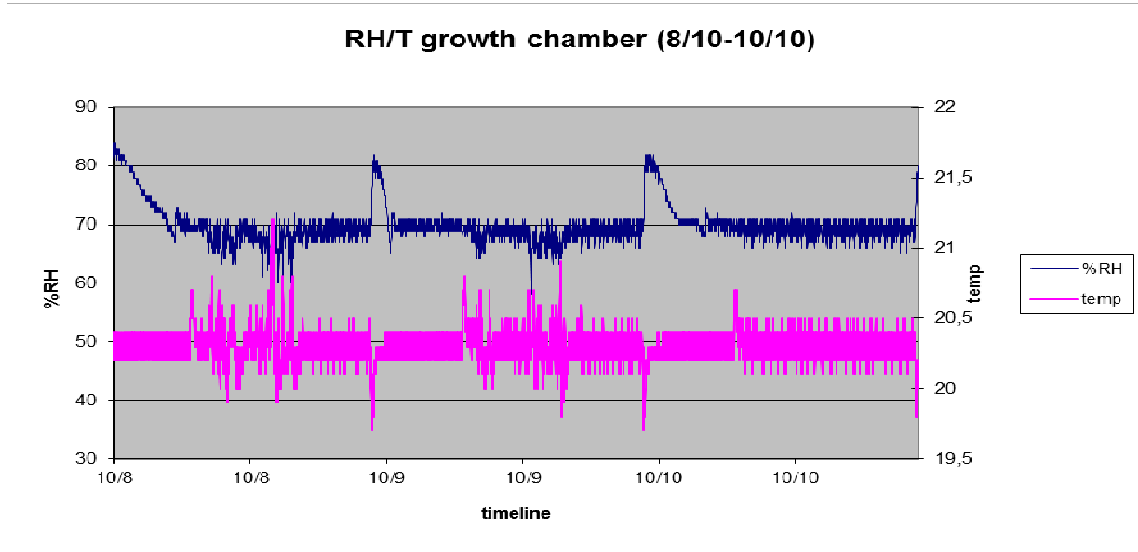


Fig. 36 UGent - RH/ T growth room detail 8/10 – 10/10

4.2.3 Chamber CO₂ level

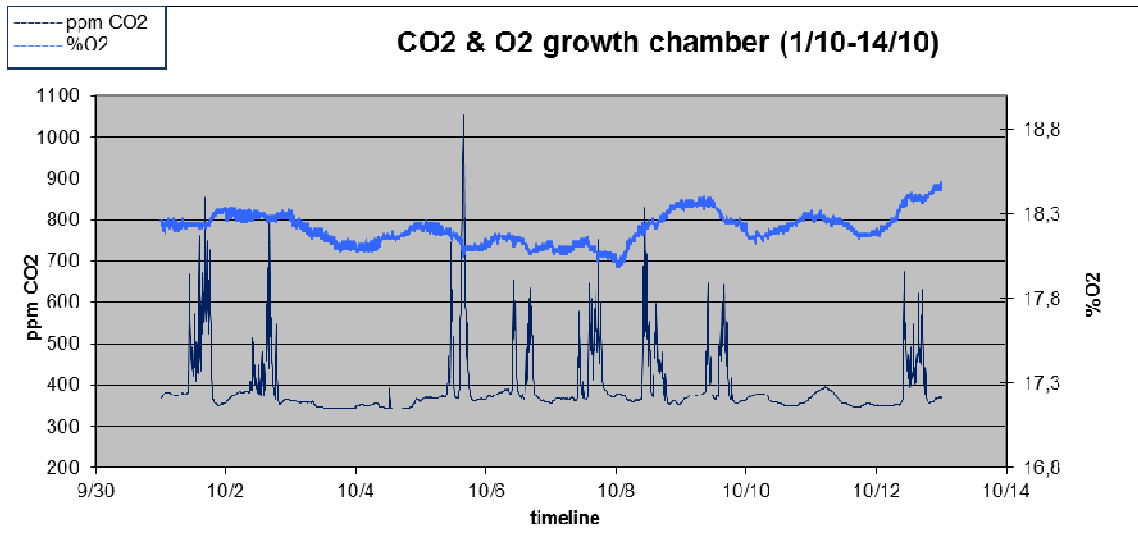


Fig. 37 UGent - CO₂/O₂ logging growth room for a long period

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CO₂ levels corresponded to ambient values. Operator presence induced peaks of CO₂. The O₂ sensor shows considerable sensor drift, and needs calibration in order to readout the ambient value.

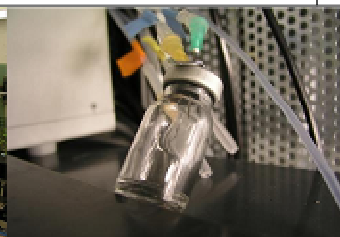
4.2.4 Ethylene production

On-line determination of ethylene emanation levels in flow-through cuvettes with the Sensorsense photo acoustic system didn't reveal any increase with the used flow-speeds (see 4.3.4), measurements were carried out concurrently on the same cuvettes as for the gas exchange determinations.

Small vials were put in the chamber used for acclimation and pre-test, which has a much lower airflow. The capped sample vials were subsequently analysed by the Sensorsense photo acoustic system. High values were recorded (50ppb is a general level known to inhibit plant growth).

positions vials in room 12 October	ppb ethylene
rack 1A	74.30
rack 1B	99.10
rack 2A	126.45
rack 2B	58.41
average	89.56

10 ml vials placed in room



vials measured by a photoacoustic detector

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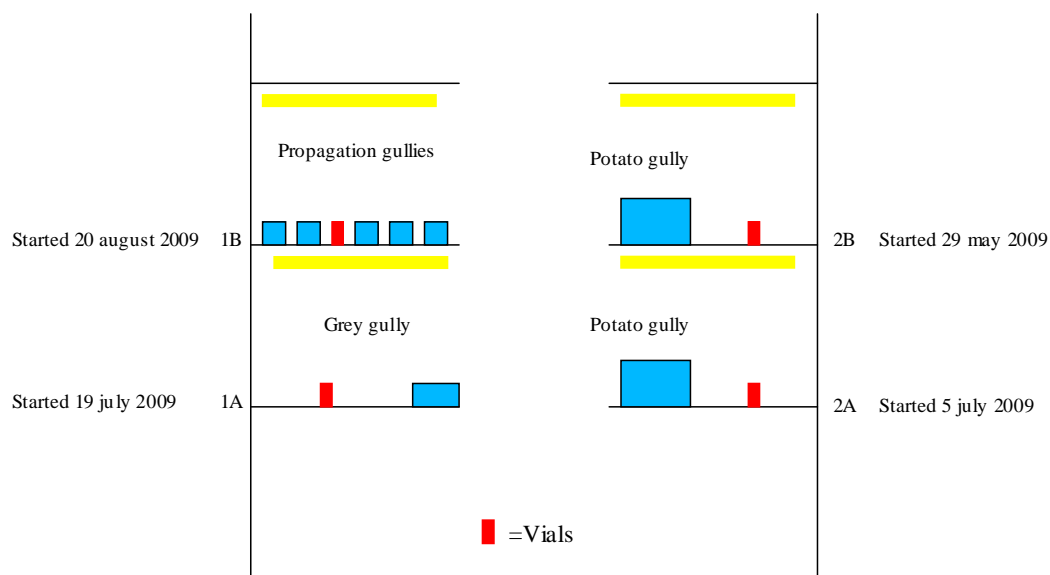


Fig. 38 UGent - Ethylene production: placement of the vials in the growth chamber

4.2.5 Nutrient Solution Environment

4.2.6 pH and EC evolution

At the start of the culture alcalinisation of the medium was compensated by H₃PO₄ addition. After nutrient exchange to tuberisation solution, KOH was used to compensate the acidification.

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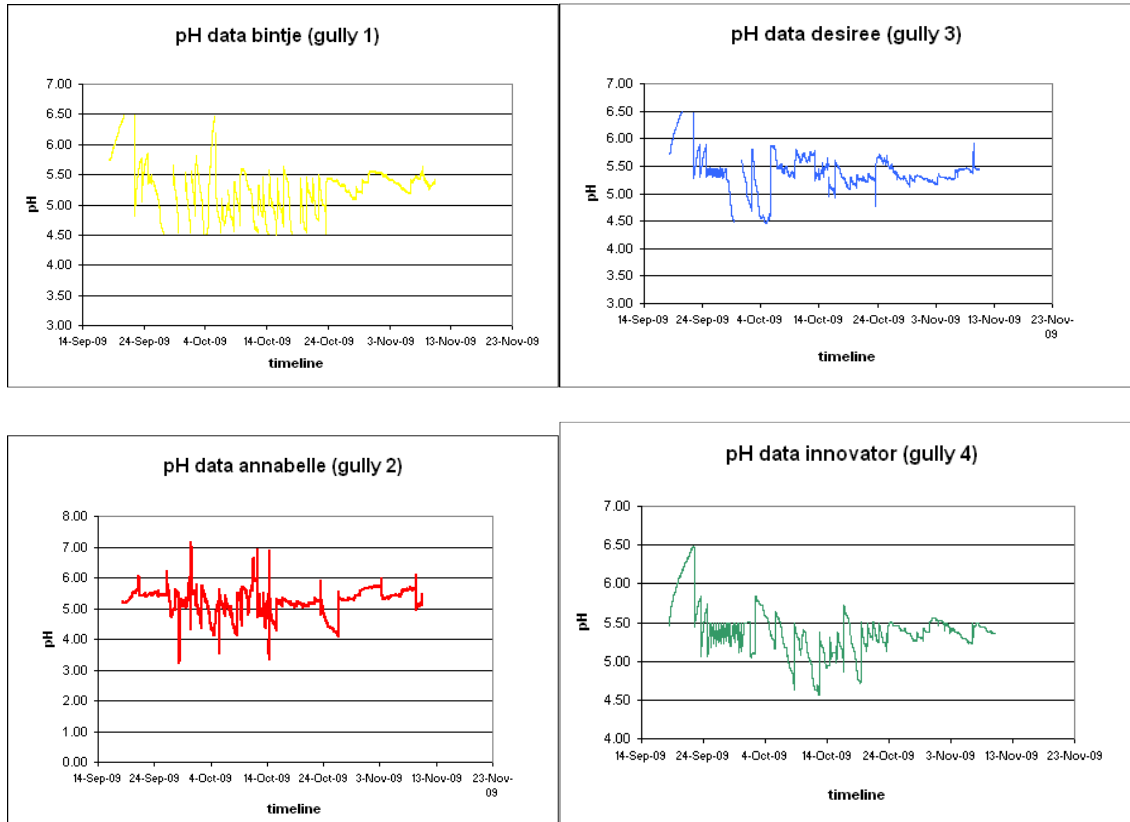


Fig. 39 UGent - pH data of each cultivar

Automatic control was only used with H₃PO₄ Compensation with KOH was carried out manually, since the magnitude of the effect of additions of Ca-nitrate was unknown, and automatic control was limited to either acid or base addition.

The amounts needed were small, hence deviations were within the foreseen range (Fig. 399)

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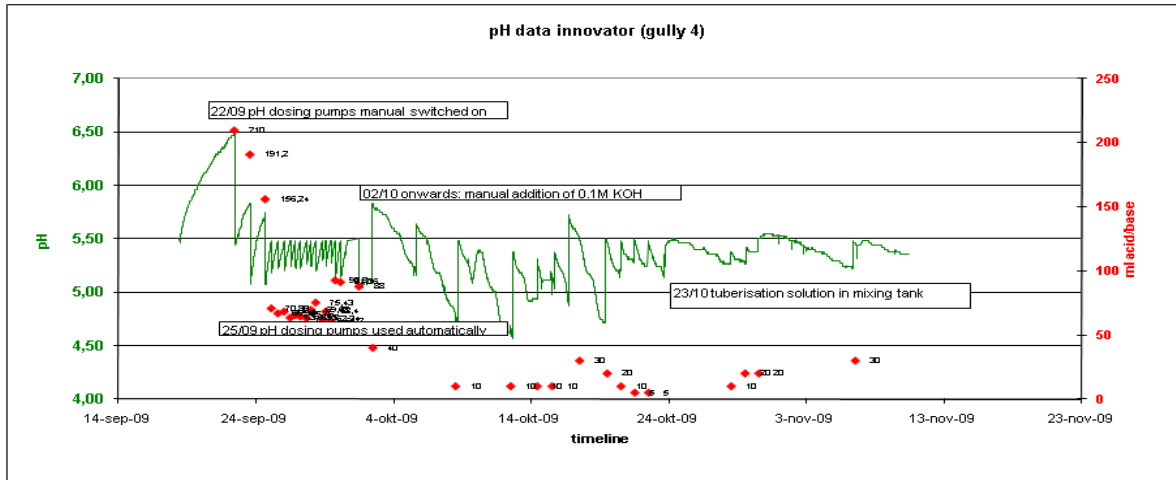


Fig. 40 UGent - Detailed pH evolution of innovator cultivar

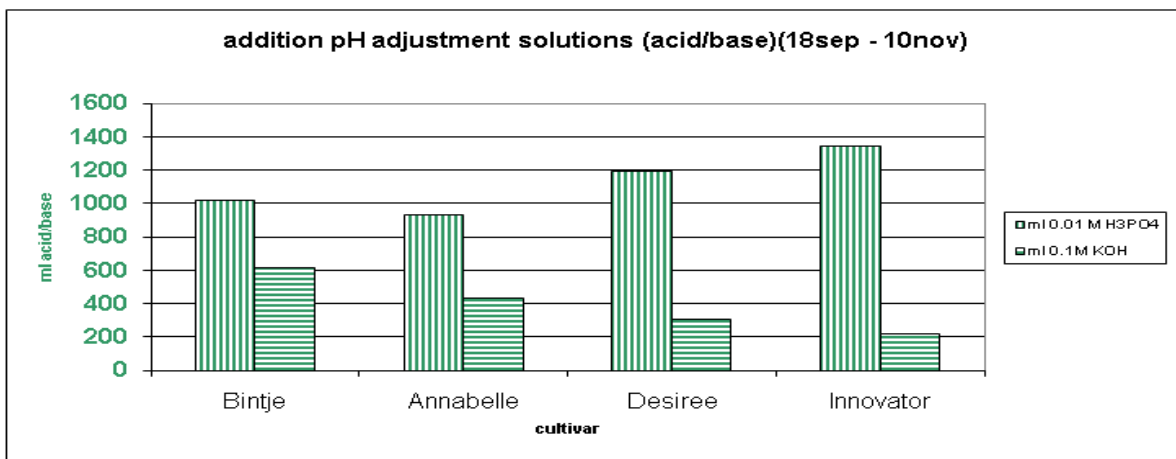


Fig. 41 UGent - Total amount of added pH-adjustment solutions

EC control was carried automatically for the whole duration of the experiment. EC compensation solution (K₂SO₄ during start-up growth) and K₂SO₄ and KH₂PO₄ in equal amounts during tuberisation) addition was triggered by automatic level compensation with distilled water (the amounts of liquids added are shown in Fig. 434).

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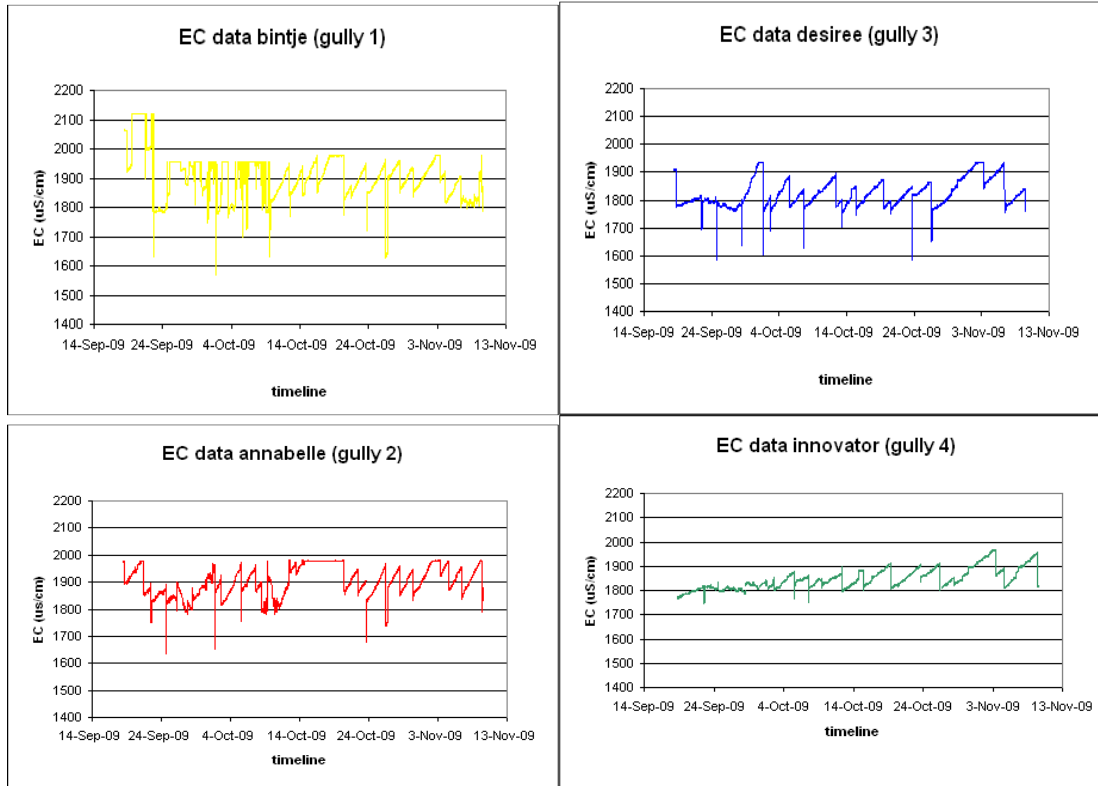


Fig. 42 UGent - EC data of each cultivar

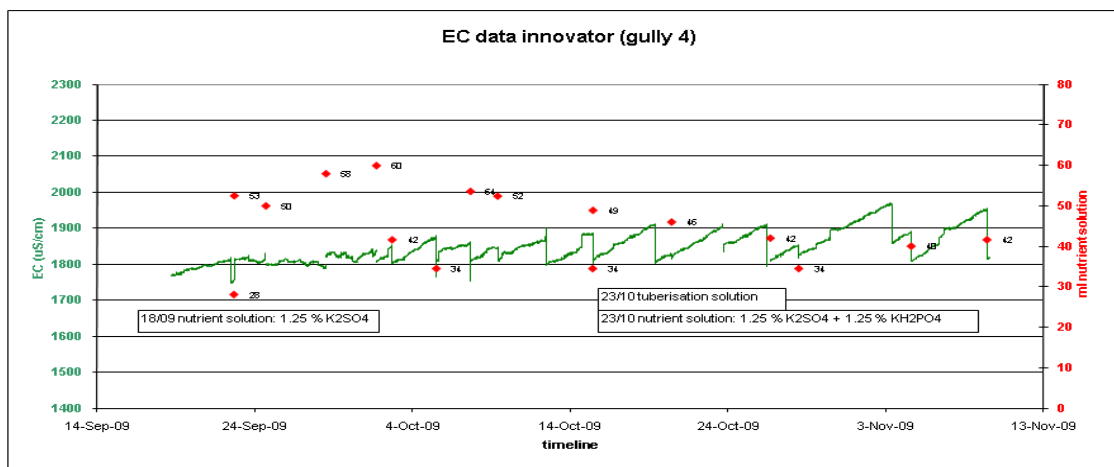


Fig. 43 UGent - Detailed EC evolution from Innovator cultivar

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At mid-November most plants stopped growing and some died (see 4.3.3), hence uptake graphs were not updated.

Plant water uptake is an integrated measurement of transpiration.

