MELiSSA

TECHNICAL NOTE

TECHNICAL NOTE 85.2

Baseline Data for beet and lettuce: Test Plan, Test Prediction and Test Procedure Document

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Acronyms and Abbreviations

CSA: Canadian Space Agency
CESRF: Controlled Environment Systems Research Facility
ESA: European Space Agency
GC: Gas Chromatograph
HPC: Higher Plants Chamber
HPLC: High Pressure Liquid Chromatography
HPS: High-Pressure Sodium (lamps)
IRGA: Infra-Red Gas Analyzer
MELiSSA: Micro-Ecological Life Support System Alternative
MH: Metal-Halide (lamps)
MPP: MELiSSA Pilot Plant
NCER: Net Carbon Exchange Rate
PPF: Photosynthetic Photon Flux
SEC-2: Sealed Environment Chambers (x 2)
TN: Technical Note
UAB: University "Autonoma" of Barcelona
UBP: University Blaise-Pascal
UoG: University of Guelph
V-HID: Variable-High-Intensity Discharge (lamps)
VPD: Vapour Pressure Deficit

1. Introduction

Considerable advances have been made in the last few years at the CESRF in devising management strategies and control algorithms for crops grown in sealed environments. Specifically, the utility of Net Carbon Exchange Rate (NCER) analysis has been successfully demonstrated over the last few years as a means to accurately assess higher plant responses to a range of environment (control) variables at the full canopy scale. Particular emphasis has been placed on quantifying plant responses to atmospheric CO_2 concentration and light intensity, particularly with beet, lettuce and to a lesser extent kale and soybean. The NCER approach involves the determination of plant growth responses (biomass gain) through the direct measure of net sequestration of CO_2 in a hermetically sealed plant growth chamber. This is done by integrating metered compensatory injections of bottled CO_2 into the chamber in order to replace that taken up by the canopy through net photosynthesis. The CESRF has two chambers dedicated to simultaneous NCER analysis and crop production at the full canopy scale. These chambers are designated as the SEC-2 chambers (Sealed Environment Chambers – x 2).

The NCER approach has been used to validate a canopy photosynthesis model outlined by Thornley. The model is based on incremental biomass partitioning to leaves, an increase in leaf area and, hence, effective photosynthetic area. Provision is made in the model for light attenuation within the dense canopy as Leaf Area Index (LAI) develops. The Thornley model has been validated using data collected from the SEC-2 chambers for beet and lettuce. The model has performed well, but calibration of the model tuned for staged (multiple age classes) culture is still required.

The principle of model development for the HPC is similar to other MELiSSA compartments although attention must also be duly paid to factors affecting partition of the plant biomass into edible and inedible fractions, nutrient uptake and evapo-transpiration. As part of our historical empirical production trials, including NCER analysis, we have collected a considerable amount of baseline data relating to biomass partitioning, mineral composition, proximate (carbohydrate, protein, fat, ash) composition and nutrient uptake / depletion profiles from a hydroponics reservoir (using off line HPLC) for beet and lettuce. These data have been provided to MELiSSA partners (eg. UBP) for use in global simulation and stoichiometric modelling of the HPC.

Our team has also been involved in the calibration of a steady state nutrition model which relates nutrient uptake (by individual ion) to the relative growth rate of the canopy estimated from non-destructive NCER determinations. Our early work on the steady state model proved promising but more data collected in the frame of Phase A will be required in order to fully assess this modelling approach's utility in the MPP.

The research team at CESRF accepts the requirements for Phase A related work and understands that successful integration of a Higher Plant Chamber prototype in the MPP requires the development of algorithms for the lower-level control of the HPC based on mathematical models of gas exchange response to environment variables, such as light. This is consistent with the modelling approach used in the other MELiSSA compartments. While we have already conducted a number of baseline empirical studies with beet and lettuce, work remains on the characterization of other MEliSSA crops, including wheat, with respect to mineral and nutritional composition and gas exchange characteristics. Such data are essential for evaluating the nutritional quality of foodstuffs derived from crops grown in an HPC and for developing model-driven control strategies for the HPC based on, for example, light intensity variation as a means to control photosynthetic (gas exchange) rates. Data must therefore be collected and presented in a way that are useful to the MELiSSA team member(s) working on diet formulation and modelling (UBP).

The CESRF has, for a number of years, been involved in the collection of baseline data required for crop metabolic characterization. These include the computer logging of key environment variables (CO_2 concentration, Light Intensity, Temperature, VPD, Pressure) and the regular monitoring of nutrient uptake in hydroponics (using off-line HPLC), evapo-transpiration and logistical (labour/replacement part) requirements. All of our studies include, at crop harvest, analysis of biomass and partitioning, leaf area, proximate composition, fibre content and mineral composition. All of these tissue data, with the exception of fibre content determination, are collected at internal UoG laboratories. Fibre analysis is conducted by an external laboratory in the City of Guelph experienced in such analyses for the terrestrial agriculture community.

Collection of data for wheat, first involves the identification, proposal and selection of a cultivar suitable for inclusion in the MELiSSA MPP. The CESRF has already identified potential cultivars which are German in origin, and have been used successfully in other crop science laboratories at the University of Guelph. These cultivars, which include ‘Sable’ and ‘606’ exhibit short stature, high photosynthetic potential, growth promotion through extended photoperiods and are among the leading cultivars used in terrestrial agriculture in Ontario, Canada. Some work will be required in adapting the NFT culture system used in the SEC-2 chambers to wheat culture given the possibility for increased planting density. This development/adaptation risk is expected to be minimal.

The Thornley canopy photosynthesis model has been successfully implemented in the EcoSim Pro software by ESA (RD5). The CESRF has retained a copy of this code for use in comparing existing and future data collected in support of Phase A activity. Validation of the model using empirical data collected at CESRF is therefore readily possible given our experience with the ECOSim Pro modelling software, understanding of the modelling framework and ability to collect the required data in support of the modelling endeavour.

