M ELiSSA

Technical Note

Memorandum of Understanding 19071/05/NL/CP

MELiSSA Food Characterization: phase 1

Technical note: 98.4.32

Preliminary trade-off of crop cultivars: Test results evaluation and selection methods consolidation

Bench Test 2

prepared by/*préparé par*	Benjamin Secco, Katrien Molders
reference/*réference*	Contract number 22070/08/NL/JC
issue/*édition*	1
revision/*révision*	0
date of issue/*date d'édition*	8.10.2010
status/*état*	Final
Document type/*type de document*	Technical Note
Distribution/*distribution*	

CONFIDENTIAL DOCUMENT

A P P R O V A L

Title *titre*	Preliminary trade-off of crop cultivars: Test result evaluation	issue 1 *issue*	revision 0 *revision*

author *auteur*	FC1 Consortium - PPWG	date *date*	30/09/10
UBern	Valerie Page, Urs Feller		
UCL	Muriel Quinet, Stanley Lutts		
UGent	Laury Chaerle, Benjamin Secco, Martin Weihreter, Jan Decat, Dominique Van Der Straeten		
UoGuelph	Michael Stasiak, Mike Dixon		
UNapoli	Roberta Paradiso, Stefania De Pascale		

Reviewed by (UGent)	D. Van Der Straeten	date *date*	04/10/10
approved by (UGent) *approuvé by*	D. Van Der Straeten		08/10/10

C H A N G E L O G

reason for change */raison du changement*	issue/*issue*	revision/*revision*	date/*date*

C H A N G E R E C O R D

Issue: 1 Revision: 0

reason for change/*raison du changement*	page(s)/*page(s)*	paragraph(s)/*paragraph(s)*

TABLE OF CONTENTS

Table of Figures

List of Tables

List of Abbreviations

ALS	Alternative Life Support
BT1/2	Bench Test ½
DW	Dry Weight
EC	Electro conductivity
FC	Food Characterisation
FW	Fresh Weight
GC	Gas Chromatography
NCER	Net Carbon Exchange Rate
NFT	Nutrient Film Technique
IPL	Institut Paul Louvain
PCR	Polyclonal Chain Reaction
RH	Relative Humidity
T	Temperature
UBern	University of Bern
UCL	Université Catholique de Louvain
UGent	University of Gent
UNapoli	University of Napoli
UGuelph	University of Guelph

1 Introduction

This document evaluates the **performance of the cultivars** pre-selected in TN 98.3.1, according to the selection method established in TN 98.3.1 and developed into the measuring plan as described in TN 98.4.12.
Experimental performance as reported in TN 98.4.22 is evaluated and compared to the results from BT1 (TN 98.4.31), remaining critical points discussed.
If needed, suggestions for adaptation of the setup and protocols will be formulated, while limiting the impact on repeatability if the first test was successfully completed.

Ranking of the cultivar performance will still be preliminary, given the fact that only data from two repeated experiments (BT1 and BT2) are available. Moreover, given the occurrence of unanticipated problems in some of the setups, BT1 cannot be considered as an experiment under optimal growth conditions.
The selection method as presented in TN 98.3.1 will be assessed per crop, in a preliminary way and in tabular form.

Bench test evaluation includes the following key parameters:
Bench test setup performance. Measures needed to counteract culture-technical problems are discussed under 1.1, 2.1, 3.1, 4.1, 5.1 and 6.1.

Nutrient solution composition evolution. This includes observations on element depletion or accumulation (see 1.2, 2.2.7, 3.2.7, 4.2.7, 5.2.7, 6.2.7). Emphasis should be put on the fact that elemental nutrient composition is the key to optimal plant production.

Cultivar edible yield, and comparison with reference field crop data (as reported in TN3.1)
(See 1.4, 2.4, 3.4, 4.4, 5.4 and 6.4).
Cultivar harvest proximate composition, and comparison with data obtained from commercial agriculture (see 1.4, 2.4, 3.4, 4.4, 5.4 and 6.4).

Additional Parameters that were proposed as of particular interest for the plant bench test evaluation were:
- Crop size
- Harvest index or ratio edible/inedible biomass
- Harvest composition regarding anti-nutritional compounds
- Water use efficiency
- Stress resistance preliminary observations
- Choice of the crop initiation procedure

These topics are discussed under the appropriate headings below, where relevant.

Limitations of the used protocols (sensitivity and timing) are indicated.
This evaluation will lead to the proposal of a consolidated crop cultivar selection method.

1.1 Experimental Layout

UBern carried out the bread wheat trials without nutrient solution cooling.
UoGuelph added nutrient solution cooling capacity for the BT2 experiment with the durum wheat cultivars Commander and Eurostar. BT1 was carried out without cooling.
UNapoli also cultivated soybean without control of the solution T.
For potato this parameter is more critical, since T>20˚C inhibits tuber induction, hence nutrient solution cooling was foreseen in UGent and UCL.

Active humidification is used in the UGent and UNapoli setups, which have air exchange with the outside atmosphere (in addition to condensation in the cooling system).
The UoGuelph setup relies on extra dehumidification capacity to keep the RH setpoint in the sealed chambers.
In the UBern chamber, the installed extra dehumidification, kept the chamber conditions close to the setpoint, avoiding the excessive RH values that occurred during BT1.
The RH in the UCL setup remained stable for BT1 and BT2 by means of the condensation in the cooling system.
Dehumidification needs for further tests will depend on the level of crop development on the available cultivation surface, versus growth chamber volume.

In the sealed UoGuelph chambers, ethylene and oxygen accumulation were counteracted by appropriately scheduled venting.

1.2 Evaluation of growth environment follow-up

The foreseen loggers for each of the setups were adequate to follow-up on chamber T and RH (VPD).
Frequent manual adjustment of pH and EC are time-demanding and the setpoint is difficult to obtain with precision (mixing being critical during adjustment in small volume tanks). The automatic pH adjustment setup at UGent was not used for BT2, as it proved to suffer from sensor stability problems in BT1.
Nutrient solution elemental analysis can give an indication on accumulation of elements. Depletion of elements can be a consequence of efficient uptake by plants (characteristic for N P and Mn), hence this parameter has to be considered per element in relation to uptake characteristics.

1.3 Evaluation of monitoring of plant development

Access to the plants is for the closed, sealed UoGuelph setup logically limited, it was hence problematic to obtain time-lapse data when plants grow tall, largely obscuring viewing window and monitoring camera placed inside.
Free access to one side of a gully is needed for efficient assessments of plant physiological parameters. BT2 Bern setup was hence started with 4 gullies / 4 racks (instead of 8 gullies in BT1).

For potato tuberisation assessment, with the aim to develop a nutrient delivery strategy for optimal tuber development, a gully setup with easily removable lid proved workable. However tuber greening remains a concern, e.g. Innovator tubers were visibly green at harvest. Hence the light-tightness needs further improvement. This can be accomplished by:

- Improving the gully covers
- Ensuring the gully lids are properly fastened to avoid light contamination
- Avoiding to open the gullies
- Reducing the time of opening of the lid during the observations.

1.4 Evaluation of crop harvest

Yield

Crop yield is based on cultivated surface, defined as illuminated surface where the foliage can develop: whole chamber area in the closed Guelph chambers, shelve or table areas covered by canopy and gullies in other setups.

BT1 durum wheat trials gave an average edible yield of 0.5kg/m^2 yield for 2 cultivars. During BT2, 2 different cultivars were tested and provided edible yields between 0.4 and 0.75kg/m^2.

For bread wheat, 3 cultivars, yielded 0.4 kg/m^2, while the fourth performed worse with 0.3 kg/m^2 in BT1. Results of BT2 were quite similar, all four cultivars yielded between 0.44 and 0.5kg/m^2.

The potato yield obtained by UGent-consultant HZPC in hydroponic greenhouse culture attains a highest value of 3kg FW/m^2. Potato on average has 20% DW content, hence a value of 0.6 kg/m^2 is also attained for DW/m^2. During BT2, UCL yielded 1.96 kg FW/m^2 with Annabelle, and UGent 1.44kg FW/m^2 with Bintje which correspond respectively to 0.39 and 0.29kg DW/m^2.

The suboptimal start or growing conditions and subsequent phyto-sanitary problems which severely limited growth in BT1 at UGent, UCL (potato), and UNapoli (soybean) were avoided and solved for BT2.

The repeat experiments (BT2) showed a real improvement as potato harvest was doubled in UGent and UCL.

Nutritional analysis

In proximate analysis, carbohydrate content is obtained by difference between starting sample weight and water, protein, lipid and ash determinations. The sum of these values yields the FW (harvest weight) of the starting sample.