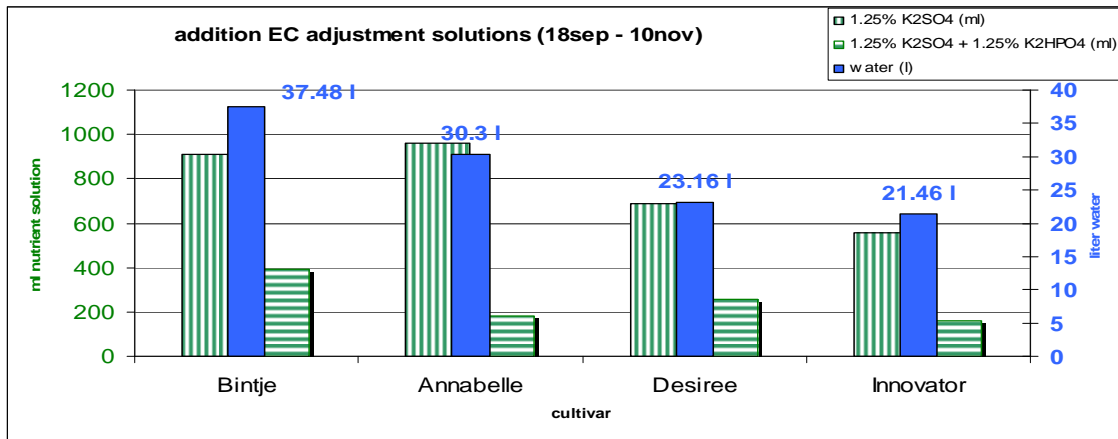


Fig. 44 UGent - Total amount of added EC-adjustment solutions

4.2.7 Nutrient solution T

Temperature of the nutrient solution was controlled to approximately 20 degrees. The 2 coolers had a different output, likely due to their position in the chamber. Setpoints were matched to better coincide (see end of graph Fig. 45).

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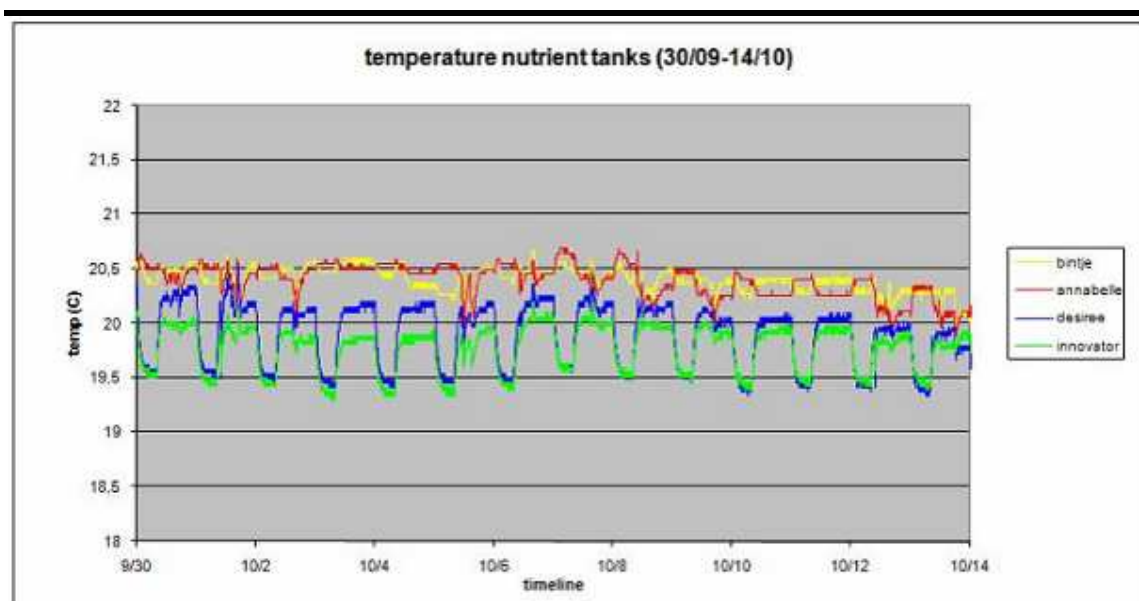


Fig. 45 UGent - Temperature nutrient solution in mixing tanks (setpoint chillers 18,5°C)

4.2.8 Nutrient solution analysis

Na levels were found to be 20 times higher than expected in the UGent pre-bench test tubers by IPL (see harvest 4.5), but in the analysis of the bench test samples the levels was corresponding with levels from food databases.

N levels were rapidly depleted; UGent added half the amount of the HZPC recipe in order to minimize shoot growth.

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Tab. 26 UGent - Overview nutrient solution analysis

		pH	EC	mmol/l										umol/l						
				K	Mg	Ca	Na	NH4	N	P	Cl	SO4	HCO3	Fe	Mn	Cu	Zn	B	Mo	
Start sol.	18/09/2009	5.6	1770	9.8	1.5	2.4	0.4	0.1	5	1.7	0.1	5.5	0.4	79.2	34.6	6.8	7.8	38.9	0.8	
Bintje	14/10/2009	5.2	1658	11.6	0.3	0.9	0.3	0	0	1.8	0.1	6.3	0.3	45.2	5.4	29.3	43.6	24.1	0.1	
	14/10/2009	+Tenso cocktail	5.3	1776	12.3	0.2	0.9	0.6	0	0	1.7	0	6.5	0.4	96.1	22.9	48.3	73.7	56.4	0.6
	23/10/2009	End growth sol	5.6	1700	13.2	1.3	0.2	0.6	0.1	0	9.1	0.1	3.2	0.5	96.9	42.1	11	13.1	51.8	1.1
	06/11/2009		5.6	1651	13.1	1	0.3	0.5	0	0	9.4	0	3.5	0.5	85.9	39.8	22.8	40.3	50	0.9
	16/12/2009	End tuberisation sol	5.7	2397	19.6	0.9	0.7	0.7	0.1	0	12	0.1	5.1	0.6	104.3	54.4	39.2	85	74.9	1.1
Annabelle	14/10/2009	5.2	1811	13.3	0.8	1.4	0.3	0.1	0.1	3.8	0.1	7.1	0.4	45.7	11.3	54.2	111	24.1	0.2	
	14/10/2009	+Tenso cocktail	5.9	1780	12.1	0.7	1.2	0.5	0	0	3.1	0	6.2	0.6	45.9	25.5	60.9	121	49.1	0.9
	23/10/2009	End growth sol	5.7	1701	13.2	1.3	0.2	0.6	0.1	0	9.2	0.1	3.1	0.6	96.4	42.9	12.5	23.1	52.7	1
	06/11/2009		5.4	1714	13.4	1.2	0.3	0.6	0	0	9.3	0	3.5	0.5	110.1	36.9	27.4	81	57.4	0.9
	01/01/2010	End tuberisation sol	5.7	1859	14.3	1.1	0.7	0.8	0	0	9.4	0.1	4	0.6	75	45.9	62.7	183	71.2	1.2
Desiree	14/10/2009	5.4	1730	11.2	0.7	1.3	0.3	1	0	1.9	0.1	7	0.3	33.8	9.8	38.2	180	25	0.2	
	14/10/2009	+Tenso cocktail	5.7	1792	11.6	0.7	1.2	0.6	0	0	1.6	0	6.8	0.5	43.7	27.3	49.5	225	49	0.9
	23/10/2009	End growth sol	5.5	1760	13.7	1.4	0.3	0.6	0	0	9.3	0.1	3.4	0.5	99.1	44	13.2	40.4	53.7	1.1
	06/11/2009		5.8	1710	13.5	1.1	0.3	0.6	0	0	8.6	0	3.6	0.6	68	28.5	26.3	141	53.7	1
	16/12/2009	End tuberisation sol	3.2	4191	32.4	2.4	2	1.3	0.1	0	27	0.1	9.7	0	404.4	86.3	187	651	148	0.2
Innovator	14/10/2009	5.3	1682	11.5	0.8	1.6	0.3	0.1	1.6	2.3	0	7	0.3	39.1	10	35.8	116	24.1	0.3	
	14/10/2009	+Tenso cocktail	5.4	1735	11.1	0.7	1.3	0.6	0	0	1.8	0	6.7	0.5	76.4	25.9	55.9	123	51.8	0.9
	23/10/2009	End growth sol	5.5	1692	13.1	1.3	0.2	0.6	0	0	9.3	0.1	3.1	0.5	97.6	42.9	10.8	18	54.6	1
	06/11/2009		5.6	1706	13.5	1.2	0.3	0.5	0	0	9.1	0	3.5	0.6	102	34	35.7	75.4	56.4	0.9
	16/12/2009	End tuberisation sol	5.8	2106	16.3	1.1	0.6	0.9	0	0	9.8	0	4.7	0.6	44.8	42.6	44.9	251	68.5	1.4
YARA Iron 6%				0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	18.1	0.0	0.0	0.0	5.2	0	
Yara Tenso cocktail				0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2	32.4	22.1	3.9	3.9	30.7	0.5	

4.3 Monitoring of plant development

The in vitro plants were obtained after 21 days of in vitro and 7 days of propagation culture.

The potato plants at UGent were grown for 134 days, of which 107 in the BT room.

The first 4 weeks the plants were grown in the propagation room for size increase.

By 90 days (starting with in-vitro plants), tuber growth was halted due to plant growth problems.

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4.3.1 *Photographic follow-up*



8Oct. Bintje



8Oct. Desiree



8Oct. Annabelle



8Oct. Innovator

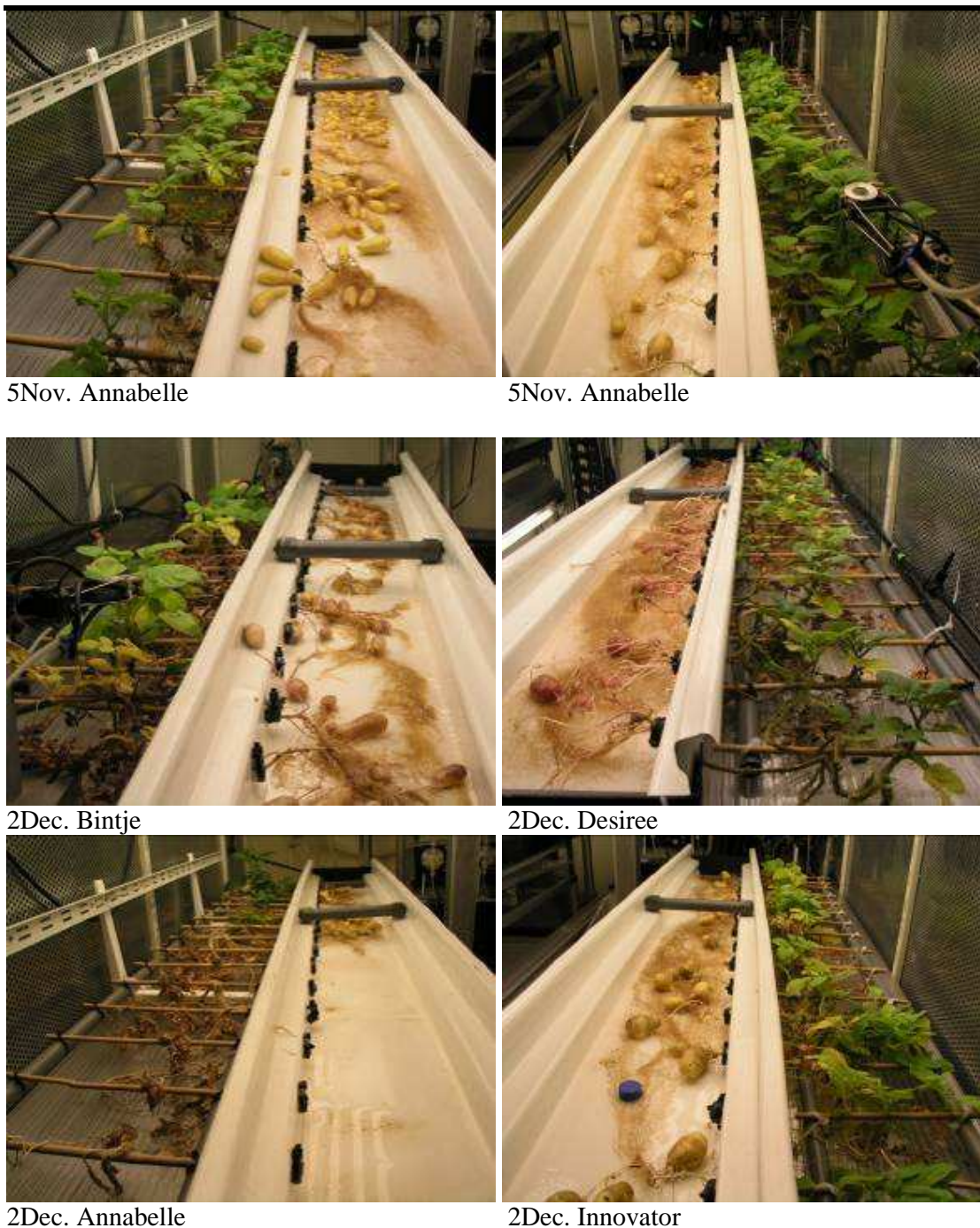


5Nov. Bintje



5Nov. Desiree

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5Nov. Annabelle

5Nov. Annabelle

2Dec. Bintje

2Dec. Desiree

2Dec. Annabelle

2Dec. Innovator

Fig. 46 UGent - Photos growth evolution

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The Annabelle plants at the drain side of the gully all wilted and died by this time point. To avoid rotting of the tubers, they were already harvested.

4.3.2 Detailed photographic observations



17Nov Bintje yellow leaves



17Nov. Desiree



17Nov Annabelle yellow leaves



17Nov. Innovator yellow leaves

Fig. 47 UGent - Photos leaf size

Leaf size as shown in Fig. 47 was small as compared to the HZPC test setup with the same in-vitro starting material.(see 4.4).

As can be seen in Fig. 488, plants of all cultivars were affected by yellowing of younger leaves and gradual drying of the older ones 3 months after start of the culture. Some plants rapidly wilted and completely died. This indicated a likely phytopathogenic problem spread by the nutrient solution. Both microscopic and PCR analysis of the solution was carried out.

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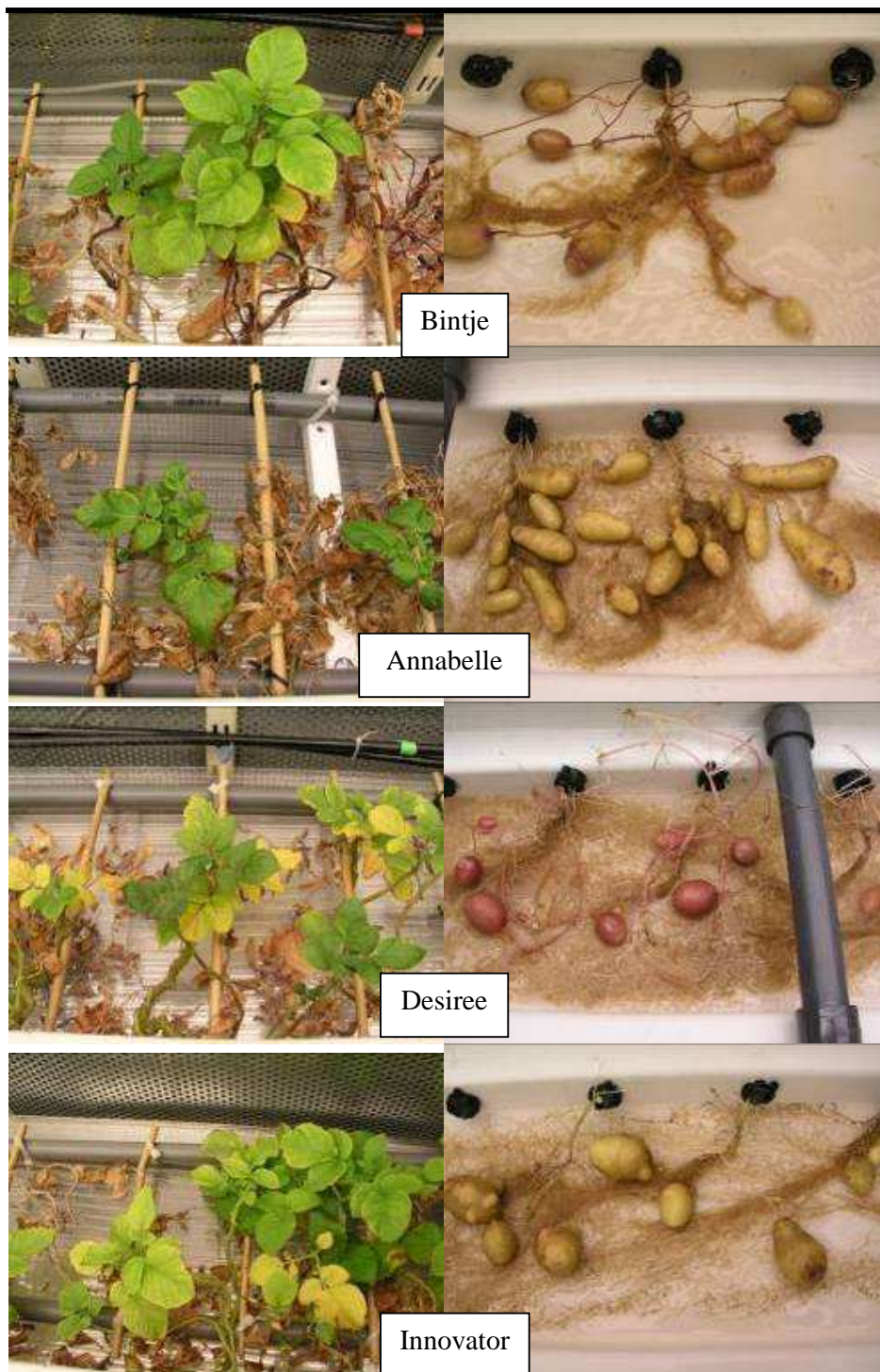


Fig. 48 UGent - Photos plant and tuber appearance (2dec.)

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On December 14th 2009 the results of a PCR analysis (DNA Multiscan, from Scientia Terrae Diagnosecentrum) of a sample of nutritive solution from the Annabelle gully (most affected, sampling 26/11/2009) of the Bench test 1 were received. It revealed the presence of significant levels of four pathogens:

- Colletotrichum accutatum, medium infection
- Colletotrichum coccodes, medium infection
- Fusarium oxysporum, medium infection
- Pythium dissotocum, strong infection

By microscopical observation of the nutrient solution, Colletotrichum coccodes spores were revealed. Colletotrichum coccodes infection of stems was confirmed by microscopy (Congo Red staining, laboratory of Mycology).

A previous PCR analysis on samples collected by Christel Paillé/ESTEC the 9th November revealed the presence of

- Fusarium oxysporum
- Cladosporium
- Enterobacter

Cladosporium and Enterobacter are common non-pathogenic fungal respectively bacterial genera in hydroponic culture.

The oomycete Pythium is a typical hydroponics pathogen.

During the experiment, the gully liquid was infested by *Clogmia* mothflies (Species: *Clogmia albipunctata*, Common Name: Mothfly, Order: Diptera, Family: Psychodidae). Apparently it is not a harmful insect (saprophage, scavenger) but still it is a possible vector of plant diseases.

Flies belonging to the Family of *Sciaroidea* were also present. Larvae of these species live in the nutrient solution and some species feed of roots, although some are harmless. The exact species was not identified. As their common name fungus gnat suggests, they are a vector of fungal diseases.

The tubers that developed were limited in size due to the early die-off of the crop, but corresponded to the typical appearance for each cultivar.

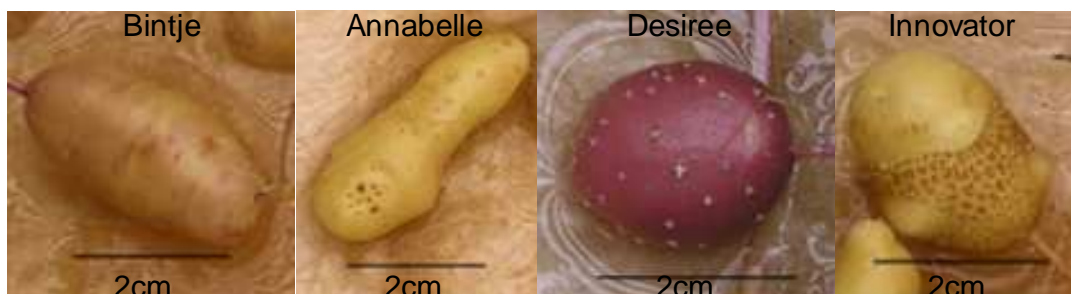


Fig. 49 UGent - Representative tuber of each cultivar 5/11/2009

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4.3.3 Growth assessment

In this section the development of the plants is documented.

Fig. 5050 illustrates the effect of the phytosanitary problems, Annabelle being most susceptible.

Shoot and tuber development were mostly halted after 3 months of development.