Given the CESRF's successful history in collecting baseline data for MELiSSA candidate crops, such as those required in Work Package A, and given the history of successful collaboration between the CESRF and other MELiSSA partners working on issues of HPC modelling, the general risk to deliverables under Phase A of the proposed research is negligible.

The purpose of this TN is to provide, in as much detail as possible, the planned experiment procedures for generating baseline data for wheat. It includes the procedures for selection of a suitable wheat cultivar and the methods for its production in batch and staged culture.

2. Review of Facilities for Data Collection at the CESRF

2.1. SEC-2 Higher Plant Chambers

The sealed environment chambers at CESRF (SEC-2) are now used exclusively for the generation of baseline data for MELiSSA activity. These chambers will be used for Phase A activity as originally proposed.

The SEC-2 chambers are constructed primarily of relatively inert materials such as glass, Teflon® and stainless steel. This minimizes off-gassing into the sealed environment. Typically, leakage rates of the chambers ranges from 4 – 7% per day. Leakage rates are calculated from the depletion of CO_2 as a marker gas in an empty, sealed chamber, over time. The SEC specifications and capabilities are detailed in Table 4.1.1.1.

Table 2.1-1 Sealed Environment Chambers Specifications and Capabilities

1. Dimensions:
   - a: Volume = 29 m^3 (430 ft^3) (4.5 m x 2.8 m x 2.3 m) per chamber
   - b: Plant Growing Area = 5 m^2 (54 ft^2) (2 m x 2.5 m) per chamber
2. Materials used:
   - a: Stainless Steel 316 (walls, floor, valves, plumbing)
   - b: Tempered Glass (roof)
   - c: Teflon (tubing, gas expansion bladders)
   - d: Polypropylene (tubing, valves)
   - e: Heresite (oxidation barrier on fans, heat exchangers, motor parts)
   - f: Viton (O-rings, solenoid seats)
   - g: Silicone sealant (DOW-Corning RTV 732) and silicone grease
   - h: PVC (This material is temporary and only used externally
3. Analyzers:
   - a: LiCor LI6262 Gas Analyzer for CO_2/H_2O vapour
   - b: Instrumar Custom Gas Analyzer for CO_2/O_2
   - c: Gas Chromatograph/Mass Spectrometer (HP-5890/HP-5971)
   - d: Dionex DX500 HPLC Ion Chromatograph (offline)
4. Lights:
   - a: The glass topped chambers have externally mounted lighting with 9 x 600 W High Pressure Sodium (HPS) and 6 x 400 W Metal Halide (MH) lamps to provide a light level between 400 and 450 µmoles m^{-2} s^{-1} PAR at stand height.
5. Sensors:
   - a: Hydroponics system
     - 1) Electrical Conductivity (one per chamber)
     - 2) pH metres (two per chamber)

3) Flow switch (one per chamber)

b: Chamber

1) Aspirated Air Humidity sensors (2/chamber)
2) Aspirated Air Temp. sensors (2/chamber)
3) Root Zone Temp. sensors (4/chamber)
4) LiCor Quantum Sensor (one per chamber)

6. Environment Control:

a: Temperature (10 to 40 °C) $\pm$ 0.2 °C
b: CO_2 Concentration (10 to 5000 ppm) $\pm$ 10 ppm
c: O_2 Concentration (18 to 25 % without special security equipment)
d: Relative Humidity (50% to 95%) $\pm$ 5%

A photograph of the interior of one of the SEC-2 chambers to be used in Phase A studies follows.

Figure 1 Photograph of one of the SEC-2 Chambers be Used in Phase A studies. The chamber production area is 5m^2 .

2.1.1. Air Handling

In the SEC-2 chambers air is conditioned for temperature and humidity and is re-circulated inside the chambers. Externally supplied chilled water and steam are circulated through sealed and "heresite" coated (baked oxidation barrier) heat exchange coils mounted in an internal plenum at the rear of each chamber. Condensate from the chilled water coil is measured and collected in the hydroponics nutrient reservoir. Heresite coated fans and fan motors with silicone covered wiring are also mounted in the plenum and distribute the air through stainless steel ducts with baked enamel louvres. Modulated steam and chilled water valves effect temperature and dehumidification control of the aerial environment.

2.1.2. Hydroponics Nutrient Solution

The nutrient requirements for the plants are supplied in a hydroponics medium stored in a 200 L Teflon® liner located inside the chamber or a 200 L steel reservoir. The solution is pumped into the chamber in polypropylene tubing to the head of a sloped stainless steel trough. The 2.5 metre long trough, of which there are usually ten in each chamber, is designed to accommodate a variety of root media as a substrate for the hydroponics solution. These include glass beads, rockwool, lecca (expanded clay particles), silica sand, etc. Gravity assists the return of the solution to the external reservoir. The condition of the solution with respect to pH and electrical conductivity is monitored and adjusted continuously through measured injections of acid, base and/or various nutrient mixes. Alternatively the spent solution is replaced with fresh solution at regular 5 day intervals (without breaking an atmospheric seal)

2.1.3. Volume and Pressure Control

Each SEC-2 chamber is fitted with ten 200 litre double sealed Teflon® liners (Now Technologies Inc., Minneapolis, MN) manifolded on a 50 mm diameter stainless steel tube which protrudes through the rear wall of the chamber. This provides a total expansion volume potential of 2.0 m^3 or $\pm$1.0 m^3. Given the 29 m^3 internal volume of the chamber, this represents about 7% or $\pm$3.5% volume expansion/contraction in response to possible temperature fluctuations inside the chamber. The total temperature range influencing gas volume in the chamber represented by this capacity is about 20 Kelvin degrees ($\pm$10 degrees).