Fiber content determination: IPL and UNapoli according to the same AOAC 985.29 protocol

1.5 Cultivar selection method and ranking

Preliminary ranking of the tested cultivars of BT1 and BT2 can (for all the crops) as a minimum be based on:

- Edible harvest yield/m^2
- Growth period (maturation time) = Yield can be expressed as a function of time
- Harvest index (ratio edible/total yield DW)
- Mature plant height
- Light use efficiency = Yield as a function of time and light level
- Nutritional analysis
- Total amount of water transpired

2 Bread Wheat (UBern)

2.1 Evaluation of experimental layout

2.1.1 Measuring plan

The setup with 1 cultivar per shelf (0.6 m^2 illuminated growth area) generates a better access to follow up the growth of the plants.

2.1.2 Setup

A plant density of 100 plants / m^2 (60 plants/0.6m^2) is considered adequate based on the bench test 2 results.
Seed germination, as carried out in closed boxes at 100% humidity (for 3 days) allowed a pre-selection of synchronously germinated seeds (seedlings of the same early developmental stage), and allowed a 100% plant survival till harvest.
The density was reduced to 100 plants/m^2 (one gully instead of two gullies per shelve) in BT2.
In BT2 the concentration of macro and micronutrients in the nutrient solution was step-wise decreased after flowering to reach an EC of 400µS/cm and the pH compensating acid was changed (H_2SO_4 replaced HNO_3). This change in the nutrient medium diminished the number of extra side-stems (tillers) for CH Rubli and Greina.

2.2 Evaluation of growth environment follow-up

2.2.1 Settings

RH was reduced to some extent in BT2 as compared to BT1 using the added dehumidification capacity.

2.2.2 Chamber T/RH evolution

Extra dehumidification was installed and functionality proven to reduce the RH to the 60% to 80% (depending on developmental stage of the wheat) regardless of time of day.

2.2.3 Chamber CO_2 level

Only a minor depletion of CO_2 concentration was observed throughout the day (values close to 380 ppm).

2.2.4 Nutrient solution environment

Adjustment of the nutrient level needs frequent manual additions given the high transpiration rates of the crop.

2.2.5 pH and EC evolution

EC was used as the setpoint (1200µS/cm) when adjusting the gully reservoir water level. The concentration of macro and micronutrients in the nutrient solution was step-wise decreased after flowering to reach an EC of 400µmS/cm.
The pH rise of the nutrient solutions was compensated by acid additions (HNO_3 at beginning and H_2SO_4 after flowering).
For subsequent tests, the amounts of N and S from acids used to adjust pH have to be included in the nutrient level adjustment strategy as linked to development and maturation.

2.2.6 Nutrient solution T

The solution T was presumably higher than optimal. Plants yielded normal ears and kernels, hence this parameter is not the most critical.
It is logistically difficult to setup a cooling system for all separate gully reservoirs.

2.2.7 Nutrient solution analysis

Phosphate, Copper and Manganese were nearly completely depleted after 4 weeks. They are rapidly taken up by plants, hence this is expected.
The 4 week nutrient exchange cycle likely permitted to avoid limitation, final confirmation can only be obtained by the analysis of plant material.

2.3 Evaluation of monitoring of plant development

2.3.1 Photographic follow-up

Crop development and differences in maturation between the cultivars was described. CH Rubli and Greina matured first, Aletsch was intermediate, and Fiorina was the latest maturing cultivar.

2.3.2 Detailed photographic observations

The ripening of the ears, on which harvest time was based, was documented.

2.3.3 Growth assessment

Resistance to lodging: Fiorina suffer for lodging already before stem elongation and some threads were placed around the gully to maintain the plants. During BT2, CH Rubli did not suffer from lodging.

Fig. 1 UBern - Thread placed around Fiorina Gully A1 to maintain the plants

2.3.4 Gas exchange data

Not foreseen in the measuring plan for bread wheat.
Global plant water usage determined volumetrically from the nutrient solution usage was correlated with length of the growth period of the cultivars, the first maturing cultivars having the lowest water consumption.
The length of the growth period explains a part of the water usage. But the water usage is also related to the cultivar. Greina and CH Rubli have more or less the same length of growth period but CH Rubli used more water than Greina (Fig. 2). Aletsch was harvested 3 weeks before Fiorina, but Aletsch used more water than Fiorina. Transpiration during maturation was presumably also affected by late tillering caused by excess nutrient availability.
The water evaporation from gully was different for gullies B1 and C1. These two gullies were run without plant during 12 days. The evaporation from gully B1 was 5L while it was only 3L for gully C1. Gullies A1 and D1 were not tested. The place of the gullies in the growth chamber may have an influence on the water evaporation. Gully B1 is (more than the other gullies) in the air flow of the ventilation.

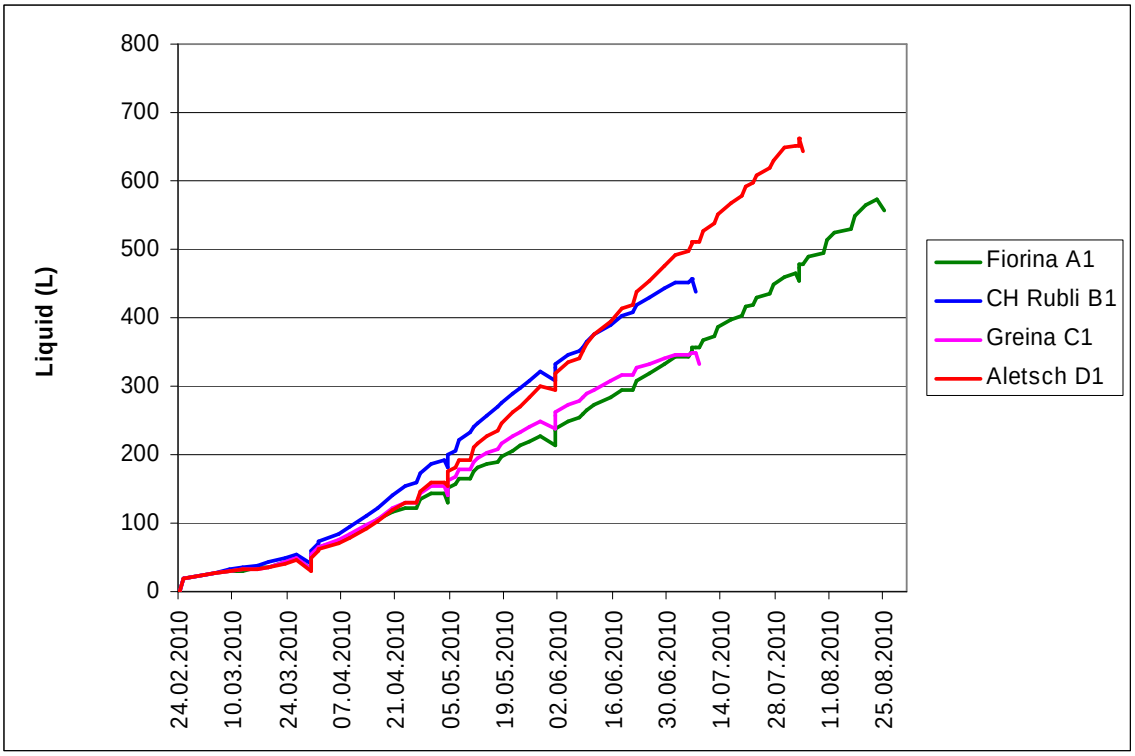

Fig. 2 UBern - Liquid (water, start-up solution and replenish solution) cumulative uptake by the plants

2.4 Evaluation of crop harvest

Separation of the kernels from the rachis (central 'stem' of the wheat ear) and the glumes was carried out by a dry separation method.

The Fiorina and Aletsch cultivars proved the most difficult to manually separate kernels from the ears.

Greina produced 299 g of kernel, CH Rubli 278.46 g, Fiorina 276.72 g and Aletsch 267.95g. With the growth condition of BT2 (modifications on the nutrient solution and 100 plants per m^2 instead of 200 plants per m^2 for BT1) the 4 cultivars had a higher yield than in BT1. Greina was still the best cultivar with a yield of 498 g/m^2 followed by CH Rubli (464 g/m^2), Fiorina (461 g/m^2) and Aletsch (446 g/m^2). The harvest index [DW kernels/(DW kernels + DW straw + DW roots + DW threshing debris)] was 0.41 for Greina, 0.30 for CH Rubli, 0.25 for Fiorina and 0.24 for Aletsch. For all cultivars, the harvest index was higher in BT2 than in BT1, most likely related to the change in the nutrient solution concentration and the lower density of the plants. In literature, the harvest index is defined as the ratio of grain yield to aboveground biomass (Li et al., 2011), the roots are therefore not counted in the dry weight. When comparing the harvest index shown in Tab. 4 (which is calculated with the dry weight of roots)

with the harvest index shown in Tab. 5 (which is calculated without the roots), the value for the harvest index (without the roots) are higher: 0.47 for Greina, 0.35 for CH Rubli, 0.28 for Fiorina and 0.27 for Aletsch. The harvest index found in the literature for winter wheat was in between 0.15 and 0.44 (Li et al., 2011), around 0.45 (McIntyre et al., 2010) or around 0.5 (White and Wilson, 2006). The green tillers (which didn't produce grains) were an important part of the dry weight of shoot, and thus decreased the harvest index.