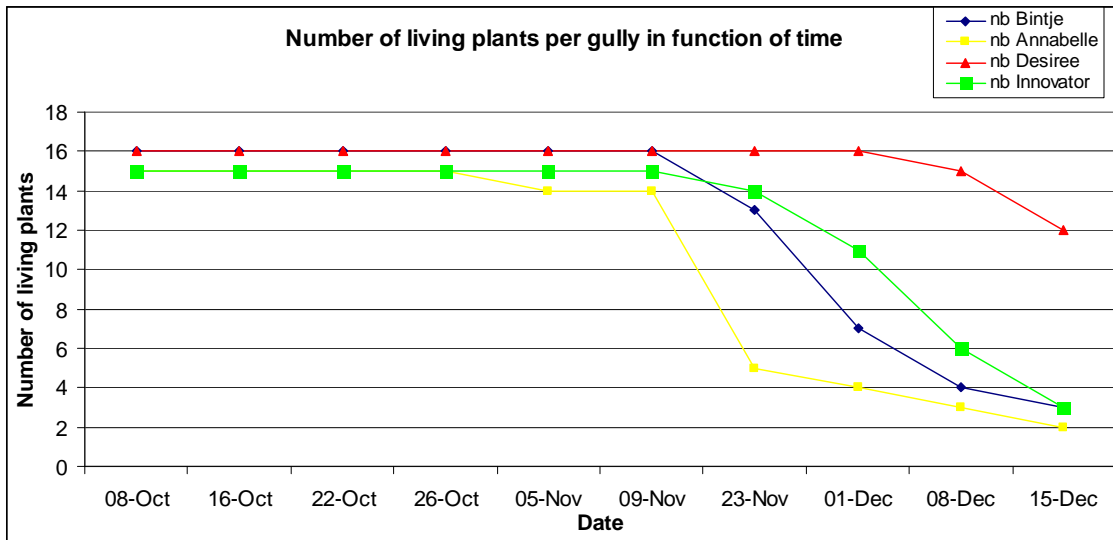
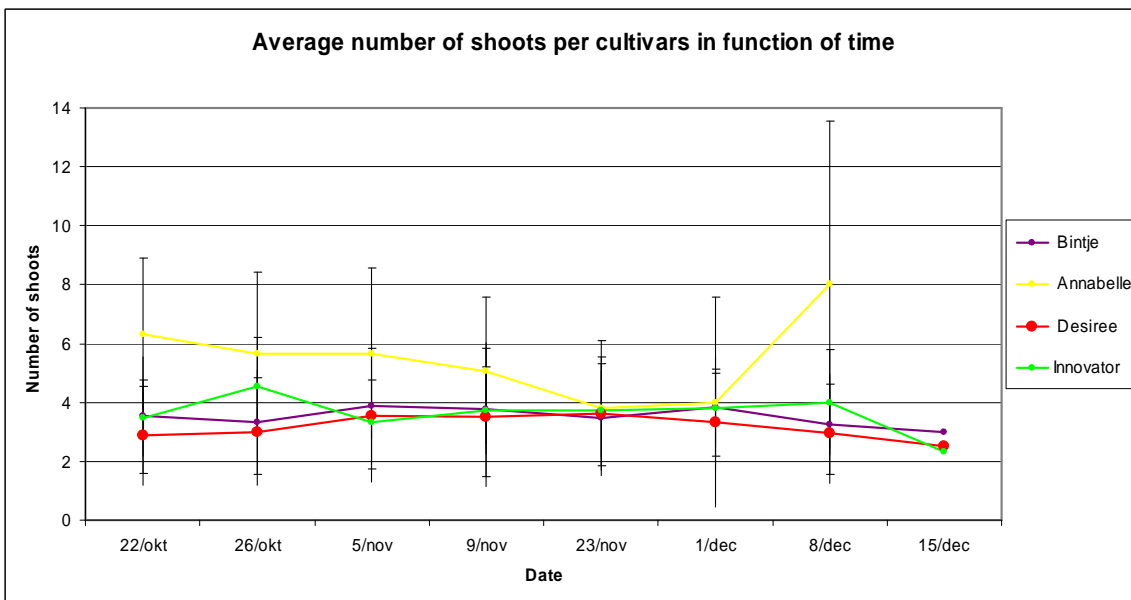


Fig. 50 UGent - Number of living plants per gully in function of time



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Fig. 51 UGent - Average number of branches per cultivar per plant as a function of time

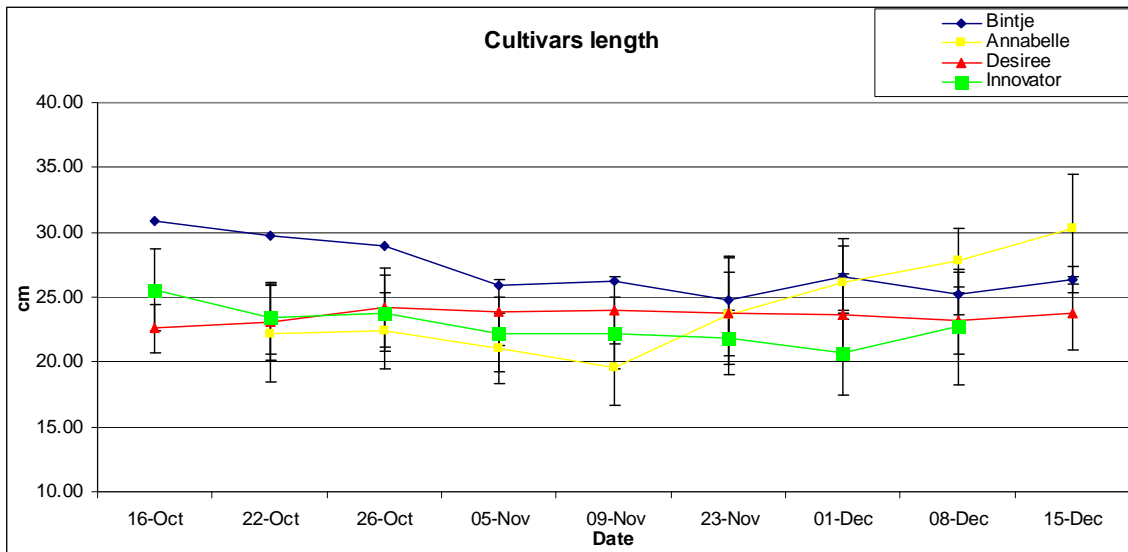
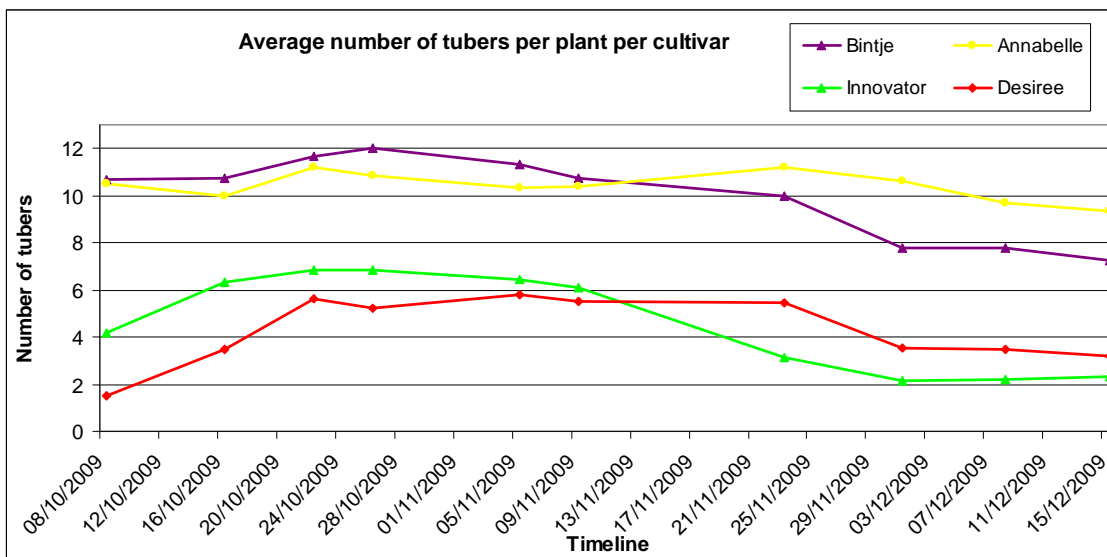


Fig. 52 UGent - Cultivars main stem length

Annabelle plants died rapidly, however from 1 December on a few plants recovered and 1 plant started to grow vigorously, developing several branches. Towards the end of December this plant died rapidly, presumably caused by the pathogens present in the nutrient solution.



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Fig. 53 UGent - Number of tuber per cultivars

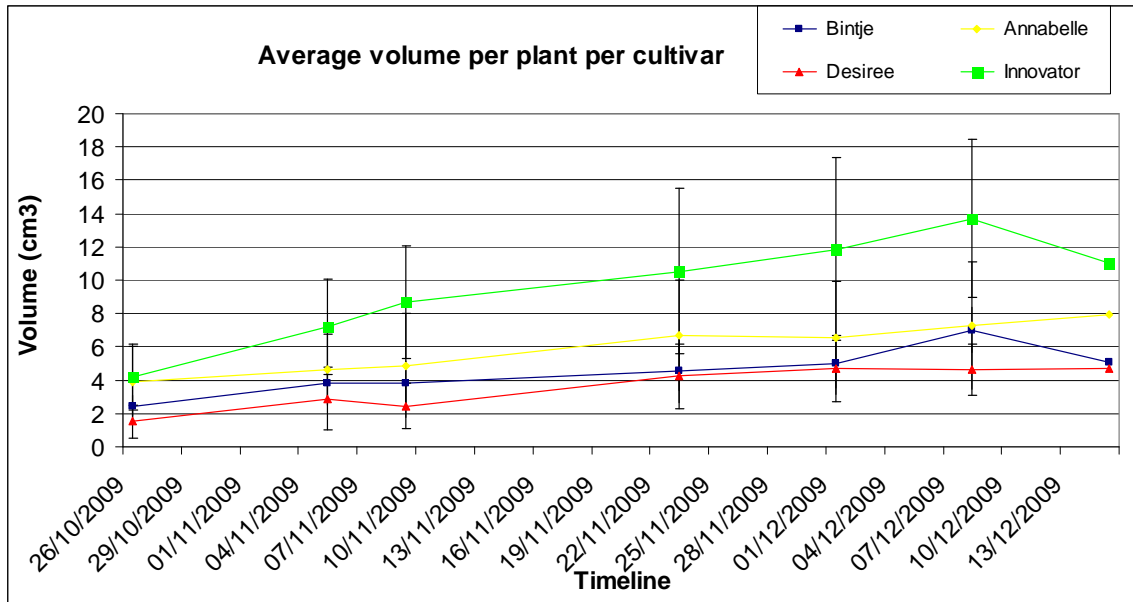


Fig. 54 UGent - Average tuber volume per cultivar

4.3.4 Gas exchange data

The CO₂ gas exchange of the plants was measured using an ADC 2550 gas exchange equipment. The goal was to obtain concomitant measurements on 2 cultivars by means of 2 attached cuvettes, and 2 continuous flow exits that could also be measured by the ethylene monitoring system. Such a setup precluded the use of auto-calibration of the CO₂ signal.

The chamber CO₂ level was measured by a PPSsystems WMA4 IRGA analyser (recorded by the dl2 data logger), with continuous hourly autocalibration.

First results proved sensor drift of the ADC system to be too important to further use this experimental setup. Therefore only single plant cuvette measurements are reproduced below for the Annabelle and Bintje cultivars.

The green and dark grey lines indicate the CO₂ assimilation during the day and a proportionally smaller CO₂ production through respiration at night (0AM to 8AM).

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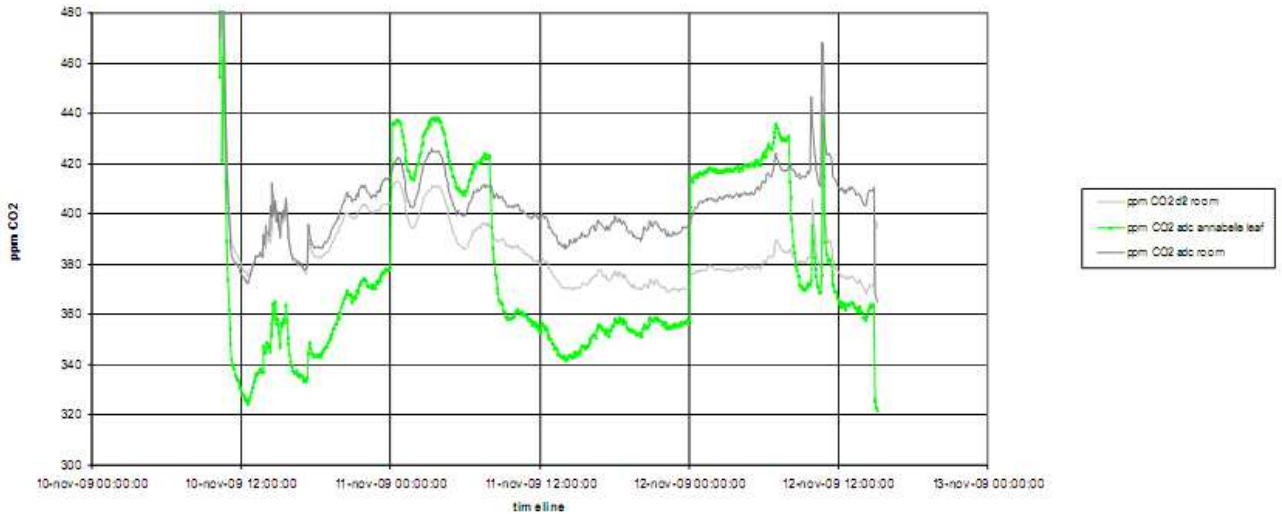


Fig. 55 UGent - Annabelle gas exchange

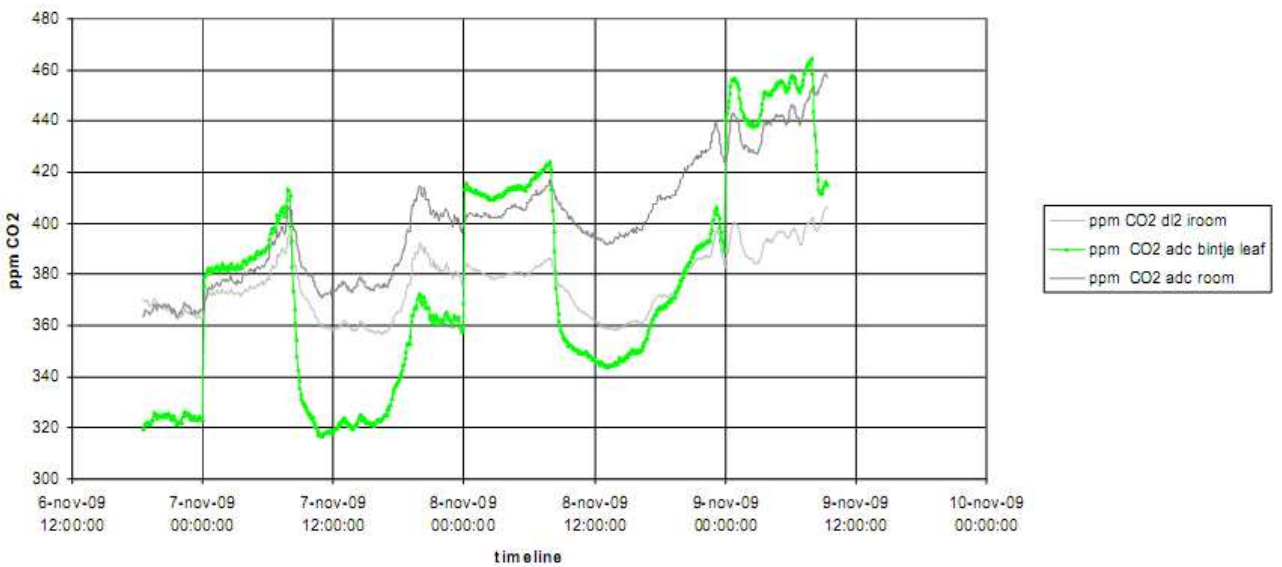


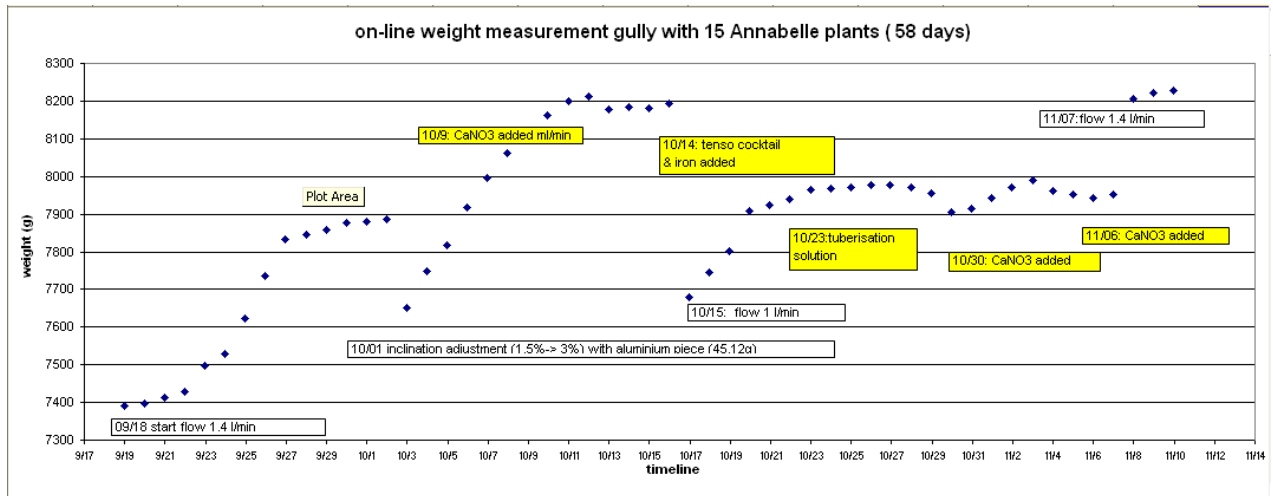
Fig. 56 UGent - Bintje gas exchange

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4.3.5 Plant weight determination

The independent NFT gully system with the Annabelle cultivar provided an online weight measurement through load-cells supporting the gully.

An 800g total biomass increase was recorded; changing of gully inclination and nutrient solution flow rate lead to immediate weight changes of maximum 500g due to a change of amount of liquid present in the gully.



biomass increase after 58 days	
flow (1.4l/min)and NFT layer thickness (1mm) same at start- and endpoint	
weight startpoint:	7390 g
weight endpoint	8229 g
total biomass increase	794 g

Fig. 57 UGent - Weight Annabelle

4.4 HZPC greenhouse test

UGent consultant HZPC ran a parallel experiment in their greenhouse using the same starting material from the same batch of in-vitro plants.

The setup (Fig. 58) has 2 independent NFT recirculating systems, each being composed of
 -100 liter nutrient solution tank

-9m long PU-coated stainless steel gully (Meteor Systems, NL), 20cm width

-the 5 cultivars pre-listed in the measuring plan were organised in blocks of 12 plants, the gullies have 10cm interplant distance holes through the side.

The width of the setup is 75cm. Plant stems are manually attached to a trellis made of overhead metal wires and per-plant trellis twines as used in commercial horticulture (e.g. tomato).



HZPC1 Annabelle, VanGogh, Innovator, Desiree, Saline

HZPC2 Innovator, Saline, Bintje, Annabelle, Desiree

Fig. 58 HZPC - Set up

The first test HZPC1 was used as a guideline for the selection method elaborated in TN98.3.1. The second test HZPC2 is reported here as a comparison with the UGent/UCL results.

The greenhouse tests took 84 (HZPC2) respectively 49 days (HZPC1), starting with 21 day old in vitro plants.

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Fig. 59 HZPC - Greenhouse test (photographs after 41 days)

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Annabelle



Desiree



Innovator

The typical size of fully developed leaves as seen in Fig. 60 is increased due to the lower light level in fall (September-November) in comparison with spring tests at HZPC.

Annabelle 102.9 cm²

Desiree 130.5 cm²

Innovator 101.3 cm²

See 4.3.2 for comparison with UGent grown plants, and 5.3.3 for leaf surface results at UCL.

Fig. 60 HZPC - Leaf sizes

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4.5 Harvest results

This section summarizes the harvest results from the NFT hydroponic potato experiments: bench test 1 at UGent and UCL, the greenhouse experiments at UGent consultant HZPC.

The edible part harvest is summarised for UGent, UCL and HZPC in Tab. 27. The tuber yield obtained in pre-tests carried out in the UGent propagation room is included for comparison. The pre-test growth periods were 180 (1) respectively 150 (2) days, starting with tubers.