2.2. Test Equipment and Analytical Facilities

The CESRF has an array of test equipment to support Phase A related research. They are summarized below. Computers for data acquisition and control are readily available.

The CESRF and University of Guelph has site/individual licences for various software to be used in model validation and statistical analyses, including ECOSim Pro, Matlab/Simulink and S-Plus. Staff have dedicated computers with standard word processing, presentation and spreadsheet software installed. The CESRF also has Microsoft Project Management software for maintaining records of project progress.

3. Summary of Historical Crop Production Methodology and Data from CESRF – Beet and Lettuce

At the MELISSA general working meeting held 29/30 November 2001, it was decided, initially, that three crops be selected for production trials within the MELiSSA Pilot Plant. The selected species were wheat (*Triticum aestivum* L.), lettuce (*Lactuca sativa* L. cv. Grand Rapids) and beet

(*Beta vulgaris* cv. Detroit Medium Red). These crops are representatives of plants with varying harvest index (edible biomass/total biomass, dwb) and mineral composition. As such, they each provide a unique challenge to the first compartment.

Since the November 2001 MELiSSA meeting, the UoG has been involved in the collection of baseline data sets for two of the three candidate crops; beet (*Beta vulgaris* cv. Detroit Medium Red) and lettuce (*Lactuca sativa* L. cv. Grand Rapids). Empirical production trials have included replications in batch culture (single seeding date) using either a deep water or an NFT hydroponics system. The deep water culture trials were conducted in 2002 at a lower planting density than those reported for NFT (17.6 and 24 plants m^{-2}, respectively). Since these NFT data, collected in 2004, are used to scale the HPC for the Pilot Plant a description of the methodology used to collect them is warranted.

The 2004 data set was collected from three production trials of each crop at the full canopy (120 plants per chamber) scale. Experiments were completed in August 2004. The data set includes Net Carbon Exchange Rate (NCER) and nutrient uptake for a developing canopy, stand level NCER as a function of both light intensity and crop age and photosynthetic responses to CO_2 and light intensity at the leaf scale. A summary of the available data sets and key variables collected to date may be found in the tables and figures below. All experiments were conducted in the SEC-2 chambers at the UoG CESRF (see Section 7.2)

Paramater	Batch Cultures			Staged Cultures	
Identification	Rep 1 GW0204 - Lettuce-Batch-	Rep 2 GW0604 - Lettuce-Batch-	Rep 3 GW0704 - Lettuce-Batch-	Rep 1 GW0904 - Lettuce-Staged	Rep 2 GW1005 - Lettuce-Staged
Chamber used	SEC-2	SEC-2	SEC-1	SEC-1	SEC-1
Date of seeding	06/02/04	25/05/04	09/04/04	07/09/04	19/9/05
Experiment start in chamber	25/02/04	14/06/04	08/07/04	27/09/04	11/10/05
Experiment end date	29/03/04	21/07/04	12/08/04	16/12/04	20/12/05
Photoperiod (day-hours)	14	14	14	14	14
Demand temperature (ºC day/night)	25/20	25/20	25/20	25/20	25/20
Demand CO_2 (ppm)	1000	1000	1000	1000	1000
Hydroponics system	NFT	NFT	NFT	NFT	NFT
Number of plants in chamber	120	120	120	108	108
Production area (m^2)	5	5	5	4,5	4,5
Planting density (plants m^{-2})	24	24	24	24	24

Table 3-1. **Experiment summary sheet for lettuce batch and staged cultures conducted in 2004 and 2005.**

Paramater	Batch Cultures			Staged Cultures	
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2
Identification	GW0204 - Beet-Batch	GW0404 - Beet-Batch	GW0504 -Beet-Batch	GW1204-Beet-Staged	GW0106-Beet-Staged
Chamber used	SEC-1	SEC-1	SEC-1	SEC-1	SEC-1
Date of seeding	05/01/04	10/03/04	19/04/04	16/12/04	28/12/05
Experiment start in chamber	04/02/04	2/04/04	19/05/04	15/01/05	27/01/06
Experiment end date	17/03/04	07/05/04	24/06/04	24/06/05	19/04/06
Photoperiod (day-hours)	14	14	14	14	14
Demand temperature (ºC day/night)	25/20	25/20	25/20	25/20	25/20
Demand CO_2 (ppm)	1000	1000	1000	1000	1000
Hydroponics system	NFT	NFT	NFT	NFT	NFT
Number of plants in chamber	120	120	120	96	96
Production area (m^2)	5	5	5	4	4
Planting density (plants m^{-2})	24	24	24	24	24

Table 3-2. .Experiment summary sheet for beet batch and staged cultures conducted between 2004-2006.

For each of the 2004 studies conducted using NFT, beet or lettuce were germinated in a research greenhouse at the UoG, using Rockwool© (1.5 " sq, 9.4 cm^2) cubes. The plants remained in the greenhouse until there was sufficient root exposure to facilitate planting into an NFT hydroponics system (approximately 20 days after seeding) and the SEC-2 chambers. During the germination period, seedlings were watered regularly with distilled water and once weekly with a fertilizer solution (20-8-20 N-P-K commercial mix having an EC = 2.5 $dS \cdot m^{-1}$).

A total of 120 seedlings were transferred to each chamber (12 plants per stainless steel trough). The Rockwool© cubes containing seedlings were positioned in larger cubes (4" x 4" x 2.5", 625 cm^3) to improve water distribution in the hydroponics channels. Trays were covered once the blocks were in position so as to minimize the growth of algae on the surface of the Rockwool©. For most experiments, leaf area was destructively determined on the remaining (un-planted) seedlings using a Li-Cor 3100 Leaf Area Meter (Li-Cor, Lincoln, NE, USA). These initial leaf area estimates were used as input variables into the NCER models described in the sections below.