Tab. 1 UBern – Yield per m^2

Cultivar	Yield in g per m^2
Aletsch	446.6
CH Rubli	464.1
Greina	498.3
Fiorina	461.2

Tab. 2 UBern - BT2 harvest and ripening

Cultivars	Gully	Germination	Harvest	Number of days	Ripeness	Number of days for ripeness
Fiorina	A1	22.02.2010	25.08.2010	184	not all ears mature at harvest	more than 184
CH Rubli	B1	22.02.2010	07.07.2010	135	07.07.2010	135
Greina	C1	22.02.2010	08.07.2010	136	08.07.2010	136
Aletsch	D1	22.02.2010	04.08.2010	163	04.08.2010	163

Tab. 3 UBern - Amount of kernels collected per gully and cultivars

Cultivar	Gully	Rockwool Piece	Yield (g) per Rockwool piece	Yield (g) per cultivar
Fiorina	A1	a	48.33	276.72
	A1	b	126.61	
	A1	c	59.99	
	A1	d	41.79	
CH Rubli	B1	a	90.02	278.46
	B1	b	51.32	
	B1	c	40.98	
	B1	d	96.14	
Greina	C1	a	87.27	299.00
	C1	b	62.77	
	C1	c	55.88	
	C1	d	93.09	
Aletsch	D1	a	65.00	267.95
	D1	b	50.59	
	D1	c	41.06	
	D1	d	111.29	

Tab. 4 UBern - BT2 harvest index for dry matter (with roots)

	DW Kernels * in g	DW straw in g	DW roots in g	DW threshing debris* in g	Harvest index for dry matter
Fiorina	276.72	569.39	106.13	141.32	0.25
CH Rubli	278.463	424.54	127.787	82.59	0.30
Greina	299.004	253.85	90.049	86.44	0.41
Aletsch	267.947	586.28	122.42	137.71	0.24

* stored at room temperature

Tab. 5 UBern – Harvest index for dry matter (without roots)

	DW Kernels in g	DW straw in g	DW threshing debris in g	Harvest index for dry matter
Fiorina	276.72	569.39	141.32	0.28
CH Rubli	278.463	424.54	82.59	0.35
Greina	299.004	253.85	86.44	0.47
Aletsch	267.947	586.28	137.71	0.27

2.5 Cultivar selection method and ranking

As mentioned in TN 98.3.1 (section 2.2.2), the following selection criteria were considered for the bench test trials.
The first 2 criteria below are pre-test criteria, and not to be used in the ranking:

- Availability of the cultivar: 4 pre-selected cultivars
- Vernalization: excluded the use of winter wheat

Tab. 6 UBern - Cultivar overview

	Greina	CH Rubli	Aletsch	Fiorina
Shoot length (short)*	1	3	3	2
Generation time (short)	1	1	2	3
Precocity of ear emergence	1	1	2	3
Resistance to lodging	1	1	1	2
High yield	1	2	4	3
Total rank	**5**	**8**	12	13

*shoot length Greina and Fiorina ears appear at the top of the stalks (see TN4.12, 2.3)

- Disease resistance

No fungal infection observed on the plant of BT2 (most likely related to the better adapted nutrient supply).

- The plant macro and micronutrients content

The plant macro and micronutrients (K, Ca, Mg, P, Fe, Zn, Cu, Mn and Ni) content in the kernels of BT2 and the market samples are shown in the table 14 and figures 29 and 30 in TN 98.4.22. In the kernels of BT2, the macro and micronutrient contents were higher (K, Mg, P, Fe, Zn, Cu, Mn and Ni) than the content in the market samples, with some exceptions: Zn in CH Rubli, Ni in CH Rubli and Greina. The content of Ca and Mn were comparable in BT2 and MS kernels, with some exceptions: Ca in Fiorina and Mn in Greina and Aletsch. These higher contents of macro and micronutrients might be due to an inadequate supply. The difficulty of the wheat to get mature (high amount of green tillers at harvest time (see Fig. 3), flag leaves not fully senescent at the harvest (Fiorina and Aletsch) were also a sign that the macro and micronutrient supply was more adequate than in BT1, but still a bit inadequate. The EC, which was step-wise decreased after flowering, was maybe still too high. The pH was adjusted to 5.6 – 6 with acids (HNO_3 replaced after flowering by H_2SO_4). In conclusion, the macro and micronutrient contents in kernels of BT2 were different but not too far away from the content in the market samples and may become closer to the content in the market samples by a better adjustment of the EC.

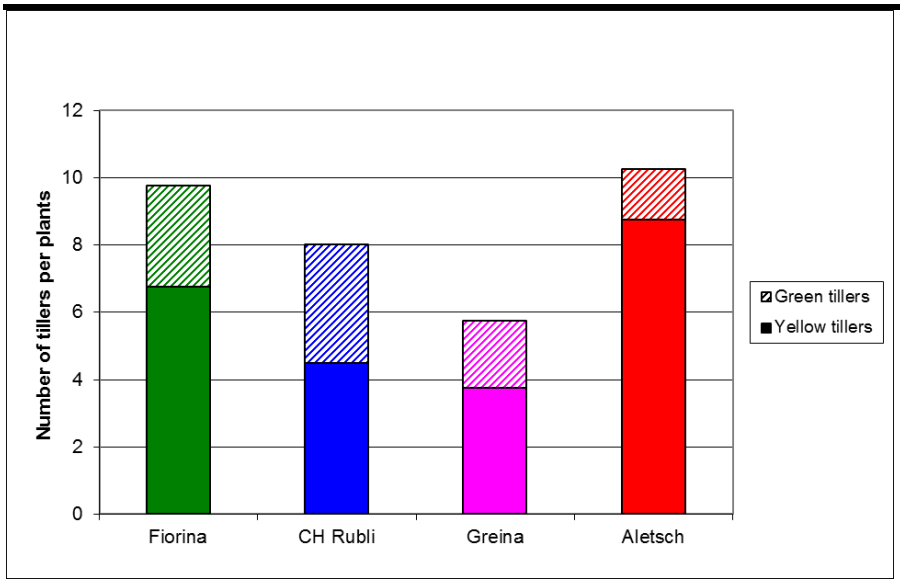

Fig. 3 UBern: yellow and green tillers at harvest

There were 2 post-harvest analysis criteria to be assessed:

- Protein content
- Processing properties

These have been assessed in TN 98.5.2 (section 3) and further evaluated in TN 98.5.3 (section 3

Specific for controlled environment chamber plant growth additional parameters were mentioned under section 2.2.1.1 of TN 98.3.1:

- High yield of edible versus non-edible parts.
- Water use (integrated transpiration rate during the entire growth period).
  This was positively correlated with the generation time, but was also related to cultivars (for instance CH Rubli and Greina had the same generation time but CH Rubli used more water). See also comment above.
- Senescence and maturation properties: linked to the abovementioned generation time. These properties will most likely be affected by modifications in the nutrient supply. CH Rubli and Greina from BT2 were harvested one week earlier than CH Rubli and Greina from BT1. This shorter generation time is most likely related to the stepwise decrease of the EC of the nutrient solution after flowering of the BT2 plants.
- Plant macronutrient (e.g. K, Ca, Mg, P) and micronutrient (e.g. Fe, Mn, Zn, Cu, Ni) accumulation in kernels: results available for BT1 and BT2.
- Photosynthetic rate and oxygen production – measurements not foreseen in the measurement plan for BT1 and 2.

2.6 References

Li HL, Luo Y, Ma JH (2011) Radiation-use efficiency and the harvest index of winter wheat at different nitrogen levels and their relationships to canopy spectral reflectance. Crop and Pasture Science 62: 208-217

McIntyre CL, Mathews KL, Rattey A, Chapman SC, Drenth J, Ghaderi M, Reynolds M, Shorter R (2010) Molecular detection of genomic regions associated with grain yield and yield-related components in an elite bread wheat cross evaluated under irrigated and rainfed conditions. Theor Appl Genet 120: 527-541

White EM and Wilson FEA (2006) Response of grain yield, biomass and harvest index in their rates of genetic progress to nitrogen availability in ten winter wheat varieties. Irish Journal of Agricultural and Food Research 45: 85-101

3 Durum Wheat (UoGuelph)

3.1 Evaluation of experimental layout

3.1.1 Measuring plan

Measuring plan was carried out as specified.
Bi-weekly leaf opening of the chambers was used to reduce the accumulation of ethylene and oxygen (and possible other compounds) in the second MFC1 growth trial.