Tab. 27 Potato - Harvest results

		Annabelle	Bin'tje	Desiree	Innovator
Tuber harvest (kg)	HZPC 2008	1.872		1.141	0.676
	HZPC 2009	4.420	1.984	3.998	0.663
	UGent pretest 1, 2009		0.4	0.283	
	UGent pretest 2, 2009		0.249	0.418	
	UGent bench test 1, 2009	0.511	0.466	0.274	0.415
	UCL bench test 1 2009	0.662	0.546	0.299	0.283
Tuber harvest (kg/ m ²)	HZPC 2008	2.5		1.52	0.9
	HZPC 2009	4.91	2.2	4.442	0.74
	UGent pretest 1, 2009		2.67	1.89	
	UGent pretest 2, 2009		1.66	2.79	
	UGent bench test 1, 2009	0.660	0.583	0.343	0.501
	UCL bench test 1 2009	0.829	0.683	0.374	0.355
Tuber harvest (g/ plant)	HZPC 2008	93.6		57.1	33.8
	HZPC 2009	184.2	82.7	166.6	27.6
	UGent pretest 1, 2009		133.6	94.5	
	UGent pretest 2, 2009		83.1	139.5	
	UGent bench test 1, 2009	34.1	29.1	17.2	27.2
	UCL bench test 1 2009	41.4	34.1	18.7	17.7
Number of (tubers/plant)	HZPC 2008				
	HZPC 2009	20.4	12.9	10.5	3.7
	UGent pretest 1, 2009		7	4	
	UGent pretest 2, 2009		6.3	3.3	
	UGent bench test 1, 2009	9.2	6.5	3.2	2.1
	UCL bench test 1 2009	4.6	4.6	3.6	1.4

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The inedible part harvest for UGent, UCL and HZPC is summarised in Tab. 28.

Tab. 28 Potato – FW (g) and DW (g) of shoots and roots

		shoot F W	root+stolon FW	Total FW	shoot DW	root+stolon DW	Total DW	%DW
HZPC 2008	Annabelle	54.805	17.04	71.845	4.18	1.06	5.24	7.293479
	Bintje							
	Desiree	39.52	19.27	58.79	2.53	1.35	3.88	6.59
	Innovator	28.91	8.38	37.29	2.125	0.5	2.625	7.03
HZPC 2009	Annabelle	140	20.29	140			9.75	6.96
	Bintje	79	8.208333	79			5.75	7.27
	Desiree	169.25	32.375	169.25			10.75	6.35
	Innovator	37.5	2.958333	37.5			3.5	9.33
UGent Bench test 1 2009	Annabelle				1.994446	0.206133	2.20058	
	Bintje				3.65125	0.232625	3.883875	
	Desiree				4.03175	0.488875	4.520625	
	Innovator				3.15025	0.207467	3.357717	
UCL Bench test 1 2009	Annabelle				2.77225	0.300938	3.073188	
	Bintje				3.120688	0.699063	3.81975	
	Desiree				5.575313	0.93775	6.513063	
	Innovator				2.08275	0.2115	2.29425	

Due to cultivation problems, at the time of harvest, the shoots of all cultivars at UGent and UCL were largely dead and desiccated. Hence only DW could be determined as a representative value.

The nutritional analysis of the harvest was carried out at IPL for all samples from UGent, UCL and UGent consultant HZPC. HZPC also provided field grown samples harvested in fall 2009, and stored for all 4 cultivars under optimal conditions.

See TN98.4.11, 4.3.10 Table 14 for experimental protocol overview.

The nutritional composition is given in Tab. 299, including

- proximate analysis (moisture, ash, protein, lipid, fiber, carbohydrates by difference)
- elemental analysis, for harmonisation with human micronutrients to be analyzed by priority in processing trials of the same harvest samples, K, P, Ca, Mg, Zn, Cu were analysed
- Na content was considered of more importance than Cl.
- cultivar specific toxic compounds: glycoalkaloids.

As a reference values from the USDA database are included “potato, flesh and skin, raw”

<http://www.nal.usda.gov/fnic/foodcomp/search/>.

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Tab. 29 Potato - IPL nutritional analysis results

Potato Cultivar	Database	Annabelle				Bintje				Innovator				Desiree				
		UCL BT1	UGent BT1	HZPC GH	HZPC field	UCL BT1	UGent BT1	HZPC GH	HZPC field	UCL BT1	UGent BT1	HZPC GH	HZPC field	UCL BT1	UGent BT1	HZPC GH	HZPC field	
Value	USDA Potato	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	
Water (%)	79,34	82,2	82,4	84,8	81,8	82,3	77,1	78,2	76,5	78,1	76,5	77,8	77,6	84,1	83,5	81,9	79,7	
Protein (%) [N x 6,25]	2,02	1,38	1,11	1,70	1,33	1,62	1,53	2,07	1,57	1,84	1,13	2,53	1,43	2,02	2,05	2,19	1,49	
Fat (%)	0,09	0,08	0,10	0,09	0,07	0,06	0,05	0,07	0,08	0,08	0,06	0,07	0,07	0,10	0,11	0,10	0,07	
TDF (%)	2,2	1,85	1,80	2,02	1,66	2,04	2,40	2,17	2,14	2,71	2,22	3,23	2,09	2,17	2,03	2,34	1,86	
Available carbohydrates (%)	15,27	13,19	13,45	10,36	14,42	12,70	17,40	16,30	18,75	16,00	18,80	15,20	17,92	10,26	10,84	12,29	15,98	
Minerals (%)	1,08	1,34	1,19	0,99	0,75	1,27	1,44	1,22	0,93	1,27	1,30	1,24	0,90	1,33	1,43	1,17	0,87	
Of which (mg/100g)	Na	73	9,20	11,70	18,00	23,00	57,00	63,00	64,00	N/A	67,00	62,00	66,00	25,90	19,40	23,70	25,90	22,50
	K	421	551	486	365	312	769	842	882	432	738	780	715	381	631	665	521	404
	Ca	12	1,82	10,20	13,00	14,90	1,80	1,10	8,70	16,40	1,30	1,24	9,48	22,90	8,10	8,10	15,60	21,00
	Mg	23	24,60	23,87	28,10	19,30	23,40	21,60	26,80	20,60	26,10	25,10	30,00	21,20	25,10	26,30	30,30	17,00
	Fe	0,78	1,34	1,50	1,00	2,40	1,30	1,50	1,48	1,90	1,30	1,54	1,57	4,70	4,10	5,40	2,30	2,50
	Cu	0,108	0,49	0,51	0,38	0,30	0,38	0,53	0,60	0,30	0,30	0,41	0,53	0,16	0,27	0,77	0,40	0,40
	Zn	0,29	0,60	1,16	2,39	0,26	0,36	0,74	2,50	0,30	0,39	0,94	3,10	0,40	0,43	0,75	0,60	0,20
	Mn	0,153	0,27	0,42	0,23	0,10	0,23	0,32	0,25	0,15	0,21	0,28	0,19	0,13	0,25	0,48	0,27	0,11
	P	57	111,8	100,9	93	28	101	126,7	114,1	32	N/A	105,4	108,6	34	108	121	102	24
N (%)	0,32	0,22	0,18	0,27	0,21	0,26	0,24	0,33	0,25	0,29	0,18	0,40	0,23	0,32	0,33	0,35	0,24	
<i>Crop specific compounds</i>																		
Solanine (mg/kg)	NA	42	34	60	0	39	59	32	0	77	67	97	0	51	45	0	0	
Chaconine (mg/kg) E Estim.	NA	54	63	71	0	78	90	68	24	107	66	123	0	69	94	0	0	
TGA (mg/kg) Sum	NA	96	97	131	0	117	149	100	24	184	133	220	0	120	139	0	0	
Energy (for 100g) kcal	77	62,7	62,8	53,2	58,3	62,1	81,1	65,9	86,3	77,3	84,8	61,2	72,3	54,3	56,6	63,5	74,3	
Energy (for 100g) kJ	321	262,3	262,6	222,6	244,1	259,8	339,4	275,6	361,1	323,5	354,9	256,1	302,5	227,3	236,8	265,6	310,7	

TGA = total glycoalkaloids, expressed here as the sum of solanin and chaconine (the latter is an estimate, since an internal synthetic chaconin standard was not available for calibration). %N is shown here is related to the protein by the standard factor 6.25.

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5 Potato (UCL)

5.1 Experimental Layout

5.1.1 Measuring Plan

Plant development weekly follow-up

- Plant height
- Number of leaves
- Number of stolons
- Number of tubers
- Date of stolon formation
- Date of tuberisation
- Date of flowering
- Number of stolons and tubers
- Estimate of percentage of gully covered by the roots

Plant physiological parameter weekly assessment

- Net photosynthesis and instantaneous transpiration (portable Infra Red Gas analyzer LCA4 ADC Bioscientific Ltd)
- Stomatal conductance (porometer AP4 deltaT):
- Kinetics of chlorophyll fluorescence (fluorescence monitoring system 2 Hansatech Instruments)
- Chlorophyll concentration SPAD (CCM-200 opti-sciences):
- Leaf area (compact portable area meter AM 300 ADC Bioscientific Ltd, scanning width 10cm)

Destructive analysis

- Fresh weight of the leaves, stems, roots, tubers (for each tuber and total per plant).
- Dry weight of the leaves, stems, roots.
- Total soluble sugar content and starch content in leaves and roots according to Yemm and Willis (1954): 1g of frozen samples (young leaf, old leaf, roots)
- carbon isotopic discrimination to evaluate the water-use efficiency on young and old leaves

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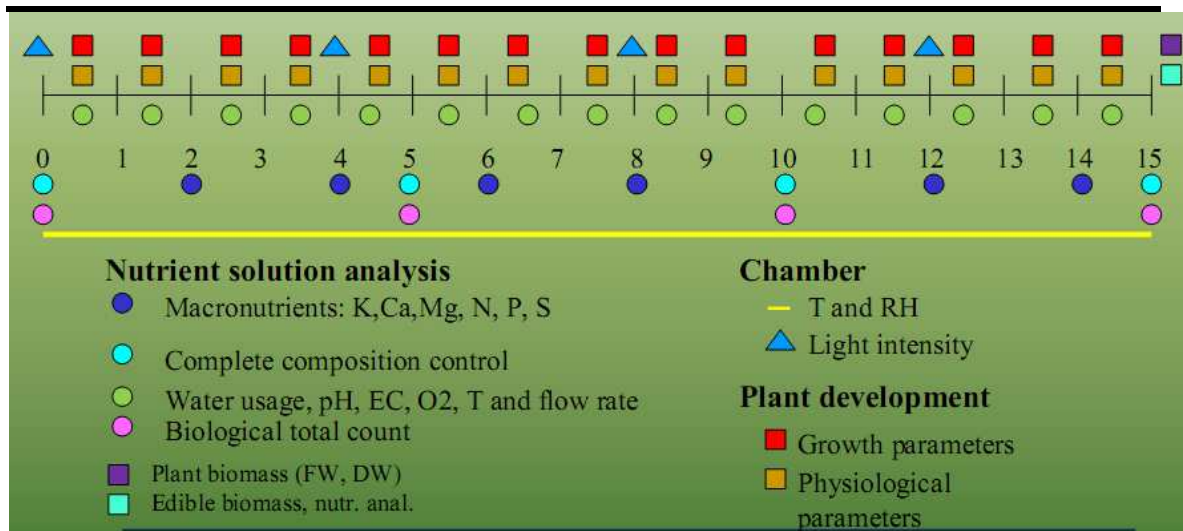


Fig. 61 UCL - Measuring plan

5.1.2 Setup

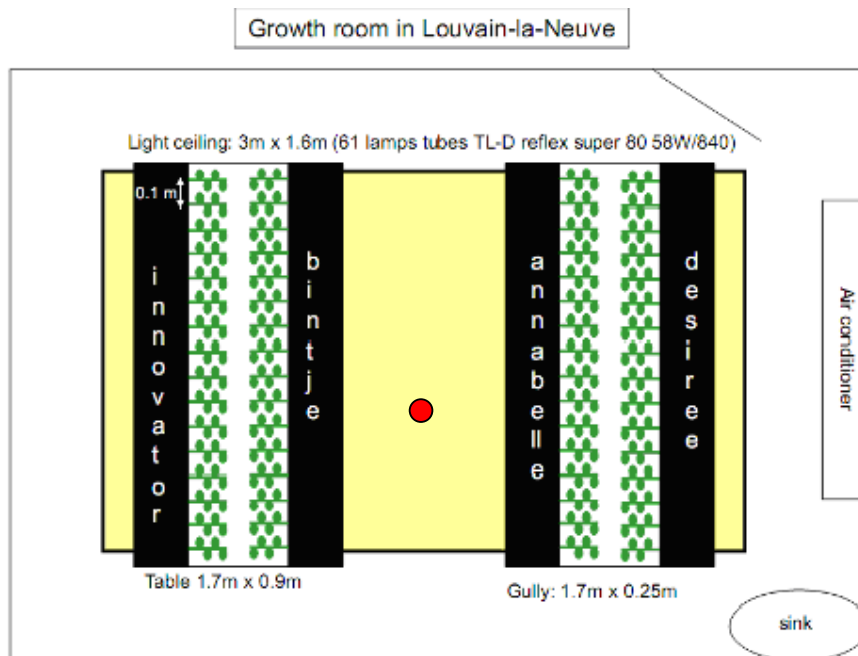


Fig. 62 UCL - Setup

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5.2 Growth environment follow-up

5.2.1 Settings

Tab. 30 UCL - Settings

Photoperiod	16h
Light intensity	200-300 $\mu\text{mol}/\text{m}^2/\text{s}$
Room temperature	22 \pm 1 $^{\circ}\text{C}$

Light intensity at canopy level was between 150 and 250 $\mu\text{mol}/\text{m}^2$ at the end of the development of the plants.

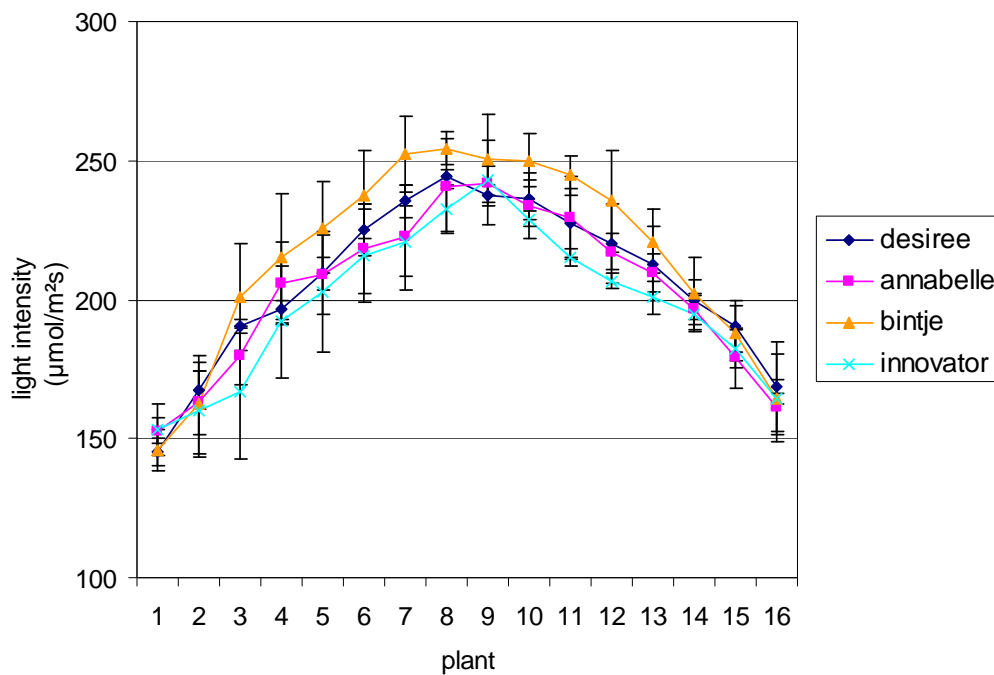


Fig. 63 UCL - Light intensity at canopy level

5.2.2 Chamber T/RH evolution

T/RH was stable according to the setpoints.

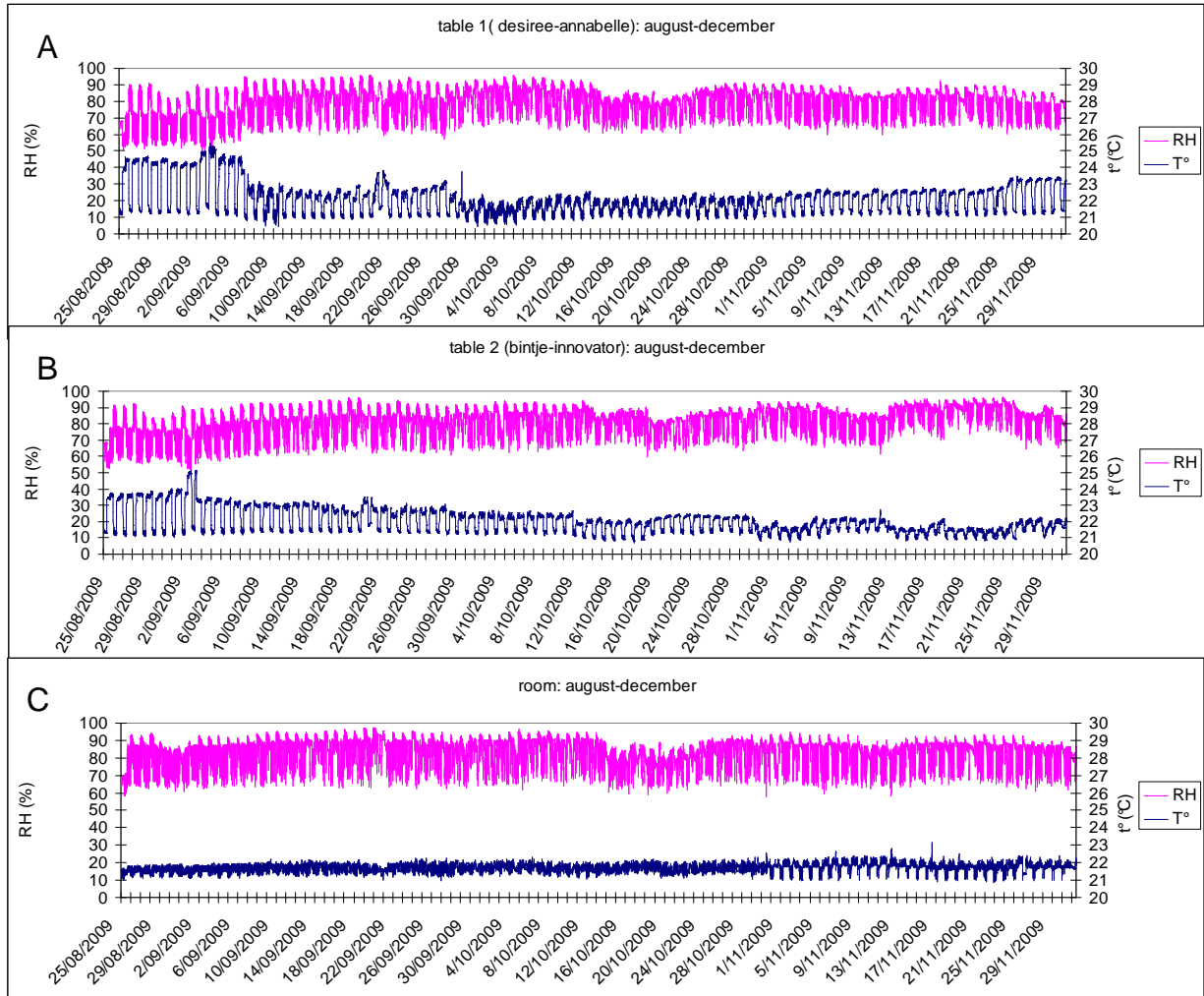


Fig. 64 UCL - Chamber T/RH

Temperature and relative humidity on (A) middle of table 1 between the gullies containing the Desiree and Annabelle plants, (B) middle of table 2 between the gullies containing the Bintje and Innovator plants, (C) between the two tables.

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5.2.3 Chamber CO₂ level

Measurements not available, only leaf level measurements using dedicated equipment. See 4.3.4.

5.2.4 Nutrient Solution Environment

As roots developed, nutrient solution flow was gradually diminished to keep the NFT layer thin.