Plants were grown under conditions of between 400-450 µmol m^{-2} s^{-1} PAR lighting at stand height as supplied by the High Pressure Sodium (HPS) and Metal Halide (MH) lamps mounted externally to the chambers. A 14/10 hr light/dark (06:00 - 20:00) photoperiod was used and coupled to a 26/20 ^oC day/night temperature. Atmospheric CO_2 concentrations were fixed in these full canopy studies at 1000 µL L^{-1} CO_2 as supplied through an external tank and computer regulated compensatory system using bottled CO_2. Average relative humidity in the chambers over all replications was 73% ± 5% with no active control.

The nutrient solution used in this study had the following composition: 1.5 mM PO_4^{3-}, 3.62 mM Ca^{2+}, 4 mM NH_4^+-N, 11.75 mM NO_3^--N, 5 mM K^+, 2 mM SO_4^{2-}, 1 mM Mg^{2+}, 0.005 mM Mn^{2+}, 0.025 mM Fe^{3+} as Fe-DTPA, 0.0035 mM Zn^{2+}, 0.02 mM B^{3+}, 0.008 mM Na^+,0.0008 mM Cu^{2+}, 0.0005 mM Mo^{6+}. This solution had an average EC of 1.9 $dS \cdot m^{-1}$. The pH of the solution was adjusted to approximately 5.5 with the addition of 1 M $NaHCO_3$ solution. At the initial transplant of the seedlings, 220 L of nutrient solution was added to the pool prior to the chamber doors being sealed. Every five days after, the solution was pumped out of the internal reservoir (without breaking the atmospheric seal) to replace it with a fresh 220 L volume having the same composition as noted above.

At the start of each solution changeover period, the total solution volume to be added was measured with a large graduated tank and three 25 mL samples were taken of the fresh solution for off-line HPLC analysis. The old solution was pumped out of the reservoir and its volume measured. Samples were also taken for HPLC analysis in triplicate. Solution volumes were measured at the start and end of the change-over periods to allow for the correction of elemental analysis results due to evapo-transpiration. During each period no amendments were made to the solution composition in any way. All solution samples were analyzed using the Dionex HPLC

Model DX-120 (Sunnyvale, CA, USA) for the ions of interest which included Cl^-, PO_4^{3-}, Ca^{2+}, NH_4^+-N, NO_3^--N, K^+, SO_4^{2-}, Mg^{2+}, and Na^+.

All plant material was harvested at the end of the study. Plant parts, with the exception of roots, were sampled at the individual plant scale. Harvested root material was pooled by each trough in the chamber. Tissue water content was measured as the difference between fresh and dry weights, obtained after at least four days in a drying oven at 60 ºC. Chamber water balance was also determined from evapo-transpiration estimates and plant water content estimates derived from dry and fresh plant weights.

Harvested tissue was also pooled for mineral content analysis. Edible and inedible fractions of each crop were analyzed using UoG Laboratory Services Protocol SNL-1020 and SNL-104.

Leaf area was measured on a sub-sample of the plants harvested using a Li-Cor 3100 Leaf Area Meter (Li-Cor, Lincoln, NE, USA).

The Net Carbon Exchange Rate (NCER) of the developing stands was determined non-destructively using a gas compensation technique. The computer controller maintained internal chamber CO_2 concentrations during the day-light hours so that any net carbon gain by the stand through photosynthetic activity was compensated for by injections into the chamber volume from an external tank. The metered flow of CO_2 injections was used to calculate day time carbon gain. During the dark period it was not possible to remove CO_2 from the chamber to achieve static conditions and as such the difference in observed CO_2 and demand was used to determine stand respiration rates (expressed as negative NCER). The sum of these signed NCER estimates over a 24 hour period (in moles C) yielded daily carbon gain (DCG).

All plant material was harvested at the end of each study. Plant parts, with the exception of roots, were sampled at the individual plant scale. Harvested root material was pooled by each trough in the chamber and sent for mineral, proximate and fiber analysis. The methods employed in tissue analyses (by an external laboratory) are outlined below.

- Proximate Analysis (Food Chemistry Laboratory – UoG): Fat, Protein, Ash, Carbohydrates, Energy, Moisture, Methods: FC-PR-109; 211 FC-CT-302; 305;306;FC-LP-203

- Mineral Analysis (Soil and Nutrient Analysis Laboratory): Total C, Ca, K, N, P, Mg, Methods: SNL-005; SNL-1020

Fiber Analysis (Agri-Food Test Services, Guelph, Ontario) :Acid Detergent Fibre, Neutral Detergent Fibre, LigninThe following tables provide a summary of data collected on tissue mineral composition. As described in the baseline data collection methodologies above, at harvest, plants were separated by part (roots, hypocotyl-beet, leaves). Tissue was pooled across all growing trays in each experiment.

Data presented in the above table sets were used to calculate nutrient and carbon dioxide uptake during the period in the chambers by multiplying the average daily plant part growth rate by the mineral content for the respective plant part. Calculation results were used in the steady state model assessment of gas and nitrogen balance described below.

4. General Research Plan for Phase A Activity

Appended to this section is the proposed Study Plan and Logic chart for Phase A of the proposed Statement of Work.

The project Work Breakdown Structure (WBS) is provided in the Project Management section of this proposal. WBS references are cited in this research plan for clarity. The WBS should be consulted for complete task lists, predecessors and labour allocation.

Phase A is dedicated to the collection of baseline crop data for wheat, lettuce and beet with emphasis on wheat. The Phase begins at project kick-off with a concurrent literature review on the metabolic characteristics of the three aforementioned MELiSSA candidate crops. Particular attention will be devoted to wheat in the literature review. Following literature review, a wheat cultivar suitable for inclusion in the MPP will be proposed to ESA for selection. WP 4 will see production trials conducted with wheat preceded by the completion of our current replications with beet and lettuce, before the formal test plan is approved (WP 3). The final WP will see the report of results from these empirical studies including data on NCER, biomass mineral composition, proximate composition, fibre content, nutrient uptake, evapo-transpiration and biomass partitioning including leaf area.