3.1.2 Setup

The same layout of plants was used with the selected cultivars.
The plant growth area corresponded to 2.5m length (gully length 2.45m) x 2m width. Gully width is 0.17m. Plants within each gully had an area of 2.5x0.4m (1 m^2) to develop.
Planting density: 3 times 45 plants per gully = 135 plants, density = 135 plants / m2, 675 total.

3.2 Evaluation of growth environment follow-up

3.2.1 Settings

Environment setpoints were identical for the 2 trials.

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. Temperature control was very good throughout the experiment. 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 as recommended by durum wheat expert Dr. Mark Jordan.

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. Humidity control was not as effective as desired and improvement requires the replacement of the current control system which is outdated and cannot be modified to improve response.

3.2.3 Chamber NCER, evapotranspiration, ethylene and oxygen production

NCER and transpiration followed typical profiles found in plant growth and development. All cultivars had similar peak productivity, however Avonlea and Commander productivity dropped off rapidly at approximately 80 days whereas Strongfield and Eurostar productivity dropped at a slower rate. As this is during the seed filling stage, the higher NCER observed at the later growth stage in Eurostar and Strongfield may be the reason for higher overall kernel yields in these cultivars.

A significant reduction in NCER was observed in all cultivars immediately after the first solution change, however the definitive reason for this is currently unknown. As the same event occurred in both chambers at chronologically different times, the observed reduction is likely directly related to the nutrient solution change. Similar reductions in NCER have been observed in soybean during growing solution changes in our laboratory in other chambers as well. The current hypothesis is that the rapid change from a differentially depleted solution to a full strength feed solution results in osmotic shock in the root zone. Increased productivity should be realized by reducing or eliminating this reaction to nutrient solution change and remedies should be investigated in future trials.

Avonlea, Commander, Eurostar, and Strongfield evapotranspiration peaked at approximately 60, 60, 120, and 90 litres per day. Unlike NCER, the first nutrient solution change had a less noticeable effect on water production, however following total productivity, the cultivars with the highest yield also produced the most water. In both cases, the highest rates of evapotranspiration were observed in chamber 2 (Eurostar and Strongfield), indicating a possible chamber effect. Recent evidence has shown that chamber 2 has a higher air velocity than chamber 1. Increased air velocity and subsequent improvements in gas exchange would likely be the cause of the differences in observed evapotranspiration, however additional studies should be performed to confirm this hypothesis.

Air samples were monitored for ethylene by GC analysis every standard working day. A sample of air was withdrawn through the atmosphere sampling ports and injected into an SRI GC. Ethylene was sampled starting the first day after closure and continued until harvest. The highest level of ethylene was observed in the Avonlea trial where of 80 ppb was observed. Commander, Eurostar and Strongfield had maximal observed ethylene concentrations of 49, 45 and 41 ppb respectively. Biweekly venting was performed in the Eurostar and Commander trials in an effort to mitigate potential ethylene effects on crop productivity.

In Avonlea, oxygen levels exceeded ambient levels and were as high as 28.5% before being reduced by chamber opening for flooding repairs. Because of the high concentrations observed with that cultivar, subsequent trials with Commander and Eurostar were vented on a biweekly basis. With venting, oxygen reached maximum concentrations of 25.5 and 23.5 percent in Commander and Eurostar respectively. Oxygen determination in Strongfield was compromised by a switching valve failure in the first trials, so only the first few weeks of data are available.

Oxygen was one of the considerations for adopting biweekly venting. In crops grown under ambient concentrations of carbon dioxide, high oxygen reduces the efficiency of photosynthesis by competing with CO_2 for the acceptor 1,5-bisphosphate (Warburg effect). However, these studies used enriched carbon dioxide levels (0.12 kPa) which can suppress photorespiration even at the high partial pressures of oxygen observed in these experiments (Maleszewski et al., 1988; Drake et al., 1996).

3.2.4 Nutrient solution environment

Intermittent irrigation was used with a circulation pump on time of 2 to 3 minutes. The on times are part of a 10 minute cycle.
Intermittent irrigation is necessary in these hydroponic systems due to the draining requirements.
The approach is likely beneficial for oxygen provision to the roots, saves energy, and likely reduces nutrient solution T increases.

3.2.5 pH and EC

pH and EC were automatically measured and adjusted on a continuous basis by the control system. Sampling of hydroponics solution was performed at the beginning and end of each 4 week nutrient solution interval. Control was excellent with deviations from setpoint only during initial operation and equilibration and during solution changes or flooding events. Observed pH and EC levels deviated from the setpoints at the end of the experiment in both chambers and were a direct result of the cessation of nutrient circulation to the plants.

3.2.6 Nutrient solution T

Suppression of nutrient solution T increases from pump friction and chamber internal heat accumulation are seen as beneficial to root zone dynamics. The nutrient solution temperature was maintained at 20C throughout the experiment.

3.2.7 Nutrient solution analysis

P, K and the micronutrient Mn were depleted at the time of nutrient solution exchange.
These elements are taken up rapidly by the plants, not necessary an indication of shortage.
Nutrient solution composition could be adjusted for the next 4 cultivar repeat test in MFC2.

3.3 Evaluation of monitoring of plant development

3.3.1 Photographic follow-up

See 3.3.3

3.3.2 Detailed photographic observations

See 3.3.3

3.3.3 Growth assessment

Limited viewing ability by integrated webcam and small chamber window.
First plants in each gully can be used to assess key development stages (flowering, grain maturation) during scheduled venting.

3.3.4 Maturity assessment

There are several methods but for general comparisons we would use the number of days from seeding until 75% of the heads have no green colour (are yellow). This is obviously not precise as the heads do not turn instantly yellow on a single day but it is practical and often used.
For carefully controlled physiological studies the exact method is physiological maturity is when the head is at 20% moisture. For this you need to take samples of heads, weigh them then dry and weigh again. If the weight difference is 20% they are mature.

3.3.5 Gas exchange data

The chamber level assimilation and evapotranspiration data per cultivar allow for the comparison of integrated values over the whole growth period.

3.4 Evaluation of crop harvest

During BT1, results from both cultivars exceeded recorded field production yields (Clark et al., 2006) by 14 and 87 percent in Avonlea and Strongfield respectively, demonstrating that the sealed environments were suitable for durum wheat growth and development. Avonlea produced over 2.1 kg of wheat kernels while Strongfield produced over 3.7 kg.
The 100 kernel weights were 4.17 with Avonlea and 4.57 with Strongfield, and are in the normal range for durum wheat.

During BT2, results from Commander and Eurostar cultivars exceeded recorded field production yields by 14 and 41 percent (Clark et al., 2005, 2009), continuing to prove that the SEC-2 sealed environments were suitable for durum wheat growth and development. In this trial, the least productive cultivar was Commander, with a kernel mass of 0.40 kg m^{-2} whereas Eurostar produced over 0.58 kg m^{-2}.

Possible chamber differences preclude drawing definitive conclusions on the most suitable cultivar for ALS use from this single replicate.

Tab. 7 UoGuelph - Harvest overview

Cultivar	Total DW (g)	Height (cm)	Roots (g)	Straw (g)	Kernels (g)	Number of Plants	100 kernel weight	kg/ha equivalent	Harvest Index
Avonlea	12 054	86	1 291	8 630	2 133	469	4.17	4 266	0.18
Commander	11 912	73	1 465	6 803	2 009	457	4.64	4 019	0.17
Eurostar	13 474	85	1 244	7 835	2 912	438	3.02	5 824	0.22
Strongfield	13 531	84	1 435	8 325	3 771	466	4.57	7 542	0.28

3.4.1 Quality tests

The current results show higher yields in the Eurostar and Strongfield cultivars, however conclusions regarding the best candidate for closed environment production cannot be made on a single case study. Both of the highest yielding crops were grown in SEC2 chamber 2, indicating a possible chamber effect. The initial consideration for the discrepancy between the two chambers was the lower rate of leakage in chamber 1 when compared to chamber 2 (<1% vs. >5%), resulting in possible negative effects from higher concentrations of oxygen and ethylene. Biweekly venting was employed in an effort to mitigate this effect in the next trials, however the highest yield was still observed in chamber 2. One of the additional variables that differ between the two chambers is air velocity. Chamber 2 airspeed is higher than that of chamber 1, which may allow improved gas exchange in the dense durum wheat canopy. Faster air velocity may also explain the large differences in evapotranspiration that was noted between the two chambers.