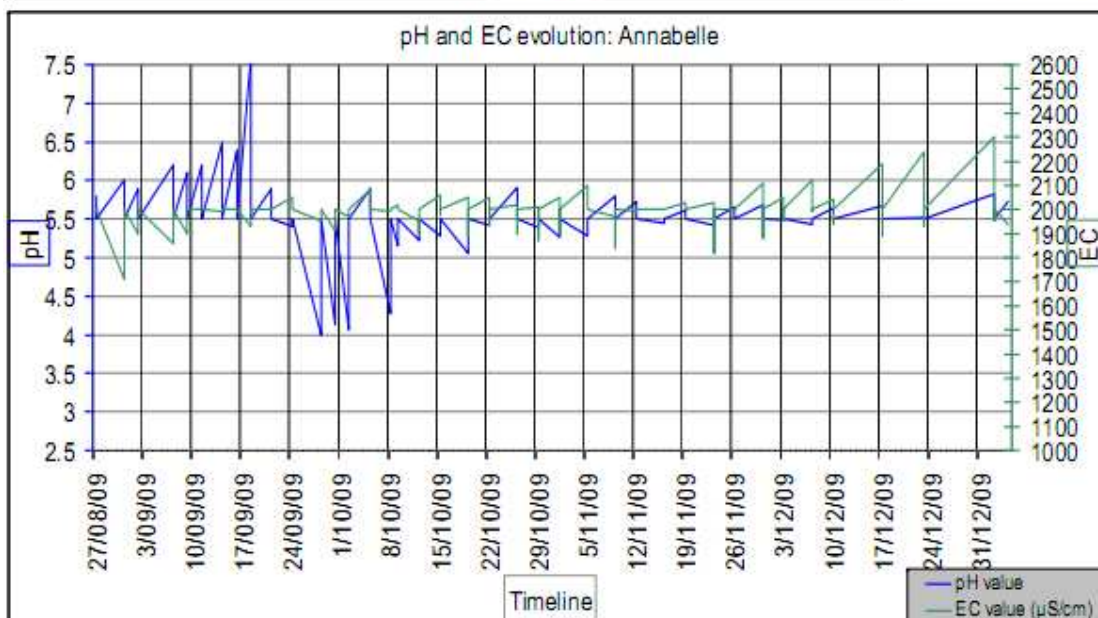
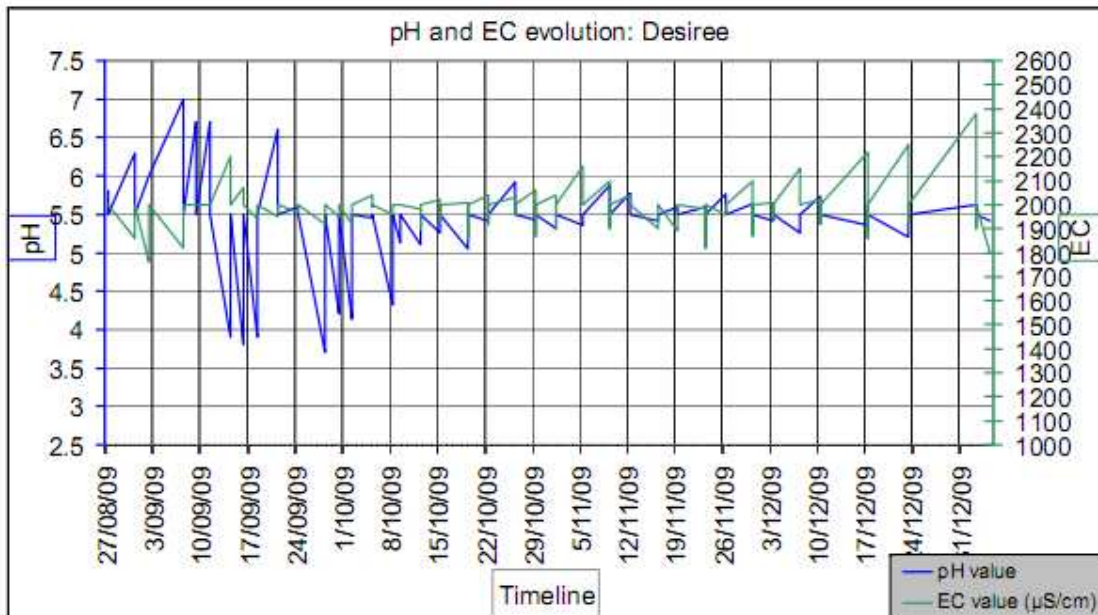
Tab. 31 UCL - Nutrient solution environment

cultivar	Desiree	Annabelle	Bintje	Innovator
Solution change	25/8, 8/10, 10/11	25/8, 8/10, 10/11	25/8, 8/10, 10/11	25/8, 8/10, 10/11
temperature	22.1°C ± 0.5°C	22.1°C ± 0.4°C	22.1°C ± 0.3°C	22.1°C ± 0.2°C
Water flow	5l/ min (25/8) 2l/min (12/10) 1l/min (12/11)	5l/ min (25/8) 2l/min (12/10) 1l/min (12/11)	5l/ min (25/8) 2l/min (12/10) 1l/min (12/11)	5l/ min (25/8) 2l/min (12/10) 1l/min (12/11)
Gully inclination	2.25 cm	2.25 cm	2.25 cm	2.25 cm
Solution thickness	4 mm	3 mm	4 mm	3 mm
EC	2000 µS/cm	2000 µS/cm	2000 µS/cm	2000 µS/cm
pH	5.5	5.5	5.5	5.5

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5.2.5 pH and EC evolution

Phosphoric acid additions were needed at the start. At the moment of tuber formation, the solution acidified, and KOH was used to further adjust.



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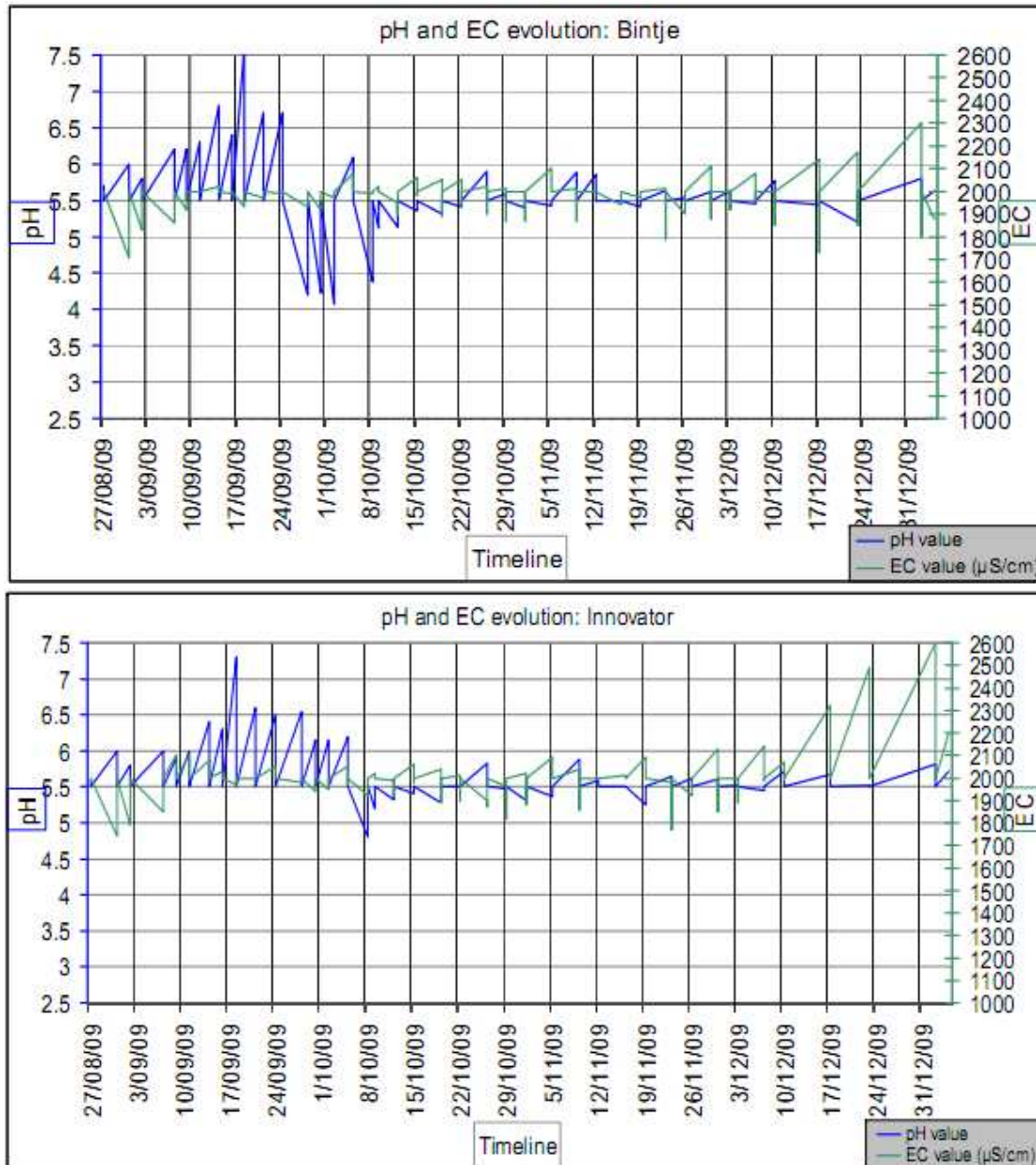


Fig. 65 UCL - pH/EC evolution

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5.2.6 Plant Water Usage

Water usage was similar among cultivars

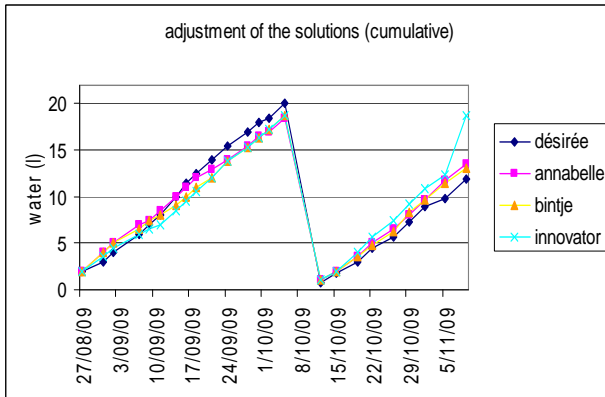
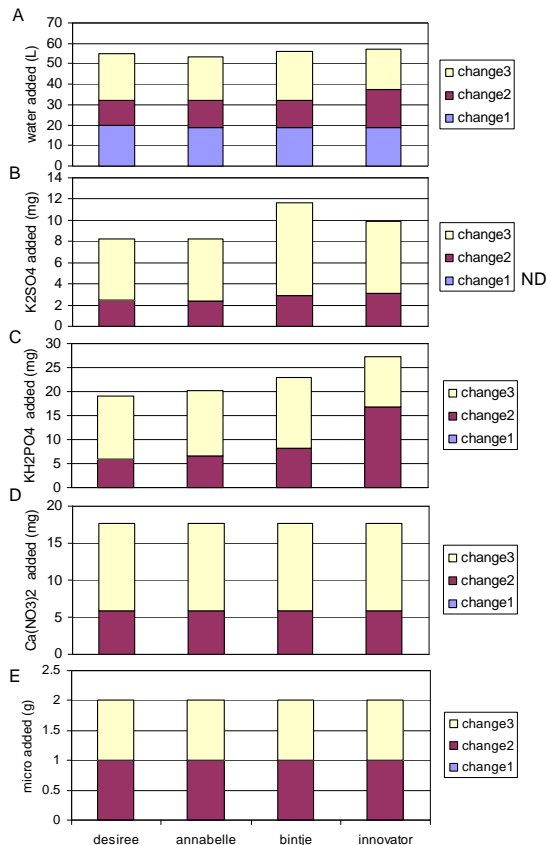


Fig. 66 UCL - cumulative water addition to the nutrient solution



Change 1 corresponds to the growth phase solution and changes 2 and 3 correspond to the changes of tuberisation solution. The amount of K₂SO₄ added during the growth phase was not determined (ND)

Fig. 67 UCL - Total amount of H₂O / K₂SO₄ / KH₂PO₄ / Ca(NO₃)₂ / microelement added

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Nitrate content was rapidly depleted. The microelement Zn accumulated.

5.2.9 Microbial count

The order of magnitude of the reported bacterial count is considered not significantly different among cultivars.

Stock solution already contained significant levels of bacteria.

Tab. 33 UCL - Microbial total count

bacteria						
date	water	stock solution	désirée	annabelle	bintje	innovator
8-oct			179000	135500	100000	114500
9-oct			140000	209000	405500	38600
12-nov		590000	810000	240000	1400000	1000000
4-janv	20		31000	230000	39000	1200000
yeast (CFU/ml)						
date	water	stock solution	désirée	annabelle	bintje	innovator
8-oct			<1	<1	40	65
9-oct			5300	435	26	400
12-nov		<1	<1	30	<1	<1
4-janv	<10		<10	190	<10	<10
mould (CFU/ml)						
date	water	stock solution	désirée	annabelle	bintje	innovator
8-oct			9000	500	46	100
9-oct			418	8	110	1000
12-nov	<10	81000	96000	450	18000	82000
4-janv			3200	190	1700	7100

5.3 Monitoring of plant development

The potato plants at UCL were grown for 134 days, starting with in-vitro plants of 28 days old. From mid-November on plants started to die because of growth problems.

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5.3.1 *Photographic follow-up*

Pictures 1/9/09



Pictures 30/9/09



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Fig. 69 UCL - Gully pictures

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5.3.2 Detailed observation

Plant die-off indicated phytosanitary problems, a *Colletotrichum coccodum* fungi was diagnosed microscopically. Plants were treated with 2 fungicides.

As described for UGent, also *Sciarioidea flies* infested the root environment in the gully.

Trips were observed , but successfully treated (Tracer, Dow chemical).

Concerning tuber appearance: Annabelle and Desiree tubers displayed more irregular forms than expected.

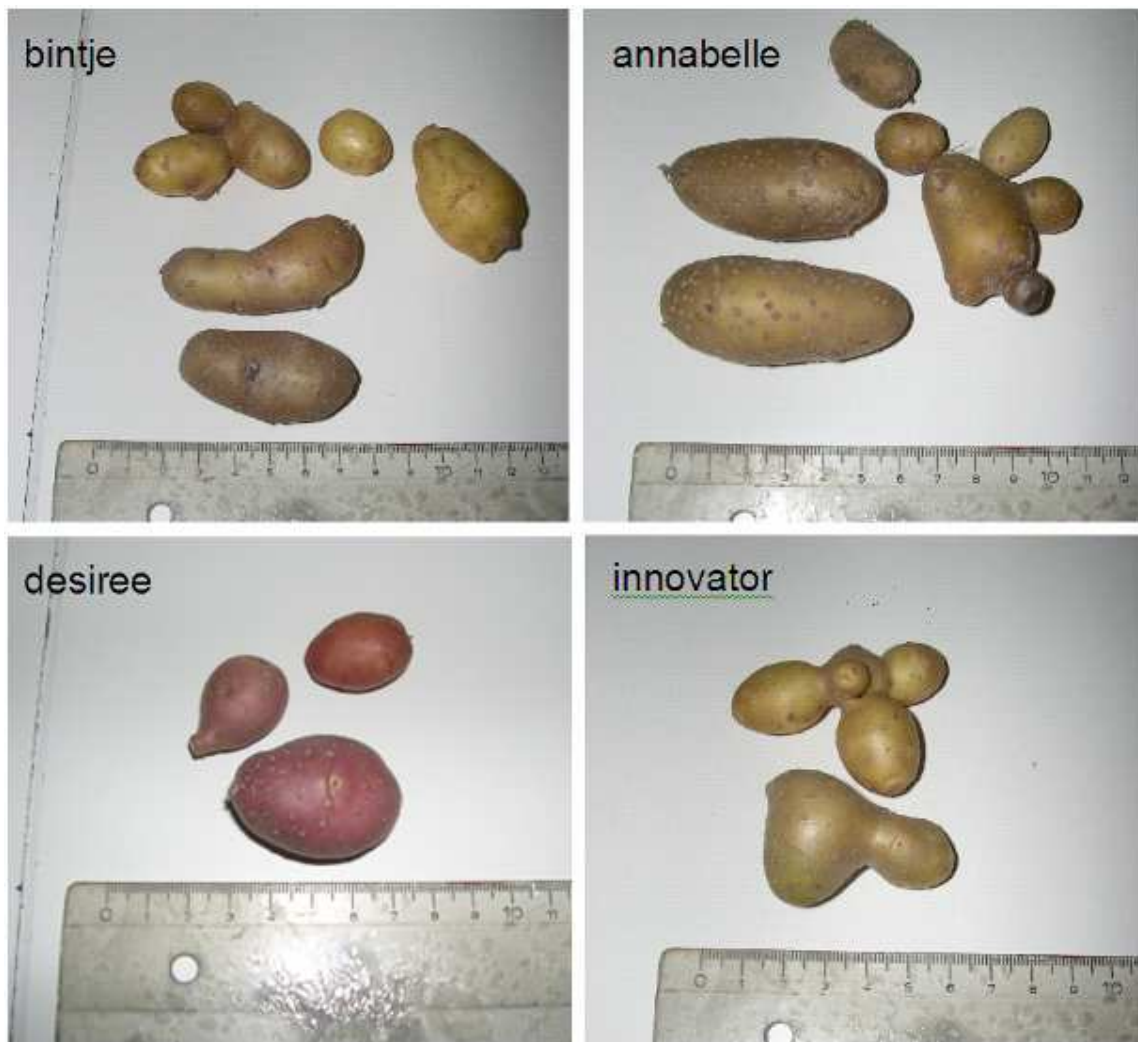


Fig. 70 UCL - Tuber detailed pictures

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5.3.3 Growth assessment

The experiment was initiated with 16 plants from each cultivar. Innovator was less robust and more sensitive to experimental handling – 4 plants were damaged by gas exchange measurements. Due to phytosanitary problems all cultivars except Desirée started to die mid-November.

Leaf surface was determined by a mobile leaf area meter.

Root growth was assessed by measuring the approximate area covered by the roots. Innovator had the lowest amount of roots developing, which were also thin and prone to damage.

Detailed developmental measurements are shown in the figure below (Fig. 72). Innovator is a slower developing cultivar, as also mentioned by HZPC. Annabelle has the fastest tuberisation induction.

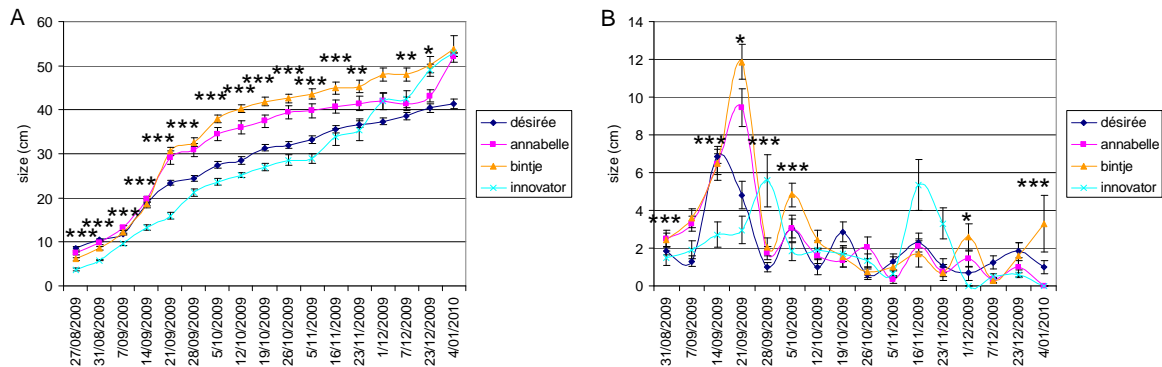


Fig. 71 UCL - Plant size evolution

Plant size evolution (A) and weekly size increase (B) for each variety. Vertical bars are standard errors. Differences between varieties are statistically significant (*), highly significant (**) or very highly significant (***) at the 5% level (ANOVA).