Work Package 2 – State of the Art (WBS 3.1)

Allied Task in WP 1: WBS 2.4.1.1 – Preparation of Technical Note TN 1

WBS 3.1.1 – Continue Current Lettuce/Beet Staged Cultures in SEC2-Chamber 1

In order to promote the timely generation of baseline data and to finalize current replications of our staged culture trials with lettuce and beet, a task will be undertaken to continue collecting data in SEC-2 Chamber 1 while TN 1 – State of the Art is being prepared and approved. These experiments will continue until acceptance of the test plan for continuation of Phase A studies with wheat in the SEC-2 chamber (WBS 3.2.2). Depending on the time of project kick-off, it is anticipated that the one additional staged beet and one staged lettuce replicate, each, will be completed prior to our investigations with wheat in the SEC-2 chambers.

This task will be conducted under the current operating protocols and experimental plans outlined in our current Canadian Space Agency contract but any modifications made by ESA will be accepted before the start of our crop cultures. Data collected include:

- Standard seedling leaf area using a Li-COR 300C Leaf Area Meter collected on a sub-set of un-planted seedlings (15 – 20 seedlings) sampled at start-up and planting of the crop in the SEC-2 chamber,

- Profiles of chamber atmospheric temperature, humidity (VPD), light intensity and solution temperature over the duration of the period of closure,

- Chamber ethylene concentrations measured at 5 day intervals using an off-line GC

- Gas-exchange (CO_2, O_2), collected at three minute intervals by an Infrared-Gas Analyzer (IRGA) and Paramagnetic analyzer and logged by the HPC controller,

- Nutrient and Water Uptake (NH_4^+-N and NO_3^--N, NO_2^--N, PO_4^{3-}, SO_4^{2-}, Fl^-, Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+} and water) as determined from sampling of the hydroponics solution over its period of closure and off-line HPLC analysis. Water uptake is determined from changes in solution volume (± 2 L) at the time of solution replacement (5 day intervals) and nutrient uptake is measured over the same interval by difference of start and end concentrations corrected for water uptake (in triplicate),

- Edible biomass, nutrient content (carbon, nitrogen, phosphorus, potassium, calcium and magnesium) and proximate composition including proteins, lipids and carbohydrates,

- Inedible biomass, nutrient content (carbon, nitrogen, phosphorus, potassium, calcium and magnesium), fibre (soluble and non-soluble), hemi-cellulose, cellulose and lignin.

Typically, the staged cultures of beet and lettuce last 60 and 90 days respectively although the timing and length of these particular production trials will be determined by the timing of the acceptance of the Phase A test plan for wheat studies in the SEC-2 chamber (WBS 3.2.2).

Throughout the duration of the staged culture, samples of the crop biomass will be taken at 10-day intervals and sent for analysis. Depending on the availability of harvested biomass, particularly in the youngest age classes, tissue is therefore sent for analysis representing range of stages in crop development, including those at maturity.

For each study, all experimental data will be compiled into an EXCEL spreadsheet, documented for clarity in data work-up and analysis and archived for availability to MELiSSA partners.

WBS 3.1.2 - Literature Review of baseline Crop Data – Lettuce, Beet and Wheat

This task will begin at project kick-off and will see a comprehensive literature review for three MELiSSA candidate crops. The literature review will update the reviews already completed by CESRF in the frame of other contracts and in support of our current and on-going studies with beet and lettuce. Focus will therefore be paid on literature review for wheat. Data collected will include, whenever available;

- Standard seedling leaf,
- Gas-exchange (CO_2, O_2), collected in controlled environments,
- Nutrient uptake from hydroponics or soil systems and tissue mineral content,
- Edible biomass proximate composition,
- Inedible biomass fibre (soluble and non-soluble) hemi-cellulose, cellulose and lignin contents

The literature review process will also be used to identify appropriate culture conditions for wheat grown in closed environments including hydroponics solution composition, culture system (NFT vs Deep Water Culture), germination requirements, temperature and humidity requirements, CO_2 enrichment tolerance and growth response, photoperiod sensitivity and the response to day length extension (i.e. 24 hrs light).

WBS 3.1.3 – Identification of a Suitable Wheat Cultivar for Baseline Studies – Literature Review and Supplemental Report

From the results of the literature review conducted above, a suitable wheat cultivar will be identified and proposed to ESA for final selection. Variables of interest in the selection of cultivars include;

- Harvest Index (edible: inedible biomass ratios)
- Gas-exchange/Air revitalization capacity
- Nutritional quality
- Small stature
- Cultivar availability and extent of use in controlled environments or terrestrial agriculture
- Non-GMO
- Disease tolerance and resistance

- Lighting requirement (intensity, spectral requirement and photoperiod)

The CESRF has already identified some potential cultivars which will meet the needs of the MPP. These include the German cultivars '606' and 'Sable'. These cultivars will be investigated in terms of their germination rates, yield and nutrient use in a standard plant growth cabinet. Depending on availability, the CESRF hypobaric chambers may be used, operating under ambient pressure conditions, to collect comparative gas exchange rates for two or three of the identified cultivars using cultures of 3 – 4 plants at a time.

At the conclusion of wheat cultivar selection, the CESRF will prepare a short communication in order to identify its findings to ESA. Formal presentation of the cultivar selection process and recommendations will be made in TN 2 – Test Plan.

WBS 3.1.4 – Compilation of Existing Empirical Data from Lettuce and Beet Production Trials at CESRF

All data collected from empirical trials conducted with beet and lettuce at the CESRF under the frame of a previous Canadian Space Agency contract will be compiled into an EXCEL spreadsheet. The sheet will be documented for clarity in data work-up and analysis and archived for availability to MELiSSA partners.