All cultivars demonstrated a marked decrease in NCER during the first nutrient solution change, demonstrating the usefulness of this measurement in advanced life support research. Study of the cause of this decrease, and methods for improved nutrient delivery should be a priority for future research to increase yields beyond those observed here.

In order to improve data capture and system control and allow for future sensor expansion, further testing on wheat cultivars requires modification of the SEC2 control system. The current system, based on MS-DOS and last updated in 1999, cannot be modified. Prior to future plant trials, a new control system provided by Argus Control Systems will be installed and tested.

3.5 Cultivar selection method and ranking

Four cultivars have been grown so far, and the selection criteria (yield and associated parameters) may have been confounded by the different closure levels of the 2 chambers used,

and the consequently different ethylene and oxygen level accumulation. Still yields were higher than for field harvests for both cultivars.

Crop quality parameters (chosen to be diverse during pre-selection) correlated with data from field crop derived data.

3.6 References

Clarke, J. M., Knox, R. E., DePauw, R. M., Clarke, F. R., McCaig, T. N. Fernandez, M. R. and Singh, A. K. 2009. Eurostar durum wheat. Can. J. Plant Sci. 89: 317320.

Clarke, J. M., McCaig, T. N., DePauw, R. M., Knox, R. E., Ames, N. P., Clarke, F. R., Fernandez, M. R., Marchylo, B. A. and Dexter, J. E. 2005. Commander durum wheat. Can. J. Plant Sci. 85: 901–904

Clarke, J.M., T.N. McCaig, R.M. Depauw, R.E. Knox, F.R. Clarke, M.R. Fernandez, AND N.P. Ames. 2006. Registration of 'Strongfield' Durum Wheat. Crop Sci. 46:2306–2307

Drake B.G., GonzalezMeler M.A., Long S.P. 1996. More Efficient Plants: A Consequence of Rising Atmospheric CO2? Ann Rev Plant Phys, 48: 609639.

Mackowiak, C.L., L.P. Owens, and C.R. Hinkle. 1989. Continuous Hydroponic Wheat Production Using A Recirculating System. NASA Technical Memorandum TM 102784

Maleszewski, S., Kaminska, Z., Kondracka, A., Mikuiska, M. 1988. Response of Net Photosynthesis in Bean (Phaseolus vulgaris) Leaves to the Elevation of the Partial Pressures of Oxygen and Carbon Dioxide. Physiol. Plant. 74: 221224.

4 Potato (UGent)

4.1 Evaluation of experimental layout

4.1.1 Measuring plan

The measurements carried out were similar as for the first bench test.
Because of the nutritient-solution-linked developmental problems encountered in BT1, emphasis was put on frequent follow up of nutrient solution management, by manual adjustments of pH and Nitrogen concentration.
Plant or leaf level gas exchange or emanation measurements were not carried out during BT2.

4.1.2 Setup

Some minor changes would be needed to ameliorate the UGent setup.
The main technical problems encountered during these 2 tests were:

- pH electrodes deviance (need of electrodes of higher quality)
- Automatic addition of water not reliable (water level sensors get stuck)
- Automatic additions of base/acid not reliable (only acids or bases can be automatically added in our setup, not both)
- Tank volume (15L) is too small (very limited buffer capacity)
- Gullies frequent overflowing via the side holes due to root development (holes need to be drilled higher, or root development must be limited)

Furthermore, imposing a difference between day/night air temperature (in a range of 20/15˚C) instead of a constant temperature should stimulate tuber production (Wheeler et al., 1997). Potatoes also have a range of maximum carbon assimilation efficiency and water use efficiency from 16 to 25˚C (Sun-Ben Ku and al., 1977).

As an explanation to the extra step of 3 weeks in-vitro culture: in-vitro plants are compared to tubers as starting material below:

<u>Tuber seed:</u>
For pre-test 1 and 2, chosen start up material were tubers obtained from the UGent consultant HZPC.
This has several inconveniences:

- Tubers have to be stored during dormancy period, sprouting can take long depending on cultivar characteristics.
- Tubers can possibly be contaminated by diseases.
- Tuber buds and shoots grow vigorously and are produced in excess numbers (dominance of the distal bud depends on cultivar) so that many need to be removed to enable efficient growth in gully systems. This procedure requires a lot of working time.

- Difference in weight and so, of nutrient reserve of mother tubers is a source of development heterogeneity.

In-vitro plants:
In-vitro plants are produced in 3 to 4 weeks' time, in large amounts on a limited surface:

- plant material is sterile
- plants are very homogenous with in theory the same genetic background.
- With the current bench test setup, *in-vitro* plants must have a stem of minimum 10 cm long to be placed through the plant-insertion openings at the side of the gully. Below this length, it is hardly possible to have both roots in the nutrients solution layer, and youngest leaves exposed to light.
- Acclimatization is a critical period that needs particular attention, but step-wise hardening and avoiding exposure to drying airflow is efficient.
- After acclimatization a 4 or 5 days elongation phase is required to obtain plants about 10cm long. This step takes place in open gullies with NFT.

For these reasons, *in-vitro* plants are easier to use and more reliable than seed tubers and were chosen for BT1 and 2.

4.2 Evaluation of growth environment follow-up

4.2.1 Settings

Tab. 8 UGent - Settings

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

4.2.2 Chamber T/RH evolution

Apart from the unavoidable short (stabilisation) day/night transients (see TN 98.4.12, section 4.1.3.2 and Table 37), no aberrations recorded.

4.2.3 Chamber CO_2 level

Ambient concentrations of CO2 were applied. No decrease was measured, only increases during crop manipulations by operator presence.

4.2.4 Nutrient solution environment

Gully nutrient solution layer thickness is regulated by gully inclination and pump flow rate.
Side holes were drilled too close (2cm) to the bottom of the gullies which is often source of overflowing.
The 15 liter tanks are too small and don't provide enough buffer capacity and flexibility.

Daily measurements of Nitrate in the nutrient solution during tubers developmental phase are important in order to keep a low but constant level of Nitrate in the solution (between 0 to 500mg/L).

4.2.5 pH and EC evolution

The observed instability of the pH sensor was presumed to be caused by the accumulation of gas bubbles at the sensor-liquid interface. The sensors were then placed in an aspirated tube connected to the circulation pump intake (similar to the setup for the manual dissolved O_2 measurement). Still, it did not solve the problem. It is now clear that pH sensors deviance is only due to their bad quality. They should be replaced by material of better quality and reliability.

4.2.6 Nutrient solution T

Temperatures of all gully system solutions were stable at 20˚C.

4.2.7 Nutrient solution analysis

In BT1 UGent added a low amount of N, UCL dosed above the recommendations; both labs obtained a similar low yield due to initial stress and progressive development of opportunistic pathogens (see TN 98.4.21 and section 4.3 below). Another mistake was that no Nitrogen was added after tuber set as recommended by HZPC. But it appeared that HZPC was refilling the nutrient tank with tap water containing nitrate instead of distilled water for UCL and UGent.

For BT2 the start solution had higher nitrate content than for BT1. After switching to growth solution, $Ca(NO_3)_2$ was daily added in order to maintain a low level of nitrate in the nutrient solution. Indeed, too high availability of nitrate, or its fluctuation induces second growth of stolons and stops tuber bulking. A complete depletion provokes peel hardening and then cracking once nitrate becomes available.

Nutritive solution goes acid rather quickly when nitrate is depleted (Wheeler et al.,1999). Adding nitrate when pH drops could be a way to control it.

Phyto-sanitary problems were identified during BT1 and 2. According to UGent consultant HZPC these pathogens can only develop when the plants are weakened by stress.
The list of organisms scanned by the DNA-multiscan PCR analysis (Sciencia Terrae Diagnosecentrum) is reproduced in Tab. 9.

Tab. 9 UGent - Pathogens present in Annabelle's nutrient solution at the end of BT1 and 2

Start of BT	BT 1 - 28/sep/09	BT 2 - 28/jan/10
sample date	**24-Nov-09**	**4-May-2010**
days after transfer to growth chamber	68	98
Botrytis cinerea	no	weak
Botrytis porri	no	weak
Botrytis tulipae	no	moderate
Colletotrichum spp.	strong	very strong
Colletotrichum acutatum	strong	strong
Colletotrichum coccodes	strong	moderate
Plectosphaerella cucumerinum	no	strong
Fusarium spp.	moderate	very strong
Fusarium oxysporum	weak	strong
Fusarium solani	no	moderate
Pythium sp.	strong	no
Pythium dissotocum	very strong	no

4.3 Evaluation of monitoring of plant development

4.3.1 Photographic follow-up

Fixed camera hourly picture logging and weekly manual photographing guarantee a sufficient level of documentation of crop development.