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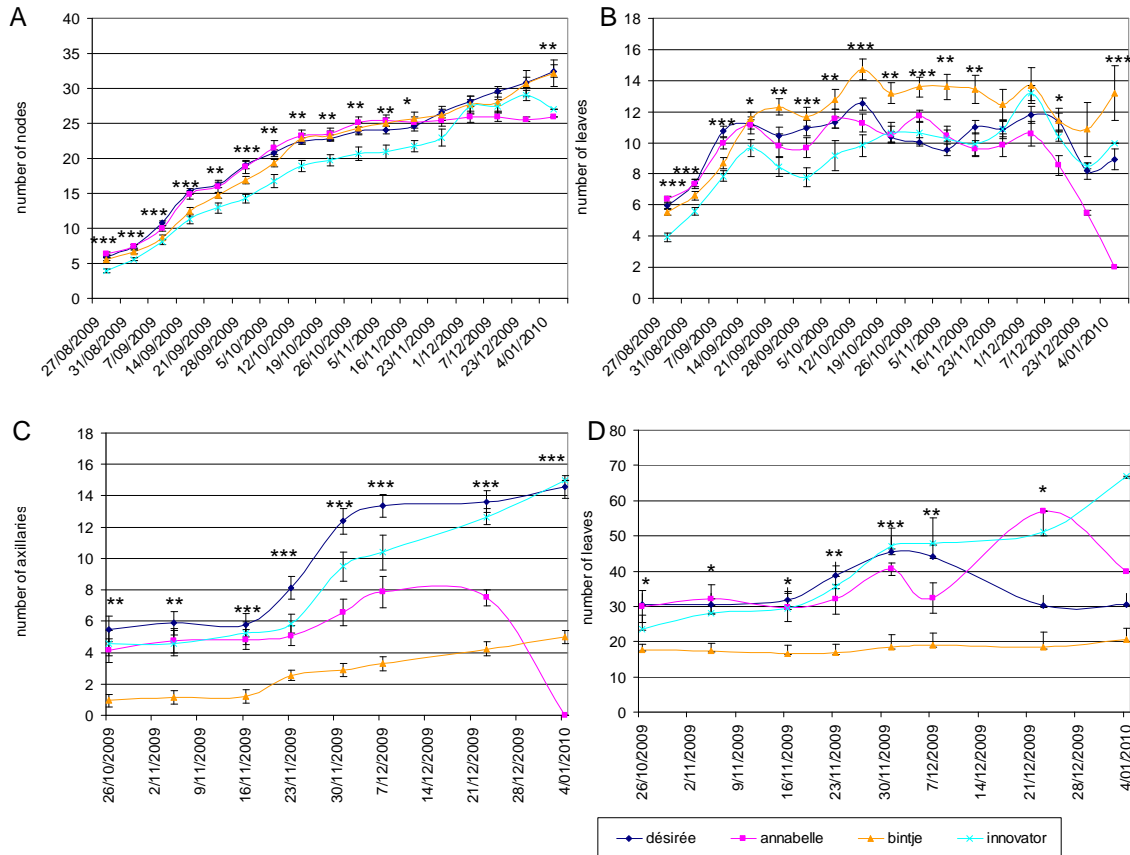


Fig. 72 UCL - Development of the plant aerial part

(A) number of nodes on the main stem, (B) number of green leaves on the main stem, (C) number of axillary branches, (D) total number of green leaves (main stem + axillary branches). Vertical bars are standard errors. Differences between varieties are statistically significant (*), highly significant (**) or very highly significant (***) at the 5% level (ANOVA).

In Fig. 73, stolon initiation is indicated as number of days after transfer of the in vitro plants to the gullies. Tuber initiation is counted from the same timepoint.

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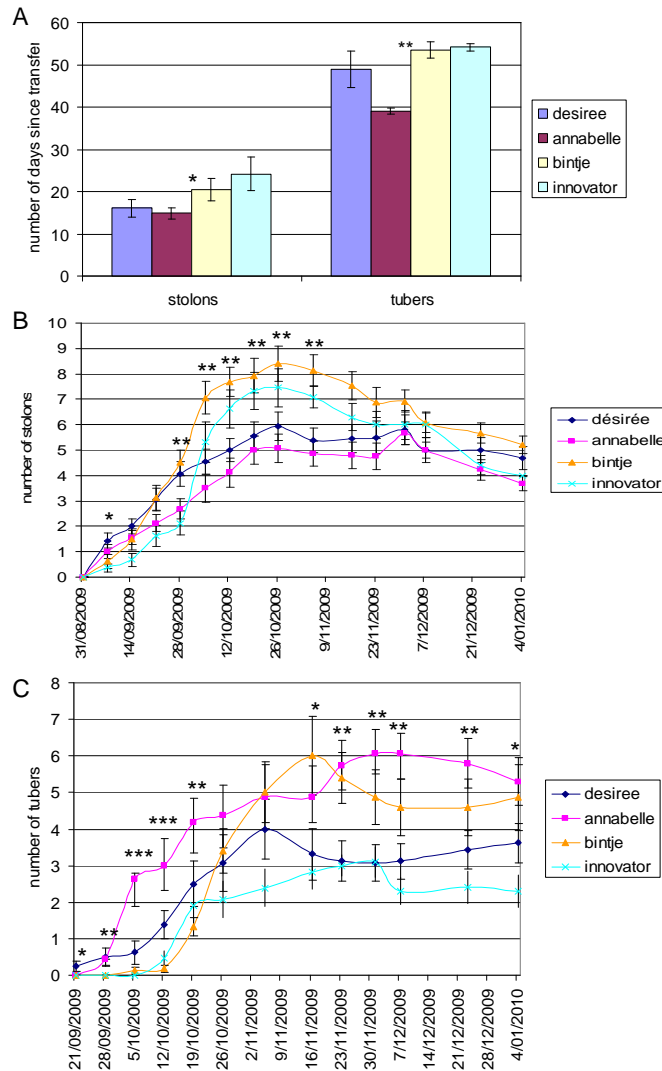


Fig. 73 UCL - Development of stolons and tubers

(A) time of apparition of the first stolon and tuber per plant, (B) number of stolons per plant, and (C) number of tubers per plant. Vertical bars are standard errors. Differences between varieties are statistically significant (*), highly significant (**) or very highly significant (***) at the 5% level (ANOVA).

5.3.4 Gas exchange data

No significant differences were seen in momentaneous CO₂ assimilation. No correlation was apparent between stomatal conductance determined by gas exchange equipment (parameter E: evaporation, Fig. 74, upper panel B) and by porometry (conductance, Fig. 74, lower panels). The first timepoint (youngest plants) showed a very high transpiration

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(large relative difference in the case of conductance measurements, compare the 2 panels) relative to the other datapoints.

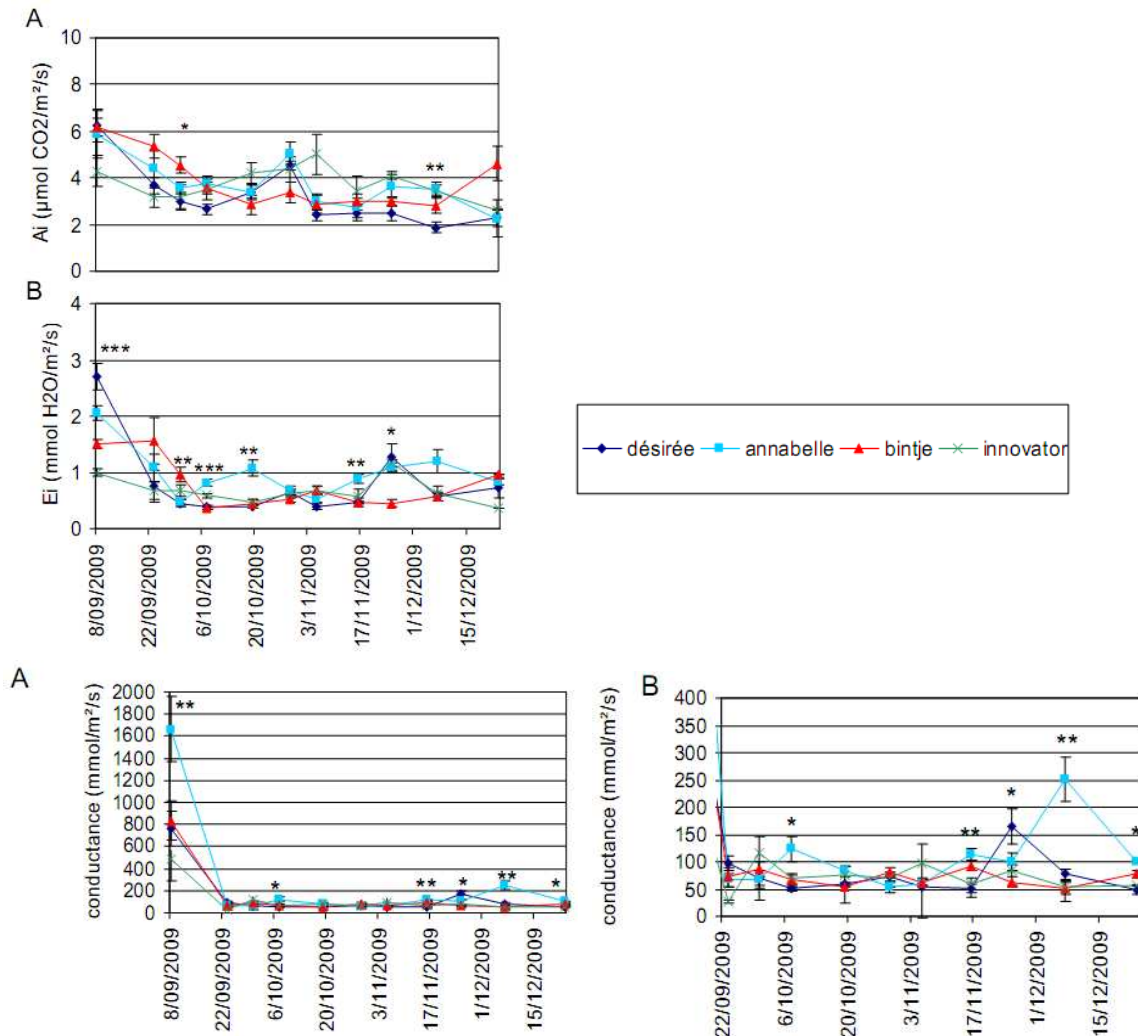


Fig. 74 UCL - Gas exchange

Instantaneous CO₂ assimilation (A) and instantaneous transpiration (E) were determined on the 5th youngest leaf (young fully expanded leaves being most photosynthetic active). Also stomatal conductance was obtained from measurements on the 5th youngest leaf. Vertical bars are standard errors. Differences between varieties are statistically significant (*), highly significant (**) or very highly significant (***) at the 5% level (ANOVA).

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5.3.5 Extra plant physiological measurements

Chlorophyll fluorescence did not reveal significant differences. Chlorophyll content was higher in Bintje.

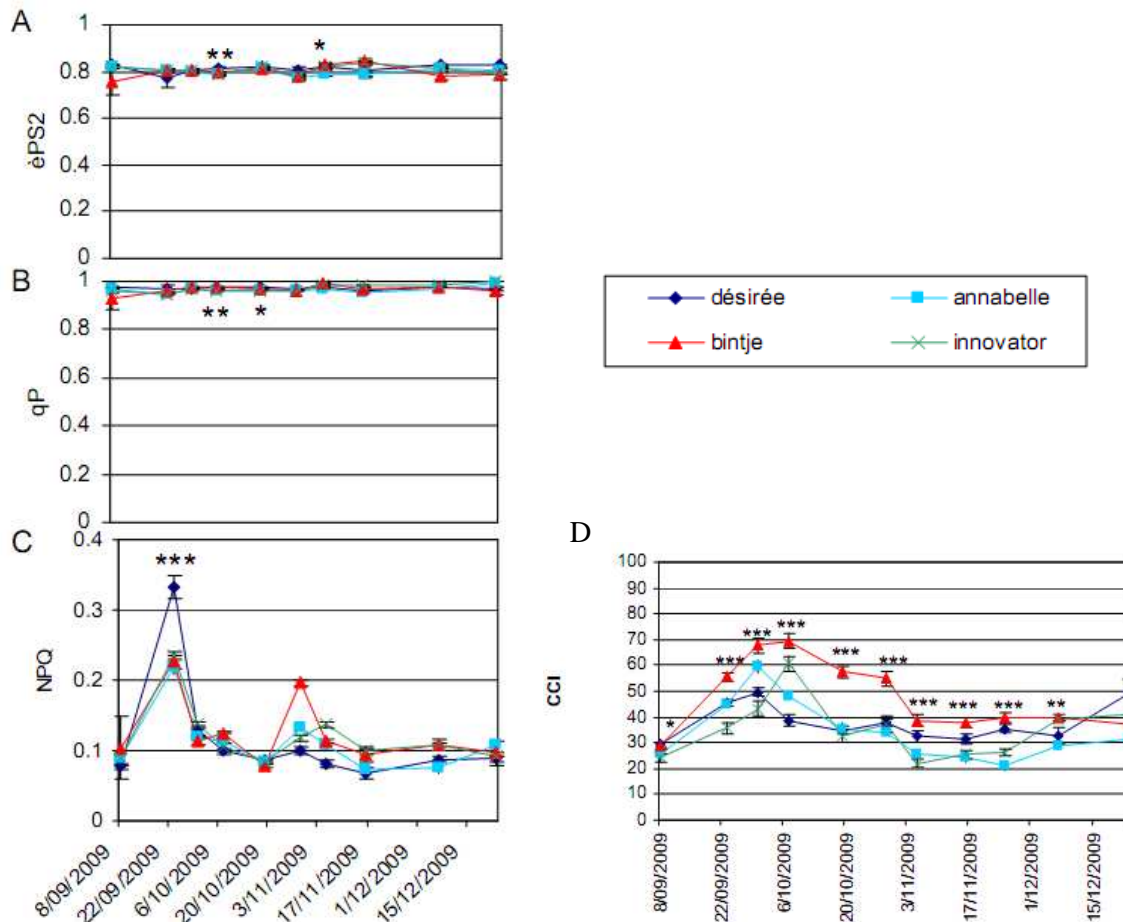


Fig. 75 UCL - Chlorophyll measurements

Kinetics of chlorophyll fluorescence of the 5th youngest leaf (young leaf photosynthetic active). (A) photosystem II quantum efficiency, (B) photochemical quenching, (C) non photochemical quenching. Vertical bars are standard errors.

Chlorophyll concentration SPAD of the 5th youngest leaf (young leaf photosynthetic active). Vertical bars are standard errors.

Differences between varieties are statistically significant (), highly significant (**) or very highly significant (***) at the 5% level (ANOVA).*

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5.4 Harvest results

See section 4.5 for an overview of the potato edible harvests (UCL, Gent and its consultant HZPC) obtained at the end of bench test 1.

The harvest was low, but tuber size distribution is also an important parameter to be considered (see Fig. 76).

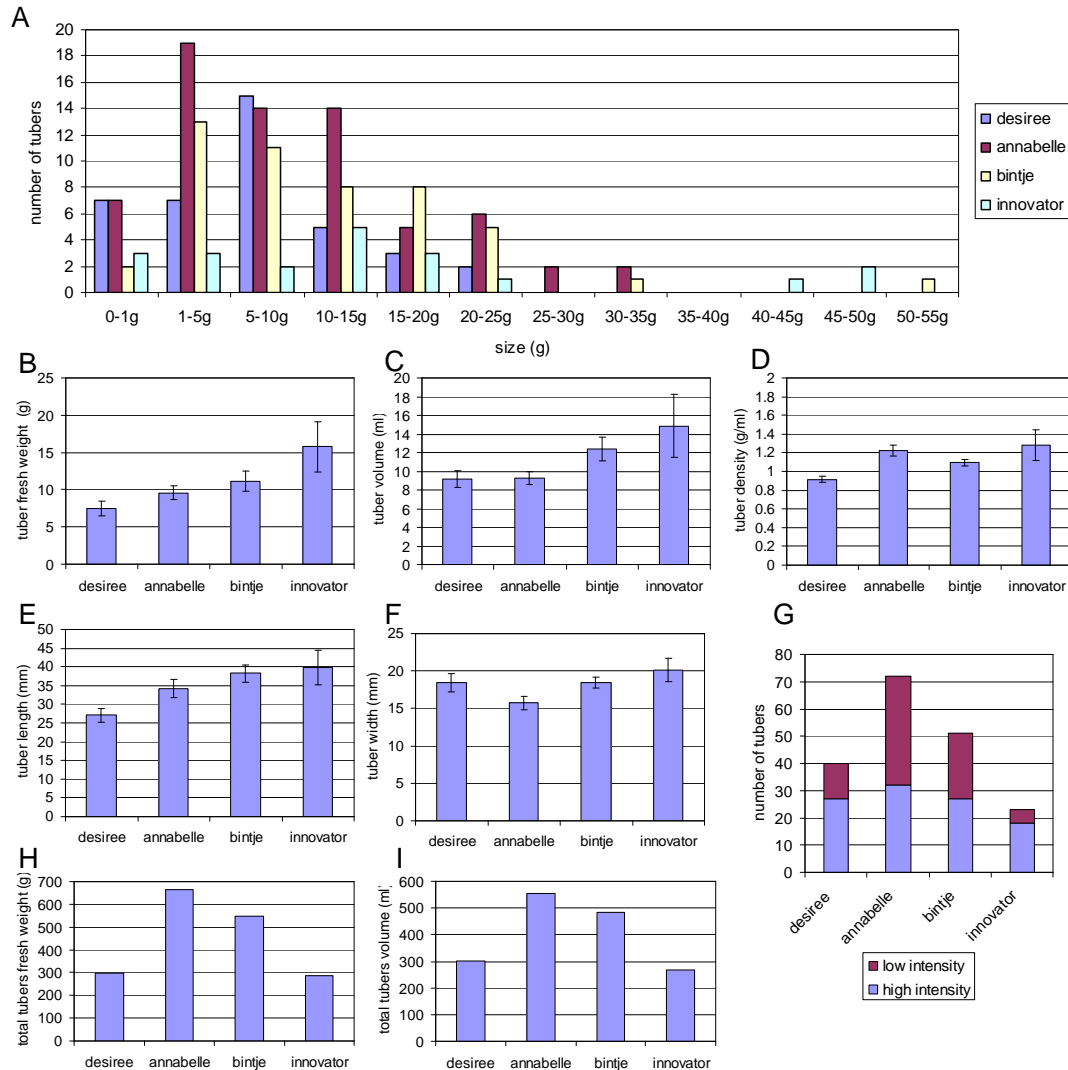


Fig. 76 UCL - Potato tuber size distribution

Number, weight and size of the harvested tubers. (A) number of tubers per variety according to grade. Average tuber (B) fresh weight, (C) volume, (D) density, (E) length and (F) width for each variety. (G) Repartition of the number of tubers harvested according to light intensity; low light intensity: 150-200 $\mu\text{mol}/\text{m}^2\text{s}$, high light intensity: 200-250 $\mu\text{mol}/\text{m}^2\text{s}$. Total harvested tuber (H) fresh weight and (I)

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volume per variety. Vertical bars are standard errors. Differences between varieties are statistically significant (*), highly significant (**) or very highly significant (***) at the 5% level (ANOVA).

The cultivar Annabelle had the highest ratio edible over inedible DW.

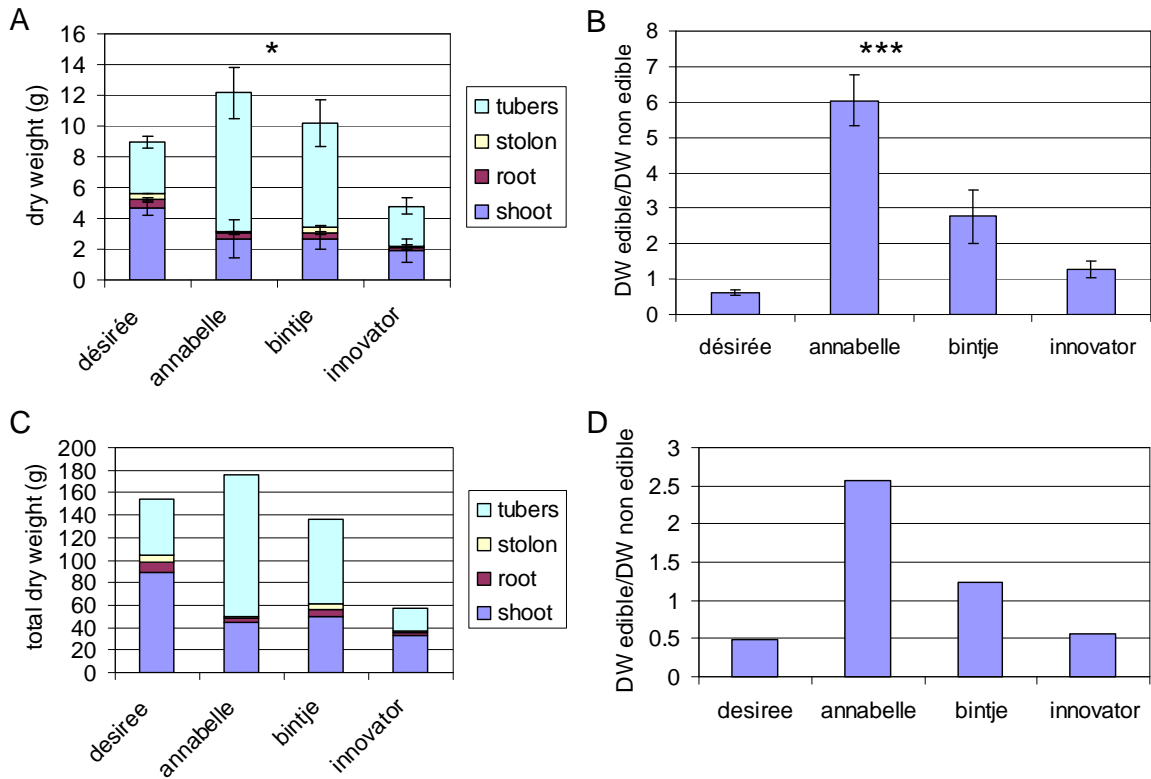


Fig. 77 UCL - ratio edible to inedible DW of potato cultivars

Biomass produced by the plants. Dry weight produced per cultivar according to the organs (A) per plant, (C) for all the plants. Ratio between total edible dry weight (tubers) and total non edible dry weight (aerial part + stolons + roots) (B) per plant and (D) for all the plants. Vertical bars are standard errors. Differences between varieties are statistically significant (*), highly significant (**) or very highly significant (***) at the 5% level (ANOVA).