Work Package 3 - Test Plan (WBS 3.2)

Allied Tasks in WP 1: WBS 2.4.1.2 – Preparation and Submission of TN 2: Baseline Data – Research Test Plan, Prediction and Procedures

It is proposed that a set of test-predictions be made using the EcoSIM pro model based on the Thornley mathematical model, already developed by ESA (RD5, RD6 and RD7). The model will be modified to account for the fusiform orientation of a wheat leaf as per the procedures documented in the original textbook (RD7). Model output from EcoSim Pro will be used to predict gas exchange rates for both batch and staged culture of wheat at the selected planting density and area.

The CESRF will dedicate at least on SEC-2 chamber (Chamber 1) to Phase A activity. This means that the test plan developed under this task will be subject to the Standard Operating Procedures of that chamber.

Work Package 4 – Test Performance – Crop Production (WBS 3.3)

Allied Tasks in WP 1: WBS 2.4.1.3-5, Preparation and Submission of TN 3-Baseline Data Chamber Performance – Beet/Lettuce Staged (TN 3.1), Wheat Batch (TN 3.2), Wheat Staged (TN 3.3)

WBS 3.3.1 – Lettuce/Beet Staged Trials

Under this task set, chamber performance data collected using the beet and/or lettuce cultures of WP 2 are analyzed and reported upon in TN 3.1. The TN will include analyzed data on chamber leakage and environmental control quality and will contain as run procedure annotations. The TN will be submitted to ESA for comment and acceptance.

Under this task set tissue samples will be prepared and sent for analysis in accordance with the test plan. These data are reported upon in TN 4.1 – Baseline Data: Metabolic Results and Evaluation: Lettuce/Beet Staged Culture.

WBS 3.3.2 – Wheat Batch Trials

Under this task set, the SEC-2 chambers will be readied for a crop production trial using wheat in batch culture. The experiment will collect data in accordance with the test plan delivered in WP 3. The production trial is scheduled to run 110 days from seed to harvest. The data collected will include:

- Standard seedling leaf area using a Li-COR 300C Leaf Area Meter collected on a sub-set of un-planted seedlings (15 – 20 seedlings) sampled at start-up and planting of the crop in the SEC-2 chamber,
- Profiles of chamber atmospheric temperature, humidity (VPD), light intensity and solution temperature over the duration of the period of closure,
- Chamber ethylene concentrations measured at 5 day intervals using an off-line GC
- Gas-exchange (CO_2, O_2), collected at three minute intervals by an Infrared-Gas Analyzer (IRGA) and Paramagnetic analyzer and logged by the HPC controller,
- Nutrient and Water Uptake (NH_4^+-N and NO_3^--N, NO_2^--N, PO_4^{3-}, SO_4^{2-}, Fl^-, Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+} and water) as determined from sampling of the hydroponics solution over its period of closure and off-line HPLC analysis. Water uptake is determined from changes in solution volume (± 2 L) at the time of solution replacement (5 day intervals) and nutrient uptake is measured over the same interval by difference of start and end concentrations corrected for water uptake (in triplicate),
- Edible biomass, nutrient content (carbon, nitrogen, phosphorus, potassium, calcium and magnesium) and proximate composition including proteins, lipids and carbohydrates,
- Inedible biomass, nutrient content (carbon, nitrogen, phosphorus, potassium, calcium and magnesium), fibre (soluble and non-soluble), hemi-cellulose, cellulose and lignin.

Chamber performance data collected during this production trial are analyzed and reported upon in TN 3.2. The TN will include analyzed data on chamber leakage and environmental control quality and will contain as run procedure annotations. The TN will be submitted to ESA for comment and acceptance.

Under this task set tissue samples will be prepared and sent for analysis in accordance with the test plan. These data are reported upon in TN 4.2 – Baseline Data: Metabolic Results and Evaluation: Wheat Batch Culture.

WBS 3.3.3 – Wheat Staged Trials

Under this task set, the SEC-2 chambers will be readied for a crop production trial using wheat under staged culture. The experiment will collect data in accordance with the Test Plan delivered in WP 3. The production trial is scheduled to run 190 days from seed to harvest. The data collected will include:

- Standard seedling leaf area using a Li-COR 300C Leaf Area Meter collected on a sub-set of un-planted seedlings (15 – 20 seedlings) sampled at start-up and planting of the crop in the SEC-2 chamber,
- Profiles of chamber atmospheric temperature, humidity (VPD), light intensity and solution temperature over the duration of the period of closure,
- Chamber ethylene concentrations measured at 5 day intervals using an off-line GC
- Gas-exchange (CO_2, O_2), collected at three minute intervals by an Infrared-Gas Analyzer (IRGA) and Paramagnetic analyzer and logged by the HPC controller,
- Nutrient and Water Uptake (NH_4^+-N and NO_3^--N, NO_2^--N, PO_4^{3-}, SO_4^{2-}, Fl^-, Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+} and water) as determined from sampling of the hydroponics solution over its period of closure and off-line HPLC analysis. Water uptake is determined from changes in solution volume (± 2 L) at the time of solution replacement (5 day intervals) and nutrient uptake is measured over the same interval by difference of start and end concentrations corrected for water uptake (in triplicate),
- Edible biomass, nutrient content (carbon, nitrogen, phosphorus, potassium, calcium and magnesium) and proximate composition including proteins, lipids and carbohydrates,
- Inedible biomass, nutrient content (carbon, nitrogen, phosphorus, potassium, calcium and magnesium), fibre (soluble and non-soluble), hemi-cellulose, cellulose and lignin.