4.3.2 Detailed observations

Leaf and tuber size were small. This is linked to the reduced stature of plants when tuberisation was initiated. Increasing Nitrogen availability during the growth phase should override this problem and produce stronger plants and so, higher tuber numbers or size. The average length of the main stem was homogeneous for the four cultivars, around 35cm.
Tuber deformation linked to variations of Nitrogen in the nutrient solution has been limited by keeping a low but stable level of Nitrate in the solution.
Manual length and width measurements of tubers during BT2 shows a constant and regular tuber size increase from tuber set till harvest.

4.3.3 Growth assessment

Fixed camera hourly picture logging and weekly manual photographing guarantee a sufficient level of documentation of crop development.
Weekly measurements of stem length, number of leaves, stolons and tubers were also done.

4.3.4 Gas exchange data

No short-time gas exchange measurements were carried out during BT2.

4.3.5 Extra plant physiological measurements

On-line plant weight determination needs further validation to guarantee more stable data. Tubers were also measured weekly and manually, allowing an estimation of the tuber biomass present in the gullies during BT2.

4.4 Evaluation of crop harvest

4.4.1 Yield

In BT1 weak plant development was induced by suboptimal starting conditions in the bench test room (non-optimal transport of the in-vitro plants, acclimatisation and nutrient solution composition), and insufficient nitrogen provision. Plants were susceptible to opportunistic plant pathogens likely present in the environment.

BT2 in-vitro elongation took place in the opened invitro-boxes instead of in a NFT layer. Still, plants reached the desired length in a few days.

The amount of harvest at UGent allowed nutritional analysis (Tab. 12). These were carried out at IPL.

Regarding the ratio edible/total plant dry weight (Harvest index, see Tab. 11) we must note that it is probably only trustworthy from the HZPC greenhouse test, since prior to harvest, the UGent bench test plants displayed severe foliage loss and disintegration. During BT2, enough plants survived until the end of the experiment to proceed to the destructive measurement on all cultivars except Desiree. Still, many leaves fell of the plants during the BT2 growth cycle which makes these results imprecise.

Tab. 10 Potato harvest results HZPC, UGent and UCL

		Annabelle	Bintje	Desiree	Innovator
Tuber harvest (kg)	HZPC 2008	1.872	-	1.141	0.676
	HZPC 2009	4.420	1.984	3.998	0.663
	UGent BT1	0.511	0.466	0.274	0.415
	UGent BT2	1.154	0.78	0.348	0.867
	UCL BT1	0.662	0.546	0.299	0.283
	UCL BT2	1.016	1.568	0.518	0.665
Tuber harvest (g/m^2)	HZPC 2008	2500	-	1520	900
	HZPC 2009	4910	2200	4442	740
	UGent BT1	660	583	343	501
	UGent BT2	940	1440	440	1050
	UCL BT1	829	683	374	355
	UCL BT2	1960	1270	650	830
Tuber harvest (g/plant)	HZPC 2008	93.6	-	57.1	33.8
	HZPC 2009	184.2	82.7	166.6	27.6
	UGent BT1	34.1	29.1	17.2	27.2
	UGent BT2	52	72.1	21.8	57.8
	UCL BT1	41.4	34.1	18.7	17.7
	UCL BT2	98	63.5	32.4	41.6
Total productivity ($g/m^2/d$)	HZPC 2008	-	-	-	-
	HZPC 2009	-	-	-	-
	UGent BT1	4,78	4,22	2,36	3,63
	UGent BT2	7,40	11,39	3,03	8,27
	UCL BT1	6,26	5,19	2,82	2,67
	UCL BT2	14,96	9,69	4,96	6,34
Number of tubers per plant	HZPC 2008	-	-	-	-
	HZPC 2009	20.4	12.9	10.5	3.7
	UGent BT1	9.2	6.5	3.2	2.1
	UGent BT2	8.1	6.3	3.3	2.8
	UCL BT1	4.6	4.6	3.6	1.4
	UCL BT2	13.2	11.4	8.7	18.5

Tab. 11 Potato: FW and DW (g/plant) of shoots, roots, stolons and tubers; harvest index based on DW

Measure-ment / Cultivar	Tuber FW (g/plant)	Shoot FW (g/plant)	Root FW (g/plant)	Stolon FW (g/plant)	Total FW (g/plant)	Tuber DW (g/plant)	Shoot DW (g/plant)	Root DW (g/plant)	Stolon DW (g/plant)	Total DW (g/plant)	% DW	Harvest index (based on DW)
HZPC 2008												
Annabelle	-	54,81	17,04		71,85	-	4,18	1,06		5,24	7,29	-
Bintje	-	-	-		-	-	-	-		-	-	-
Desiree	-	39,52	19,27		58,79	-	2,53	1,35		3,88	6,60	-
Innovator	-	28,91	8,38		37,29	-	2,13	0,50		2,63	7,04	-
HZPC 2009												
Annabelle	-	140,00	20,29		160,29	-	-	-		9,75	6,09	-
Bintje	-	79,00	8,21		87,21	-	-	-		5,75	6,59	-
Desiree	-	169,25	32,38		201,63	-	-	-		10,75	5,33	-
Innovator	-	37,50	2,96		40,46	-	-	-		3,50	8,65	-
UGent BT1												
Annabelle	34,10	-	-	-	-	6,00	1,99	0,21		8,20	-	73,18
Bintje	29,10	-	-	-	-	6,66	3,65	0,23		10,54	-	63,20
Desiree	17,20	-	-	-	-	2,84	4,03	0,49		7,36	-	38,57
Innovator	27,20	-	-	-	-	6,39	3,15	0,21		9,75	-	65,55
UGent BT2												
Annabelle	52,00	-	-	-	-	11,34	2,34	0.62		14,30	-	79,29
Bintje	72,10	49,24	3,01		124,35	13,63	4,82	0,30		18,75	-	72,67
Desiree	21,80	47,94	10,40		80,14	3,44	7,11	0,86		11,42	-	30,16
Innovator	57,80	26,99	1,73		86,52	12,77	3,05	0,20		16,03	-	79,70
UCL BT1												
Annabelle	41,40	-	-	-	-	9,04	2,64	0,38	0,06	10,53	-	85,85
Bintje	34,10	27,80	6,80	4,80	73,50	6,82	2,68	0,35	0,34	8,07	-	84,51
Desiree	18,70	30,01	11,3	3,37	63,38	3,33	4,63	0,57	0,35	8,70	-	38,28

Innovator	17,70	-	-	-	-	2,57	1,92	0,19	0,11	3,52	-	73,01
UCL BT2												
Annabelle	98,00	27,70	2,59	0,83	147,30	15,51	4,13	0,26	0,11	20,01	-	77,51
Bintje	63,50	45,90	6,79	1,25	141,30	17,50	4,76	0,61	0,20	23,08	-	75,82
Desiree	32,40	50,10	11,53	18,30	112,40	4,99	7,43	0,99	1,49	14,90	-	33,49
Innovator	41,60	128,60	1,93	0,97	189,10	10,90	6,44	0,24	0,10	17,70	-	61,58

4.4.2 Anti-nutritional compounds – alkaloid levels

Highest levels measured in BT1, were 77 mg/kg of solanine, and 107 mg/kg of chaconine all cultivars confounded. For BT2 these compounds were below the level of detection (Tab. 12). In both cases, values were lower than the total glycoalkaloid content (maximum set level of 20 mg / 100g fresh weight).

Tab. 12 Potato - IPL tuber nutritional analysis results

		BT2 UGent				BT2 UCL			
Cultivar		**Annabelle**	**Bintje**	**Desiree**	**Innovator**	**Annabelle**	**Bintje**	**Desiree**	**Innovator**
Water (%)		78.2	81.1	84.2	77.9	80,8	73,90	84.7	76.8
Protein (%)		1.62	1.20	1.58	1.39	1,39	2,16	1.47	1.95
Fat (%)		0.06	0.04	0.08	0.04	0,04	0,03	0.08	0.07
Available carbohydrates (%)		14.23	14.40	10.79	14.15	15,5	18,15	10.83	17.93
TDF (%)		1.53	1.80	2.2	1.79	1,47	1,95	1.82	2.2
Minerals (%)		1,16	1,18	1.13	1,08	0,88	1,27	1.07	1.07
Of which (mg/100g)	Potassium	504	507	477	440	365	495	470	447
	Calcium	5,5	7.5	7.4	8.7	62	12.5	4.9	7.9
	Magnesium	29,4	22.2	22.6	26.7	24.9	26	20.2	25.6
	Iron	0,7	0.8	0,4	0,6	0,6	0,7	0.5	0.6
	Copper	1,1	0.5	0,7	0,8	0,3	0.5	0.3	0.4
	Zinc	1,1	0.9	1	1.9	0,5	0.6	0.4	0.5
	Manganese	0.18	0.11	0.13	0,13	0,22	0.26	0.23	0.22
	Phosphorus	108	87	89	90	-	110	195	248
Solanine (mg/kg)		< LOD*	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Chaconine (mg/kg)		< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Energy (for 100g)	kcal	67.0	66.4	54.6	66.1	62.4	72.8	53.6	84.6
	kJ	280.1	277.8	228.48	276.8	261.1	304.5	224.1	353.8

* LOD: level of detection.