A preliminary elemental analysis was carried out for the different plant parts.

Na proved not to be present at elevated levels in the tubers.

The micronutrient Zn accumulated also in the shoot part, as a consequence of the high levels in the nutrient solution (see 5.2.8).

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6 Soy Bean (UNapoli)

6.1 Experimental Layout

6.1.1 Measuring Plan

Plant development

Weekly assessment for max 3 plants per gully

- plant height
- number of lateral shoots
- number of leaves, leaf area estimation

Plant physiological parameters

Bi-weekly assessment

- Leaf gas exchanges: net photosynthesis and transpiration rate (WALZ HCM 1000)
- stomatal conductance: (Leaf Porometer AP4, Delta T Devices, Cambridge)
- Chlorophyll content (Chlorophyll Meter Konica-Minolta SPAD 502)

Destructive

- Fresh weight, dry weight, % of DM and DM partitioning are measured for the different organs.
- Plant leaf area: leaf area meter (LI-COR 3000, LI-COR, Lincoln, NE, USA)
- Leaf water potential, with a psychrometer using the dew point method (PotentiaMeter WP4 Decagon Device) needing a 12 cm² leaf sample

Nutrient solution

- EC and pH controlled manually and adjusted daily
- crop water usage
- Water depletion is measured daily and the volume of the solution is kept constant.
- main macronutrients (NO₃⁻, PO₄³⁻, K⁺) is measured weekly by spectrophotometry
- NO₃⁻ weekly using a portable reflectometer Nitracheck kit / reactive strips (Merckoquant)
- detailed analyses (NO₃⁻, PO₄³⁻, K⁺, Cl⁻, Ca²⁺, Mg²⁺, SO₄²⁻, B₃⁺) are performed at the start (fresh solution), at the end of vegetative phase (approximately after 7 weeks) and at the end (harvest) of the growing cycle

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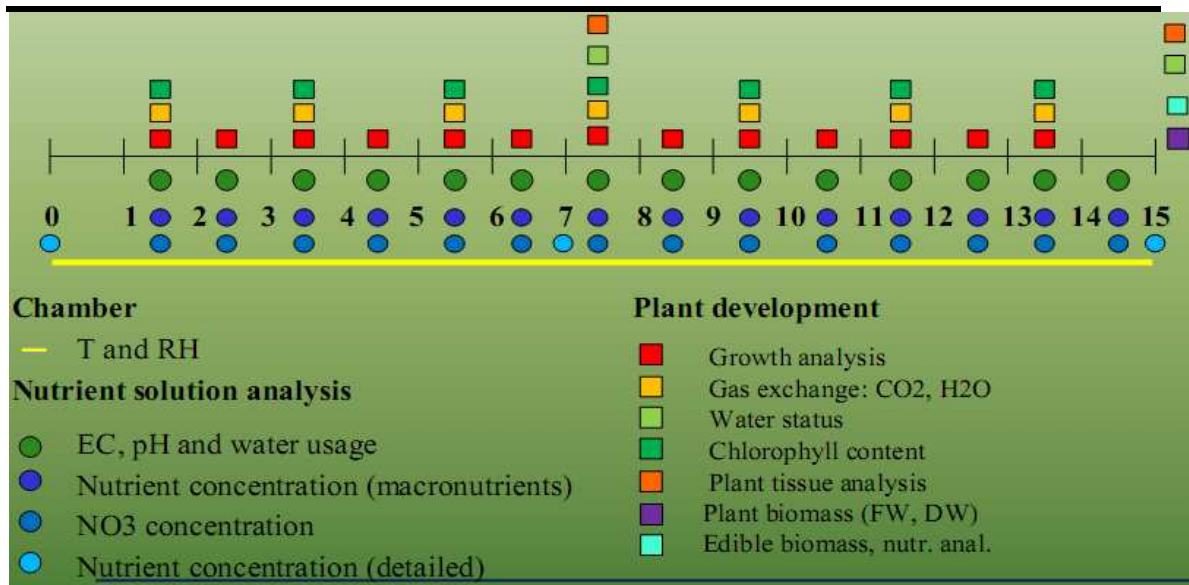


Fig. 78 UNapoli - Measuring schedule

6.1.2 Setup

The 4 cultivars as obtained from a listing following preliminary ranking in TN98.3.1 were 'PR91M10', 'Clara', 'Regir', 'Atlantic'

As the Clara cultivar had an unexpectedly low germination performance, only 3 cultivars were setup (see Fig. 79).

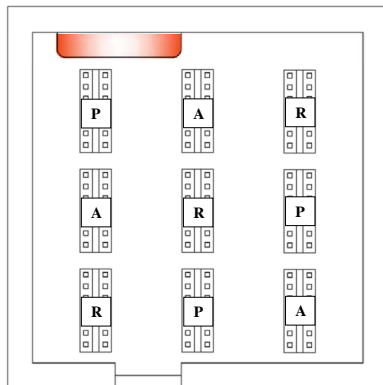


Fig. 79 UNapoli - Setup

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6.2 Growth environment follow-up

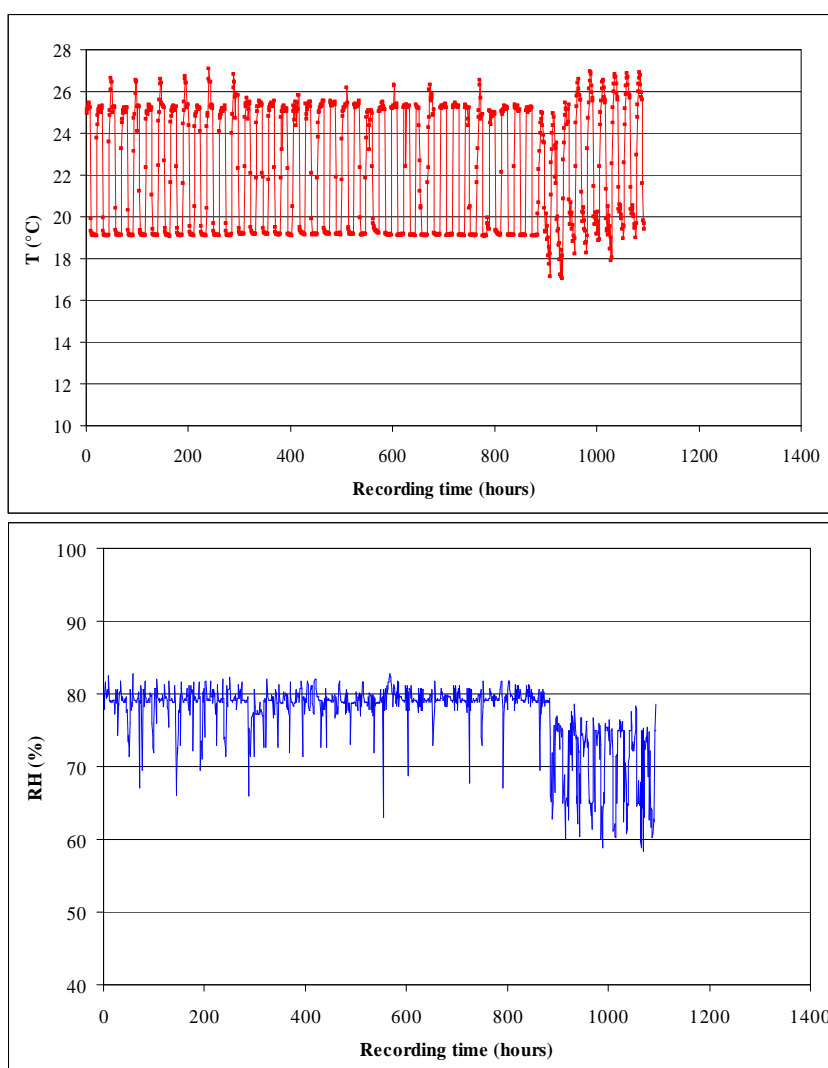
6.2.1 Settings

Tab. 34 UNapoli - Settings

Photoperiod	12-h Long Day
Light intensity	600 $\mu\text{mol m}^{-2} \text{s}^{-1}$
Room temperature	20/26 °C (Night/Day)
Humidity	65-75 (setpoint 70)

The T and humidity measurements resolve around the setpoints (see Fig. 80)

6.2.2 Chamber T/RH evolution



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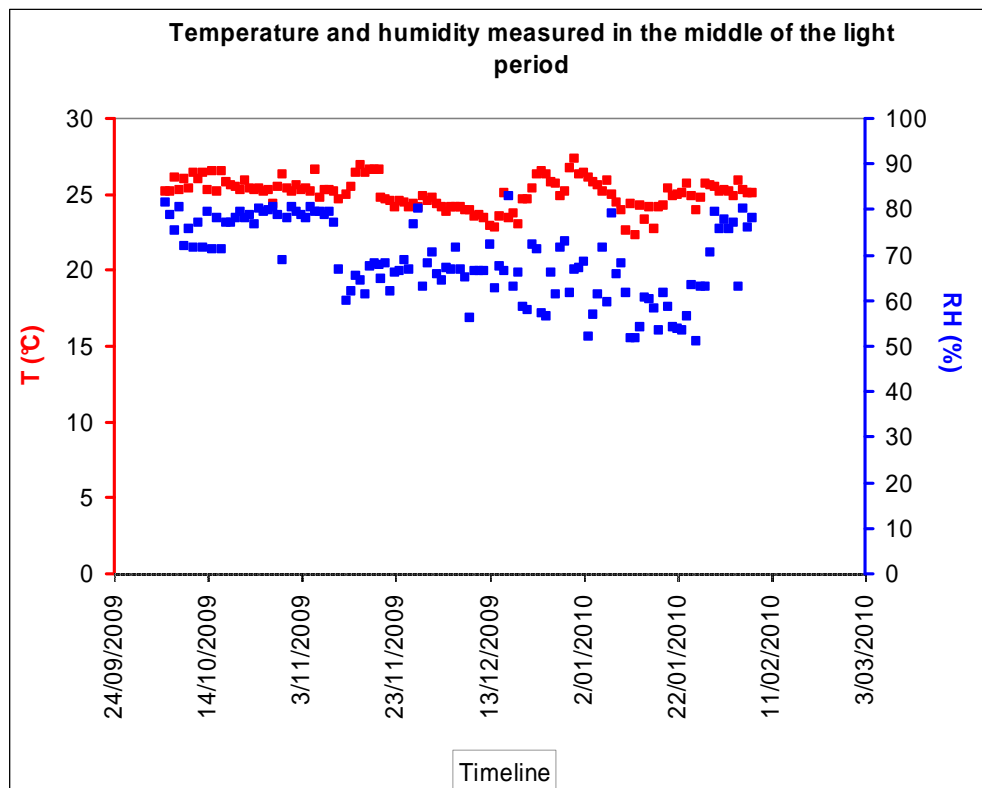


Fig. 80 UNapoli - Chamber T/RH

6.2.3 Chamber CO₂ level

Due to problems with plant growth during BT1, the number of physiological measurements was reduced, as we had to focus on understanding the reason of these problems. After additional chemical analyses and observations with pathology specialists, we discovered that they were determined by nutrient deficiency (probably Mn) due to pH fluctuations, even though possible subsequent infections occurred.

Immediately after performing the initial gas exchange measurements, plants started to show deficiency symptoms and necrosis, implying unreliable gas exchange measurements.

6.2.4 Nutrient Solution Environment

Gully inclination: 1%.
Nutrient solution flow rate: 2,4 l/min.

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6.2.5 pH and EC evolution

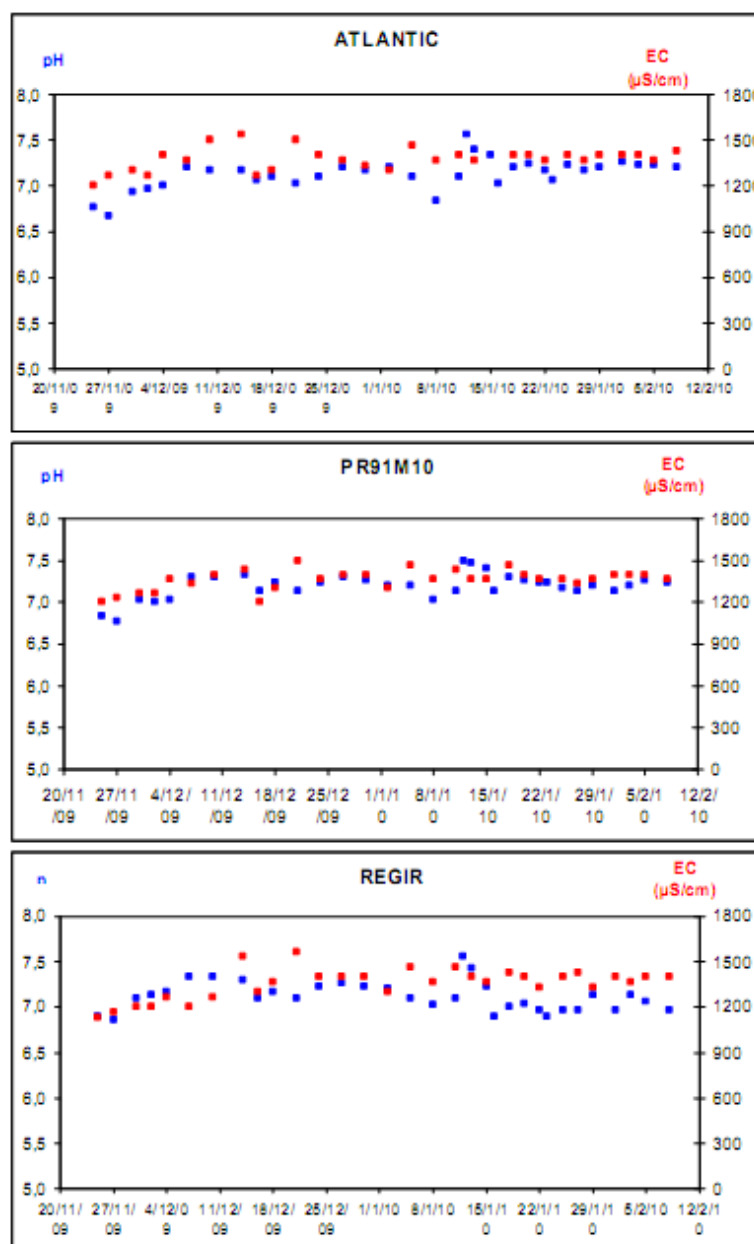


Fig. 81 UNapoli - pH/EC evolution

The datapoints indicate the values before adjustment to the setpoints pH 5.8 and EC 1200.

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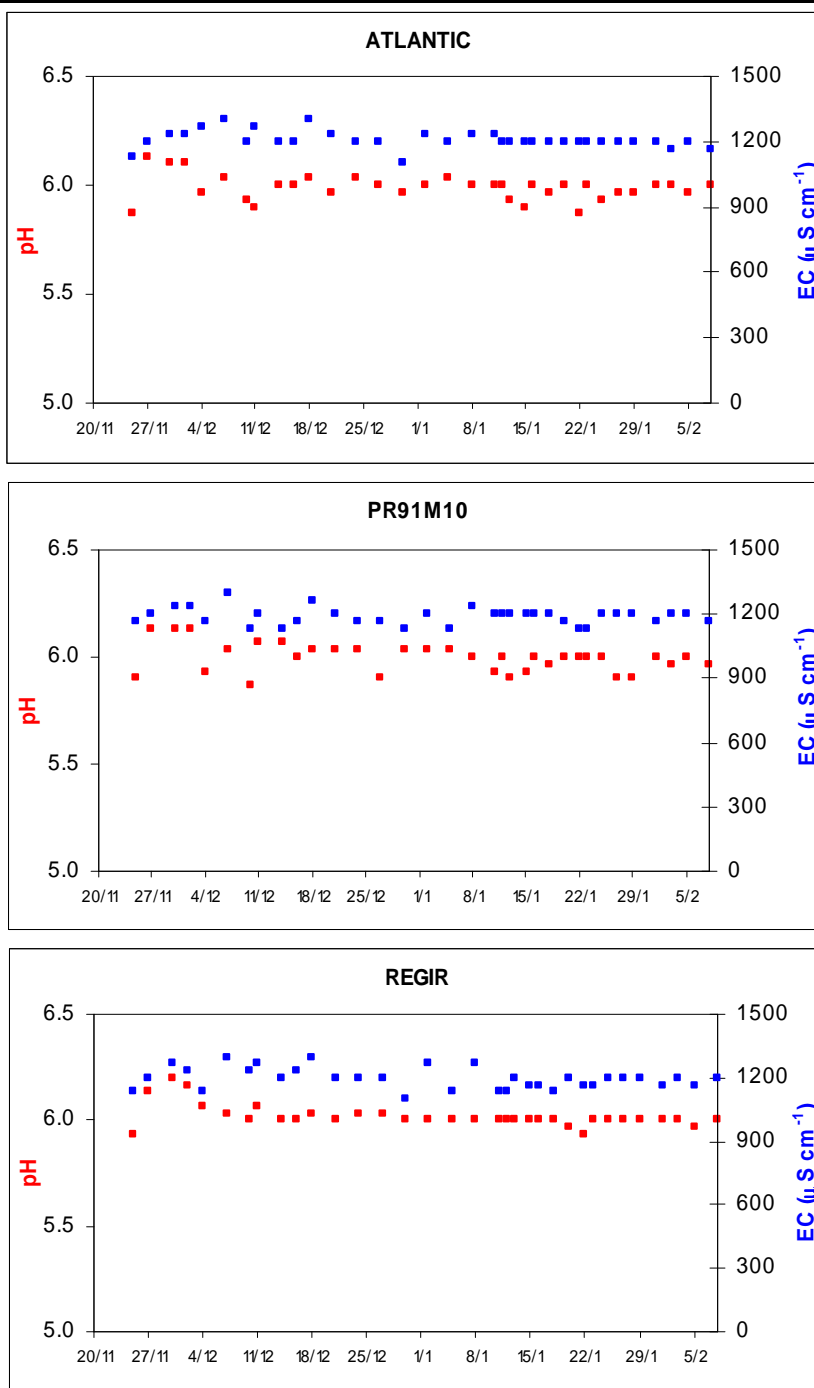


Fig. 82 Time course of pH and EC after the adjustment to the setpoints pH 5.8 and EC 1200

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6.2.6 Plant Water Usage

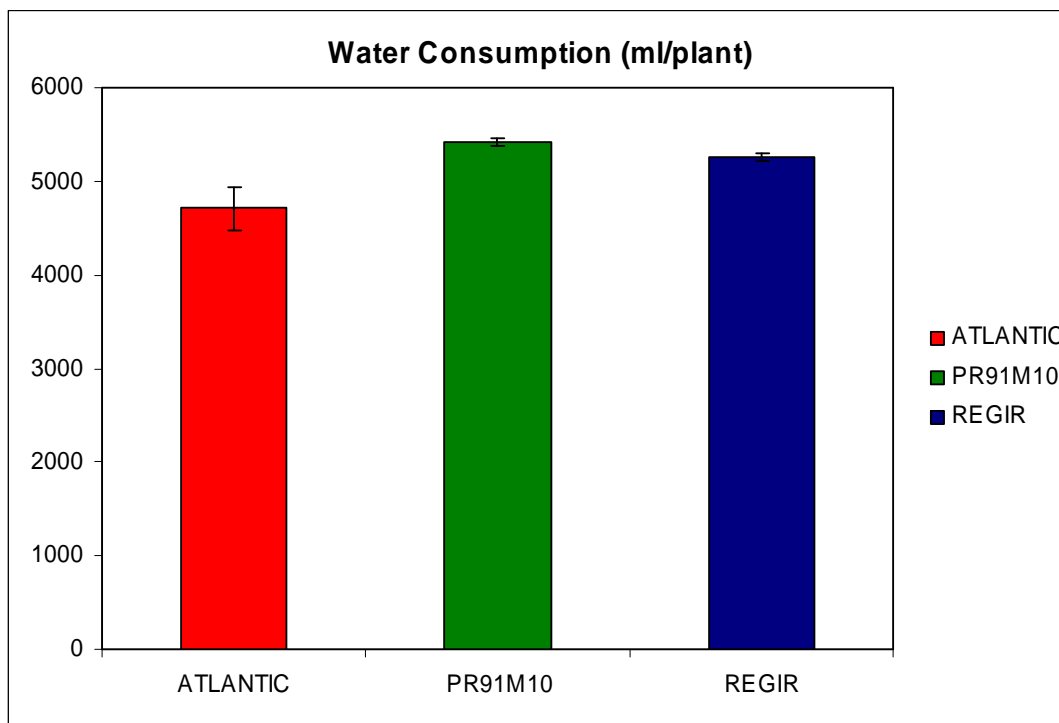


Fig. 83 UNapoli - Water consumption (6th week from sowing)

6.2.7 Nutrient solution T

18 (day) and 22 (night).