Chamber performance data collected during this production trial will be analyzed and reported upon in TN 3.3. The TN will include analyzed data on chamber leakage and environmental control quality and will contain as run procedure annotations. The TN will be submitted to ESA for comment and acceptance.

Under this task set tissue samples will be prepared and sent for analysis in accordance with the test plan. These data are reported upon in TN 4.3 – Baseline Data: Metabolic Results and Evaluation: Wheat Staged Culture.

This task set will conclude with a compilation of TNs 3.1 – 3.3 into TN 3.

Work Package 5 – Test Results Evaluation (WBS 3.4)

Allied Tasks: WBS 2.4.1.6-8, Preparation and Submission of TN 4 - Baseline Data - Metabolic Results and Evaluation – Beet/Lettuce Staged (TN 4.1), Wheat Batch (TN 4.2), Wheat Staged (TN 4.3)

At the conclusion of each empirical crop production trial and after tissue content analyses have been completed and results returned, all metabolic, tissue composition, and solution uptake data will be analyzed in accordance with the Test Plan of WP 3. The data will be presented in TNs 4.1, 4.2 and 4.3 as soon after the experiment as possible and submitted to ESA for review and acceptance. At the conclusion of all experiments and acceptance, the TNs will be compiled into TN 4.

5. Planned Experimental Protocols

Blue Box n. sol. changeover.

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1.Turn off pump distributing n. sol. to troughs.
2.Pump out end n. sol. (~ 40 min).
3.Prepare Start n. sol. with stock sol. A and B (1.3 l each); Iron 3.5 g ; up to 160 l with d H2O ; adjust pH to ~ 6.0 and take 3 samples.
4.After all End n. sol. is out record Volume(l); mix well and record ph, Ec, temp.and take 3 samples
5.Pump in Start n.sol.
6. Turn on pump distributing n. sol. to troughs.
7.Remember always to rinse pumps, houses and n. sol. containers with d. H2O after and calibrate pH and Ec meter every week.

Blue Box stock sol. preparation.

1.Stock sol. A , B and Iron is added fresh every time.
2.Stock A is only Ca(NO3)4H2O with up to 50 l dH2O
3.Stock Sol. B is rest macro : MgSO4 7H2O, KNO3, NH4H2PO4, (NH4)2SO4 and micro: H3BO3, MnSSO4 H2O, ZnSO4 7H2O, CuSO4 5H2O, H2MoO4(85%MoO3) with up to50 l dH2O.
4.Add all chemicals one by one and mix very well.
5.Container with stock sol. cover with black bags to protect from ligh.

Transferring seedlings to Blue Box.

1.Day before weight rockwool cubes : 24 cubes for beets (2 troughs and 4 age stages) and 36 cubes for lettuce (3 troughs and 3 age stages) and wet them with d H2O.
2.Nex day place them in troughs and cover with black/ white plastic (beets) or steel cover (lettuce).
3.Plant seedlings started in small cubes in to larger one and wet with start n. sol.

Blue Box – Harvest

1.Prepare paper bags separately for leaves , bulbs, roots.
2.Harvest plants in order from 1-12.
3.Separate and weigh leaves, bulbs (cut in to small cubes) and roots (collect all possible roots ,squeeze rest) and place in bags.
4.Place all bags in dryer in 50-60 C temp.

Dry samples analysis done " for" and "at".

1.Laboratory Services at University of Guelph (Soil and Nutrient Dep.)
Analysis for Total Carbon % and 5 elements (N, P, K, Ca, Mg)
2.Laboratory Services at University of Guelph (Food Chemistry Dep.)
Proximate analysis for fat, protein , ash, carbohydrates, moisture (%) and energy (Cal)
3.Agri – Food Laboratories
Analysis for Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Lignin.

Things to order from

1.BOC Canada Limited – gas cylinders
2.Canadian Hydro Gardens Ltd. – rockwool
3.Stokes Seeds Ltd. – beets (Detroit Medium Top 34) and lettuce (Grand Rapids 185 C)
4.CMS Seeds – wheat seeds (Norwell, 606, Sable)
5.Plant Products Co. Ltd. – fertilizers.

Table 5-1. Stock solution preparation

Component	mol Wt.	mmol/litre	g for stock
Stock A			
Ca(NO3)2.4H2O	236.16	3.62	5128.55
Stock B			
MgSO4.7H2O	246.48	1	1478.64
KNO3	101.1	5	3032.5
NH4H2PO4	115.08	1.5	1035.55
(NH4)2SO4	132	1	791.87
micronutrients H3BO4 (inc. w. B)	61.83	0.02	7.42
MnSO4.H2O	169.01	0.00500	5.07
ZnSO4.7H2O	289.54	0.00350	6.08
CuSO4.5H2O	249.68	0.00080	1.2
H2MoO4 (85%MoO3)	161.97	0.00050	0.49

1.3 litres of each stock solution is added to 160 litres of deionized water to make the nutrient solution. Also add 3.5 g of FeCL3 at this time. Adjust to a pH of between 5.5 and 6.0 with the addition of NaOH or another appropriate base or acid (TBD).

6. Expected Results

Recent advances have been made in the use of the Thornley canopy photosynthesis model which is an extension the rectangular hyperbola model (Thornley and Johnson, 2000). In collaboration with ESA-ESTEC, the Thornley model has been coded in EcosimPro software and the predicted responses have been compared to empirical carbon exchange data collected in the SEC-2 chambers in 2004 (Ordóñez *et al*., 2004; Favreau *et al*., 2005). Results indicate that the Thornley model is superior to the Modified Energy Cascade Model reported upon in the cited papers. Higher plant modeling efforts for space-related applications have been limited within NASA to the Modified Energy Cascade (MEC) model by Cavazzoni (Cavazzoni, 1999). However, the predictive control strategy that has been foreseen for MELiSSA imposes additional constraints to the model. A first principles model is therefore necessary to extend the capabilities of the control law to operational points beyond the limits of historical on-the-ground research. This allows a more effective control and the development of an adequate optimization strategy.