4.5 Cultivar selection method and ranking

The HZPC greenhouse NFT hydroponic results from a comparative test indicate Innovator to be the slowest developing, which is corroborated by the initial data from UGent and UCL. It is also the cultivar the most sensitive to nitrogen fluctuation and therefor to tuber deformation.

Annabelle and Bintje were the best performing of the four cultivars and are at the top of the ranking. The highest harvests were obtained by the Annabelle cultivar which yielded 1.96 kg FW/m^2 (98g/plant) during BT2 at UCL (UGent reached 0.94 kg FW/m^2 with Annabelle in BT2). Bintje is second with 1.44 kg FW/m^2 in BT1 at UGent (72.1g/plant) and 1.27 kg FW/m^2 for UCL in BT2 (Tab. 10).

Annabelle also obtained the highest tuber number with an average of 8 tubers per plants, followed by Bintje with 7 tubers per plants.

UGent obtained in a post-bench test experiment a yield of 872g for one single Bintje plant. If this were to be reproduced at a bigger scale it would in theory correspond to the excellent yield of 15.8 kg FW/m^2.

As a comparison, the maximum yield estimation for Annabelle is 4.9 kg FW/m^2 (cultivated in HZPC greenhouse). NASA studies obtained a maximum of 7 kg FW/m^2 (Mackowiak Adv. Space Res. 2007). These numbers are good indicators of the yields we are willing to reach.

It is also important to notice that cultivars do not have the same life time. Annabelle seems to have a 3 months life time, but produces a good harvest. Bintje can produce several harvests, the first one 3 months after being transplanted into the gullies, and then every two months. UGent has been able to reach two homogeneous harvests and one smaller from the BT2 Bintje plants.

Desiree provided very poor yields in both BT1 and BT2, mainly because tuber induction was not efficient (tuberisation was hardly induced). Innovator is very sensitive to nutrient solution changes resulting in tuber deformation. These are the main reasons why these two cultivars are presently in the lower part of the ranking.

Tab. 13 UGent - Potato cultivar comparison table

Cultivar	Tuber FW yield (HZPC hydroponics)	Tuber DW yield – (field)	Tuber size	Plant height	Maturity
Annabelle	Very high	Low 18,4%	Small	Medium-High	Very early
Bintje	Low	Medium to high	Medium to large	Medium	Early to intermediate
Desiree	Very high	21,40%	Large	Medium	Intermediate to late
Innovator	Medium	High 21,30%	Large	Medium to low	Early to intermediate

4.6 References

Mackowiak, C. L., G. W. Stutte, et al. (1997). "Hydroponic potato production on nutrients derived from anaerobically-processed potato plant residues." Life Sciences: Life Support Systems Studies-I 20(10): 2017-2022.

Friedman, 2006, page16; Bushway 1981.

Sun-Ben Ku and al., (1977). “Effects of light, carbon dioxide, and temperature on photosynthesis, oxygen inhibition of photosynthesis, and transpiration in *Solanum Tuberosum*”. Plant Physiology (1977) 59, 868-872.

Wheeler R. M., Mackowiak, C. L, et al., (1999). “Nutrient, acid and water budgets of hydroponically grown crops”. Acta Horticulturae: 481, ISHS.

Wheeler R. M., Tibbitts T. W., (1997). “Influence of changes in day length and carbon dioxide on the growth pf potato”. Annals of Botany 79:529-533.

5 Potato (UCL)

See section 4 (Potato UGent), hydroponic system basic components identical to UGent, starting plant material also identical, as provided by HZPC.

5.1 Evaluation of experimental layout

5.1.1 Measuring plan

The measurements carried out during the two first bench tests are feasible. Gas exchange and chlorophyll fluorescence data scheduled every month for Phase II (less manipulation of the plants).

5.1.2 Setup

No major changes needed. The pH, EC and temperature of the solution were controlled manually as well as the water level. The nutrient solution container of 20L is sufficient. Some gullies overflowing via the side holes due to roots development (holes need to be drilled higher, or root development must be limited).
An identical system as in UGent is planned for Phase II.

5.2 Evaluation of growth environment follow-up

5.2.1 Settings

The UCL settings were as described for UGent

5.2.2 Chamber T/RH evolution

No significant deviations recorded.

5.2.3 Chamber CO_2 level

Measurements were not foreseen.

5.2.4 Nutrient solution environment

NFT thickness follow up was correct. Side holes were drilled too close (2cm) from the bottom of the gullies which can be source of overflowing.

5.2.5 pH and EC evolution

pH was always kept below pH 6.5 and EC evolved between 1.5 and 2.1.

5.2.6 Nutrient solution T

Control confirmed as sufficient.

5.2.7 Nutrient solution analysis

Accumulation of Zn in the nutrient solution observed: cause unknown.

More regular follow-up of N concentration is needed. In BT1, no nitrogen was added after tuber set as recommended by HZPC. But it appeared that HZPC was refilling the nutrient tank with tap water containing nitrate instead of distilled water for UCL and UGent. At the end of the culture, nitrogen was added but UCL dosed above the recommendations while UGent added a low amount; both labs obtained nevertheless a similar low yield due to initial stress and progressive development of opportunistic pathogens (see section 4.3 above). For BT2, after switching to growth solution, $Ca(NO_3)_2$ was added every two days in order to maintain a low level of nitrate in the nutrient solution. Indeed, the amount of N added during the tuberisation phase was not yet optimal. Desiree tuber induction was delayed and low due to too high N level. Weekly addition of $Ca(NO_3)_2$ were also too high in Bintje and in less extent in Annabelle since stolons were initiated from the tubers during the tuberisation phase. This parameter needs to be better adapted in the future to find the best compromise between good yield and plant survival.

5.3 Evaluation of monitoring of plant development

5.3.1 Photographic follow-up

Weekly manual photographing guarantees a sufficient level of documentation of crop development. Installation of cameras inside the gullies in order to follow roots, stolons and tubers development is planned for Phase II.

5.3.2 Detailed photographic observations

See 5.3.1

5.3.3 Growth assessment

Weekly photographing and measurements (size, number of leaves, stolons and tubers) guarantees a sufficient level of documentation of crop development. Annabelle produced the taller plants. Bintje produced plants with a good development of leaves on the main stem and few axillaries. Innovator produced small but branched plants and few roots. Desiree produced more roots and stolons.

5.3.4 Gas exchange data, water usage

Water use efficiency, expressed as amount of biomass produced per liter of water transpired, is a critical parameter in agriculture when irrigation is limited.

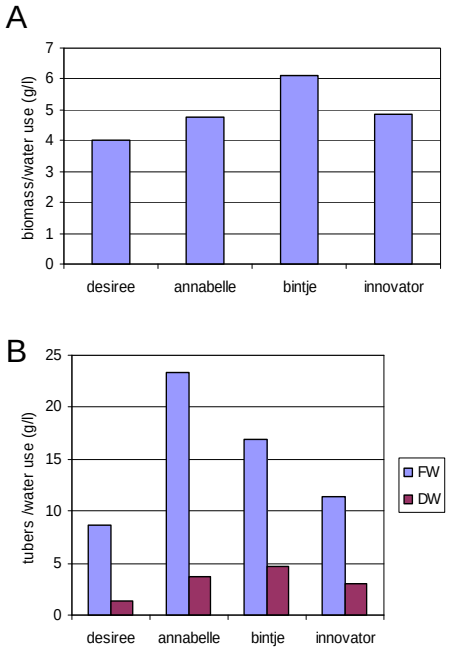

Fig. 4 UCL - Water use efficiency
(A) total plant biomass (DW) produced per liter, (B) tuber biomass produced by liter.

5.4 Evaluation of crop harvest

5.4.1 Yield

In BT1, weak plant development and yield were induced by suboptimal starting conditions (non-optimal transport of the in-vitro plants) and inadequate nitrogen provision (too late and too high, see 5.2.7). Plants were susceptible to opportunistic plant pathogens likely present in the environment.

The conditions (mainly solution composition) were better in BT2 compared to BT1 since the tuber yield increased between 2-3 times. The yields parameters are presented in Tab. 10 and Tab. 11 (UGent chapter). Annabelle produced more tubers and showed the best yield, edible to non-edible biomass ratio and water use efficiency in term of fresh weight while Bintje showed the best yield, edible to non-edible biomass ratio and water use efficiency in term of dry weight. Innovator produced the biggest tubers. Tuber initiation was delayed in Desiree so that the harvest of this cultivar was postponed. The amount of harvest at UCL allowed nutritional analysis. These were carried out at IPL.