6.2.8 Nutrient solution analysis

Data took into account the corrections on nutrient solution: the samples of nutrient solution for the analyses were taken weekly in the reservoir just after the check of water volume, pH and EC and their correction.

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Fig. 84 UNapoli - NO₃ evolution in the nutrient solution

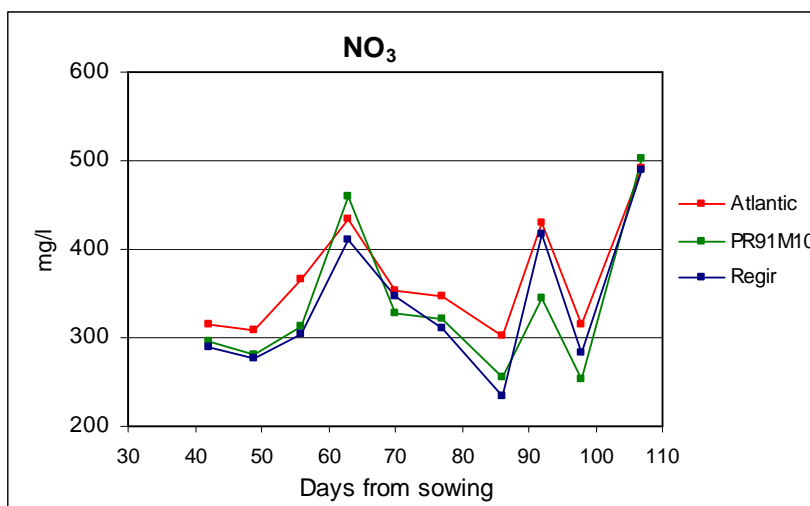
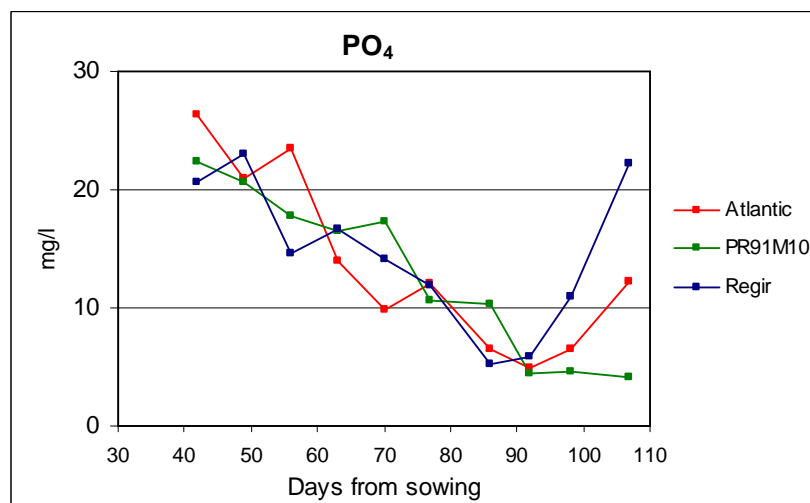
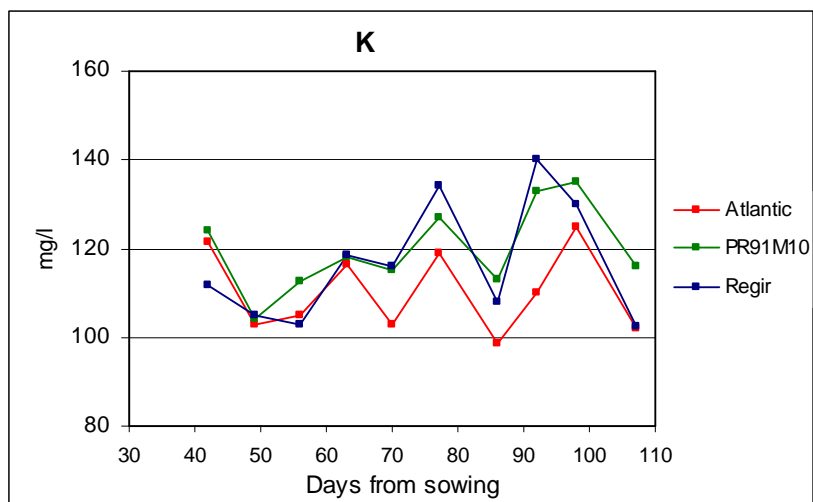


Fig. 85 UNapoli - PO₄ evolution in the nutrient solution



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Fig. 86 UNapoli - K evolution in the nutrient solution



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6.3 Monitoring of plant development

Soybean plants were grown for 127 days, developmental problems appeared around 90 days.

6.3.1 *Photographic follow-up*

October, 13
(8 days after sowing)



November, 5
(31 days after sowing)



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November, 18
(44 days after sowing)



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December, 16
(72 days after sowing)



January, 12
(99 days after sowing)



TN 98.4.21 UGent	Preliminary trade-off of crop cultivars: Test performances (Bench test 1)
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February, 8
(126 days after sowing)



Fig. 87 UNapoli - Photos growth evolution

6.3.2 Detailed observation

As evident from the January 12 pictures from Fig. 87, a combination of phytosanitary and possibly linked nutrient deficiency symptoms was observed.

6.3.3 Growth assessment

The height of 6 plants per cultivar (on a total of 42) was measured, as well as the number of leaves per plant and the number of sprouts (indicative of branching) (Fig. 88). Leaf area was estimated based on a published method (Wiersma and Bailey 1975; Lieth et al., 1986).

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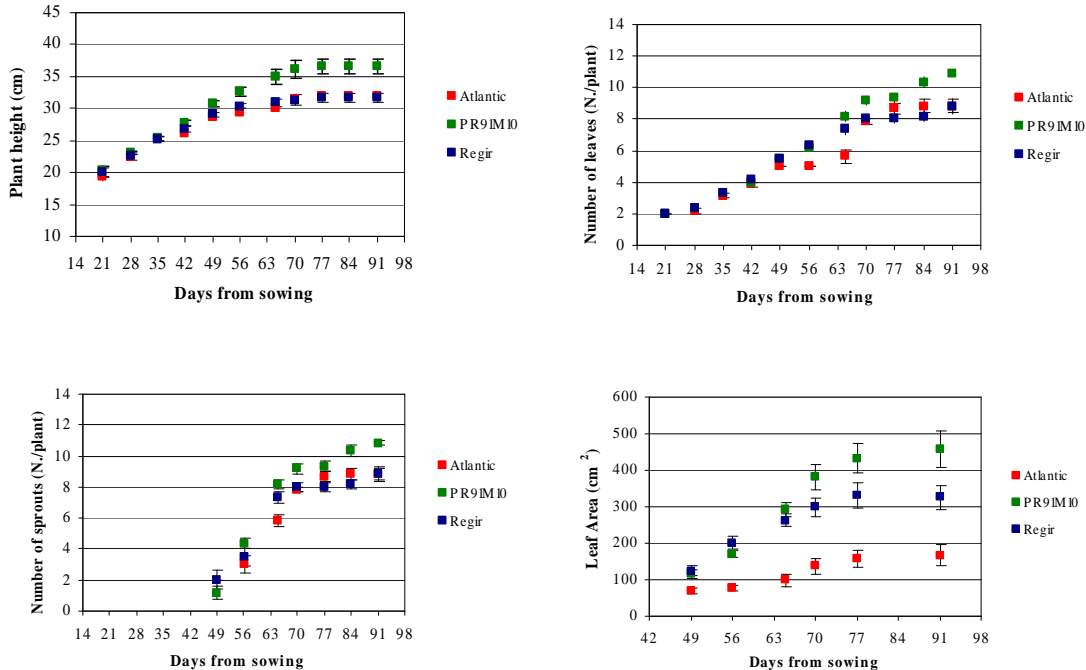
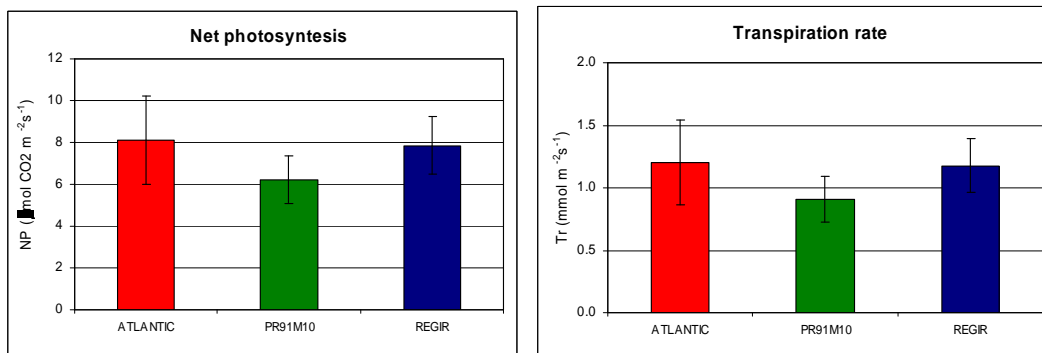


Fig. 88 UNapoli - Growth assessment

6.3.4 Gas exchange data

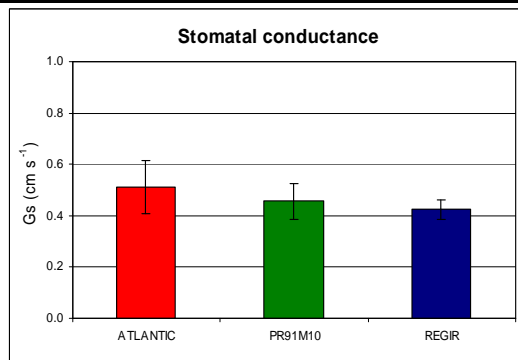
Fig. 89 UNapoli - Gas exchange



The unit of transpiration rate is $\text{mmol/m}^2/\text{s}$ and is referred to as m^2 of leaf. For NP the unit is $\mu\text{mol/m}^2/\text{s}$ and is referred to the leaf surface too.

The graphs present the average values of single measurements performed on week 10 from sowing (2 leaves per plant; 3 plants per cultivar).

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Due to problems with plant growth during BT1, the number of physiological measurements was reduced, as we had to focus on understanding the reason of these problems. After additional chemical analyses and observations with pathology specialists, we discovered that they were determined by nutrient deficiency (probably Mn) due to pH fluctuations, even though possible subsequent infections occurred.

Immediately after performing the initial gas exchange measurements, plants started to show deficiency symptoms and necrosis, implying unreliable gas exchange measurements. Therefore any analysis or conclusion on the correlation between stomatal conductance and transpiration rate and photosynthesis would be unreliable as well. We will do our best to provide this for BT2.

6.3.5 Extra plant physiological measurements

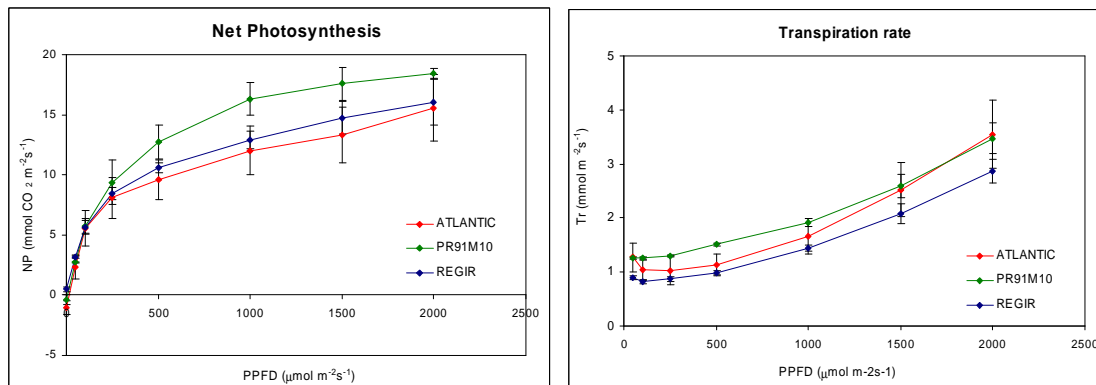


Fig. 90 UNapoli - Light response curves

The figure reports the light saturation curves of net photosynthesis and transpiration, determined at increasing levels of light intensity (PPFD 0, 50, 100, 250, 1000, 1500 and 2000 μmol m⁻² s⁻¹). Measurements were carried out during the vegetative phase (10th week of growing cycle). CO₂ concentration during the measurements was 487.5 ppm on average.

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6.4 Harvest results

Tab. 35 UNapoli – Nutritional and compositional analysis of the 3 soybean cultivars

		Dry matter (%)	Protein content (%/d.m.)	Fat content (%/d.m.)	Fiber content (%/d.m.)	Ash (%/d.m.)	Carbo-hydrates content (%/d.m.)	Phytic acid content (%/d.m.)	Total isoflavones content (g/100 d.m.) g
Soybean issued of BT1	Number of samples analyzed by cv	3	2	2	2	2	2	4	4
	<i>Atlantic</i>	80.92	36.83	16.83	27.68	0.38	18.28	1.57	207.46
	<i>PR01M10</i>	84.85	41.97	12.76	29.63	0.29	15.34	1.37	82.72
	<i>Regir</i>	81.82							115.34
Soybean from Market	Number of samples analyzed by cv	3	2	2	2	2	2	4	4
	<i>Cresir</i>	92.087	35.950	19.284	19.261	0.70	24.80	1.147	270.846
	<i>Atlantic</i>	89.808	32.479	16.498	21.716	0.68	28.63	1.404	121.644
	<i>PR91M10</i>	93.071	35.272	16.695	22.096	1.12	24.82	1.212	103.870
	<i>Regir</i>	93.474	32.519	16.985	23.680	0.57	26.25	0.894	186.077

Fig. 91 shows dry matter values in seeds samples: there are not significant differences.

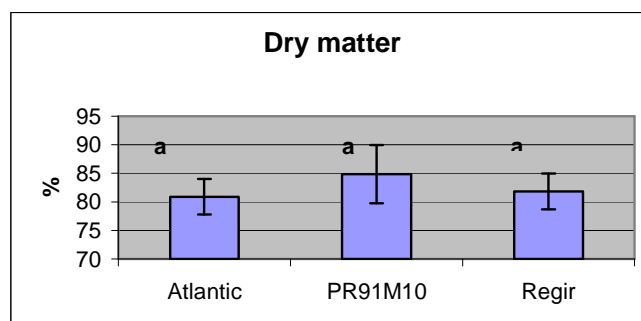


Fig. 91 UNapoli - Dry matter content (%±s.d.) in soybeans samples (confidence level=95%)

Significant differences are reported in term of total isoflavones content: Atlantic, with 207 mg/100 g dry matter in mean, is the best cultivar while PR01M10, with 83 mg/100 g dry matter in mean, is the worst cultivar (Fig. 92).

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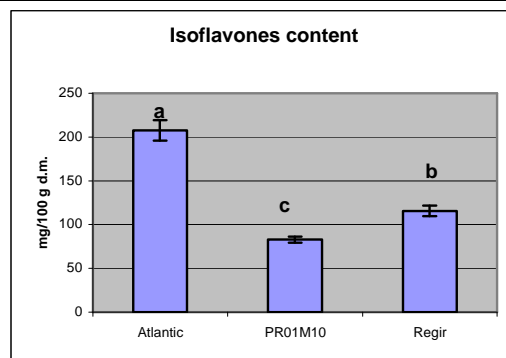


Fig. 92 UNapoli - Total isoflavones content (mg/100g dry weight \pm s.d) in soybeans samples (confidence level=95%)

Analysis on protein, fat, fiber and phytic acid were carried out only on two cultivar samples: Atlantic and PR01M10

There are not significant differences in term of fat content and phytic acid content (Fig. 93 and Fig. 94).

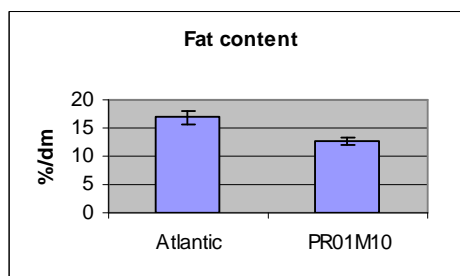


Fig. 93 UNapoli - Fat content (dry weight \pm s.d) in soybeans samples (p= 0,053)

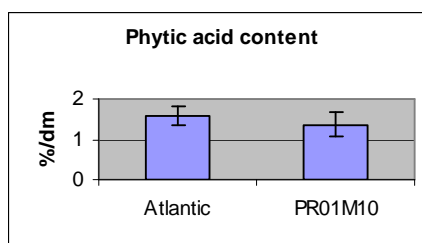


Fig. 94 UNapoli - Phytic acid content (dry weight \pm s.d) in soybeans samples (p= 0,403)

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Atlantic sample shows a lower protein content (36,8%/d.m in mean vs 41,9748 %/d.m in mean) and a lower fiber content (27,7 %/d.m in mean vs 29,6 %/d.m.) respect to PRO1M10.

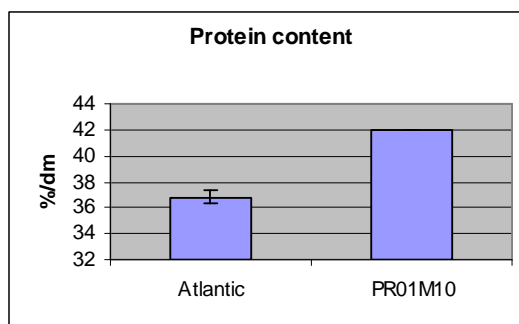


Fig. 95 UNapoli - Protein content (dry weight %±s.d) in soybeans samples (p= 0,004832)

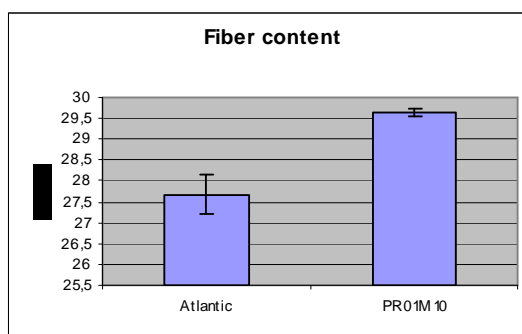


Fig. 96 UNapoli - Fiber content (dry weight %±s.d) in soybeans samples (p= 0,028303)

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6.5 References

Wiersma JV, Bailey TB (1975), Estimation of leaflet, trifoliolate, and total leaf areas of soybeans, *Agronomy Journal* 67, p26-30

Lieth JH, Reynolds JF, Rogers HH (1986), Estimation of leaf-area of soybeans grown under elevated carbon-dioxide levels, *Field crops research* 13, p193-203

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7 Summary

Bread wheat

Plants were developing normally and ears were harvested. A difference in maturation time was apparent between the chosen cultivars, with cultivar Fiorina posing problems to mature in the foreseen time.

Durum wheat

2 cultivars were successfully grown with yields higher than recorded for field agriculture. Ethylene and oxygen build up in the sealed chambers possibly led to suboptimal growing conditions. Root development was higher than expected.

Nutrient analysis was carried out at Guelph. The remaining harvest was shipped to UNapoli for further analysis and initial processing test.

Potato

Plants at the 2 locations finally revealed similar phyto-sanitary problems, and growth stopped prematurely. Tuber yield was consequently low.

A start-up with plants transported and acclimated under suboptimal conditions is assumed to be at the basis of this unexpected development. N-levels in the nutrient solution were different between UGent and UCL, yet harvest was comparably low.

Samples from both harvests are available for nutritional analysis at IPL, as well as tubers from the UGent consultant HZPC.

For initiating of the processing tests, samples from HZPC, both hydroponic culture harvest, and field-derived tuber samples with same storage history for all cultivars are available.

Soybean

Phyto-sanitary problems occurred at the time of pod development. Plants also seemed to suffer from a suboptimal nutrient solution environment that is assumed to have slowed development. Harvest was limited.

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