5.4.2 Anti-nutritional compounds – alkaloid levels

see 4.4.2 and Tab. 12 (UGent chapter)

5.5 Cultivar selection method and ranking

See also 4.5 and Tab. 13 (UGent chapter)
Observations of UCL-BT2 confirm most observations of UCL-BT1. Results obtained in UCL corroborated observations of the table that summarises the diversity of the 4 pre-selected cultivars (Tab. 13) and UGent data.
Annabelle produced several small tubers with a high FW yield and was the first to initiate tubers. Bintje produced tubers with a high DW content. They produced the best tuber harvest (Annabelle: 1.96 kg/m², Bintje: 1.27 kg/m²). Annabelle and Bintje were the best performing of the four cultivars and are at the top of the ranking.
Desiree provided poor yields in both BT1 and BT2, mainly because tuber induction was not efficient (tuberisation was hardly induced). Innovator produced big tubers but is very sensitive to nutrient solution changes resulting in tuber deformation. These are the main reasons why these two cultivars are presently in the lower part of the ranking.

6 Soybean (UNapoli)

6.1 Evaluation of experimental layout

6.1.1 Measuring plan

The measurements scheduled were similar to those reported for the BT1.

6.1.2 Setup

The hydroponics Nutrient Film Technique (NFT) system consisted of 12 independent double gullies. In BT2, one new soybean cultivar (Cresir) was tested in addition to the 3 cultivars grown in BT1 (Atlantic, Regir, PR91M10). As in BT1, 3 double gullies (corresponding to 42 plants) were used for each cultivar, in a randomized experimental layout.

6.2 Evaluation of growth environment follow-up

6.2.1 Settings

Tab. 14 UNapoli - Settings

Photoperiod	12-h Long Day
Light intensity	350 µmol m^{-2} s^{-1}
Room temperature Humidity	20/26 °C (Night/Day) 65-75% (set point 70%)

The same temperature and relative humidity conditions used in the BT1 were adopted in BT2, while the light intensity at the crop level decreased in comparison to the previous bench test (from 600 to 350 µmol m^{-2} s^{-1}), because of the reduction effect of the glass pane.

6.2.2 Chamber T/RH evolution

The T and RH monitoring showed that the conditioning system was efficient in keeping the target values, established on the basis of the optimal level for soybean.
RH set point was reduced during the last month of the growing cycle in order to improve the desiccation of soybean pods.

6.2.3 Chamber CO_2 level

UNapoli growth chamber was not sealed. On the contrary, periodic changes of the internal air were performed by two small fans (for suction and extraction), placed at the top and at the

bottom of one wall. This implies that no correlation can be done between air CO_2 concentration and assimilation rate.

6.2.4 Nutrient solution environment

Gully inclination and nutrient solution flow rate used in BT1 were confirmed. By contrast, the salt concentration (EC) of nutrient solution was increased from 1.2 to 2.0 mS/cm, because of nutrient deficiency observed during the BT1.

6.2.5 pH and EC evolution

pH and EC in the recirculating nutrient solution showed fluctuations around the target values during the whole growing cycle so that daily adjustments were required in order to maintain the proper level. In this respect, the complete renewal with fresh nutrient solution improved the nutrient solution management.

6.2.6 Nutrient solution T

Temperature of the nutrient solution varied from 18°C during the night to 22°C during the day. This parameter was found to be not critical for soybean.

6.2.7 Nutrient solution analysis

Nutrient solution analyses showed a progressive depletion of P and K during the experiment. This pattern could be taken into account in the fertigation management in subsequent experiments, even though, in our experience, it did not determine nutrient deficiency.

6.3 Evaluation of monitoring of plant development

6.3.1 Photographic follow-up

The photographic overview of plant growth at 15-day interval helped in monitoring the crop development.

6.3.2 Detailed photographic observations

None.

6.3.3 Growth assessment

Soybean plants grown in NFT system did not show any phytosanitary and nutritional problem during the BT2. The nutrient deficiency observed during the BT1 was prevented by increasing the salt concentration of nutrient solution (EC from 1.2 to 2.0 mS/cm).

The flowering occurred during the 7th week from sowing in all the cultivars tested. A wide leaf fall was observed starting from the 13th week, when the pods were completely developed. Differences in the earliness of pod ripening were found among the cultivars: Cresir plants were the earliest in reaching the seed maturity.

6.3.4 Gas exchange data

Measurements of photosynthesis, transpiration rate and stomatal conductance did not show relevant differences in physiological behaviour in the 4 soybean cultivars.

6.4 Evaluation of crop harvest

The harvests of soybean pods started from the third to the fourth week of June, depending on the different 4 cultivars, and lasted until the end of July. Cresir and Regir were the earliest and the most productive cultivars (450 g of seeds on average; total yield), followed by Atlantic (about 420g) and PR91M10 (about 320g).

6.5 Cultivar selection method and ranking

Parameters not considered in BT1 and BT2:
- Volatile organic compound (VOC) and Root exudates production
- Resistance to stress (plants during bench test should be cultivated under optimal conditions)

From Table 23 from TN 98.3.1, page 68 the selection criteria applicable to the bench test data are shown below:

Tab. 15 UNapoli - Soybean cultivar bench test selection criteria

Criteria	Major parameter(s)	Associated parameter
Crop cultivar stature	Growth space	Handling (harvest)
	Growth period length	Crop senescence
Cultivar harvest index	Waste production	Waste degradability
	Influence of plant growth system	
Cultivar nutritional composition	Absence of anti-nutritional compounds	Pro-nutritional compounds
Cultivar edible part composition	Processability	Possible conflict with levels of pro-nutritionals
	Storage stability	Storage time
Water use efficiency	Maximum water use Increases growth efficiency	High water turnover rate – regeneration rate

7 Conclusion

The general conclusions from the second bench test are:

- The NFT-systems function properly; nutrient control management has been improved since BT1 but needs further optimisation.
- Harmonised phyto-pathological and pest management protocols would enable efficient and reproducible control of the most common stresses in future test trials.
- Sealed chamber tests at UoGuelph deliver continuous assimilation and transpiration data, but ethylene and oxygen accumulation need to be limited. With the current hardware, the available approach consists of scheduled venting (opening of the access door) of the chamber every 2 weeks.
- The conventional walk-in room setups can obtain a total transpiration amount per cultivar based on water usage, as recorded from the hydroponic tank (manual) refill adjustments, as a function of time.
- Maturation of wheat crops was critical in BT1, but modulation of the nutrient solution by diminishing the EC substantially improved maturation. Some bread wheat cultivars seem relatively insensitive to the high N-levels. Cultivars don't have the same resistance to lodging.
- The need for nutrient solution composition changes upon crop development. This precludes the approach of staggered growth with a common hydroponic feeding system, where all gullies regardless of the developmental stage of the plants growing in it, would receive the same nutrient mix.
- As final conclusion, it has to be noticed that in BT2 all crops developed correctly thanks to better balanced nutrient solutions. No critical disease or significant physiological disorders were observed during BT2. All crops and cultivars fructified and harvests quantities were improved or at least kept identical to BT1. Furthermore, nutritional analysis of the edible parts did not reveal any abnormalities or toxicity.

8 References

Clark, .M., T.N. McCaig, R.M. Depauw, R.E. Knox, F.R. Clarke, M.R. Fernandez, AND N.P. Ames. 2006. Registration of 'Strongfield' Durum Wheat. Crop Sci. 46:2306–2307.

Drake et al., (1996).

Friedman, M., Potato glycoalkaloids and metabolites: Roles in the plant and in the diet. J Agr Food Chem 2006, 54, 8655-8681.

Mackowiak, C. L., G. W. Stutte, et al. (1997). "Hydroponic potato production on nutrients derived from anaerobically-processed potato plant residues." Life Sciences: Life Support Systems Studies-I 20(10): 2017-2022.

Maleszewski et al., (1988).

Sun-Ben Ku and al., (1977). "Effects of light, carbon dioxide, and temperature on photosynthesis, oxygen inhibition of photosynthesis, and transpiration in *Solanum Tuberosum*". Plant Physiology (1977) 59, 868-872.

Wheeler R. M., Mackowiak, C. L, et al., (1999). "Nutrient, acid and water budgets of hydroponically grown crops". Acta Horticulturae: 481, ISHS.

Wheeler R. M., Tibbitts T. W., (1997). "Influence of changes in day length and carbon dioxide on the growth pf potato". Annals of Botany 79:529-533.