Thornley and Johnson's work proved to be a very valuable source of information. All the aspects of the growth of plants are reviewed, giving mathematical models for photosynthesis, leaf growth, respiration, light interception, temperature effect, transport processes, root growth, and transpiration. Although not all the models proposed are based on physiology, a first principles model is proposed for photosynthesis, which is the main process driving plant growth.

6.1 Models of Gas Exchange of the HPC

The transport of CO_2 into the leaf interior is governed by the pathway conductance. Equations 8.1.1 and 8.1.2 are established considering that, at equilibrium, the diffusion rate of CO_2/O_2 into/from the leaf must be equal to the photosynthesis rate (in congruent units)

$$P_n = \frac{C_a - C_i}{r_{dc}} \qquad \textbf{Equation 6.1.1}$$

$$P_n = \frac{O_i - O_a}{r_{do}} \qquad \textbf{Equation 6.1.2}$$

Equations 8-1 and 8-2 variables have the following meaning:

P_n: Net photosynthesis rate

C_a: CO_2 concentration in the ambient air
C_i: CO_2 concentration in the leaf
r_{dc}: CO_2 diffusion coefficient from air to leaf
O_a: O_2 concentration in the ambient
O_i: O_2 concentration in the leaf
r_{do}: O_2 diffusion coefficient from leaf to air

In a simplified model of the Calvin Cycle, it is supposed that an enzyme X is activated by light. Its activated form, X*, fixes CO_2 into the carbohydrate recovering its original form. A constant dark respiration rate is assumed. Considering these three reactions as equilibrium reactions with equilibrium constants k_1, k_2 and k_3 respectively;

$$P_n = \frac{\alpha \cdot I \cdot \left(\frac{C_i}{r_x} - \frac{O_i}{r_p} \right)}{\alpha \cdot I + \frac{C_i}{r_x} + \frac{O_i}{r_p}} - R \qquad \textbf{Equation 6.1.3}$$

α, r_x, and r_p are constants derived from the equilibrium constants, the depth of the leaf (h), and the total concentration of enzyme X_0 (X_0=X+X*). This is:

$$\alpha = h \cdot k_1 \cdot X_0 \qquad r_x = h \cdot k_2 \cdot X_0 \; ; \qquad r_p = h \cdot k_3 \cdot X_0$$

R is the respiration rate and is treated below.

Given the respiration rate and the boundary conditions (light intensity, O_2 and CO_2 concentration in the atmosphere) equations 8.1.1, 8.1.2 and 8.1.3 allow solving the system for P_n, C_i and O_i.

The leaf photosynthesis model has to be extended to canopy level. Assuming a high planting density, the canopy can be considered as a murky medium. The light attenuation through a murky medium follows a Beer-Lambert law (exponential decay), given by equation 8.1.4.

$$I(l) = I_0 \cdot \frac{k}{1-m} \cdot e^{-k \cdot l} \qquad \textbf{Equation 6.1.4}$$

where:

I(l): Light intensity at leaf area index l
I_0: Light intensity at leaf area index 0 (top of the canopy)
l: Cumulative leaf area index
k: extinction coefficient

m: transmission coefficient

The leaf area index (l) represents the density of leaves in the canopy (measured as m^2 of leaf over m^2 of ground). It is supposed to be null at canopy height, and the sum of all the leaf areas at ground level. The light is thus attenuated while absorbed by the leaves. The extinction coefficient k is related to three parameters: the leaf transmission coefficient m, and two geometrical parameters ξ and ζ related to the leaf distribution and inclination within the canopy respectively (equation 8.1.5)

$$k = (1-m)\cdot\xi\cdot\zeta \qquad \textbf{Equation 6.1.5}$$

The knowledge of the light distribution within the canopy allows the integration of the leaf photosynthesis to obtain the total photosynthesis in the canopy;

$$P = \int_0^l \left[\frac{\alpha\cdot I_0\cdot e^{-k\cdot l}\cdot\left(\frac{C_{bs}}{r_x}-\frac{O_{bs}}{r_p}\right)}{\alpha\cdot I_0\cdot e^{-k\cdot l}+\frac{C_{bs}}{r_x}+\frac{O_{bs}}{r_p}} - R \right]\cdot dl \qquad \textbf{Equation 6.1.6}$$

Although a constant dark respiration could be assumed, the reproduction of the experimental results required the introduction of a respiration model. The approach consists of separating the respiration into two components. The first component is known as “growth respiration” and it is proportional to the photosynthesis rate, while the second component is the so called “maintenance respiration”, and is proportional to the total biomass,

$$R = k_p\cdot P_n + c\cdot W \qquad \textbf{Equation 6.1.7}$$

where:

R: Respiration
P_n: Net photosynthesis rate
W: Canopy dry mass

The three sub-models presented above allow the implementation of a canopy model whose results will be compared against experimental data. Three additional parameters are needed to evaluate

the leaf area growth from the net photosynthesis: the specific leaf area (m^2 leaf / g leaf), the carbon content of the plant (g C / g plant), and the percentage in weight of leaves in the plants.

$$\frac{dl}{dt} = \frac{P \cdot L_{plant} \cdot SLA}{C_{leaf}}$$ **Equation 6.1.8**

where:

l: Leaf area index
P: Photosynthesis rate
L_{plant}: Leaf content of the plant (% in dry weight)
SLA: Specific Leaf Area (m^2 leaf / g leaf)
C_{leaf}: Carbon content of leaf (% in dry weight)

Empirical data were used to validate the Thornley model with initial inputs of canopy density, initial leaf area, light intensity as a function of time, and the atmospheric conditions (pressure, temperature, atmosphere composition). The results of the comparison are shown in the figures below.

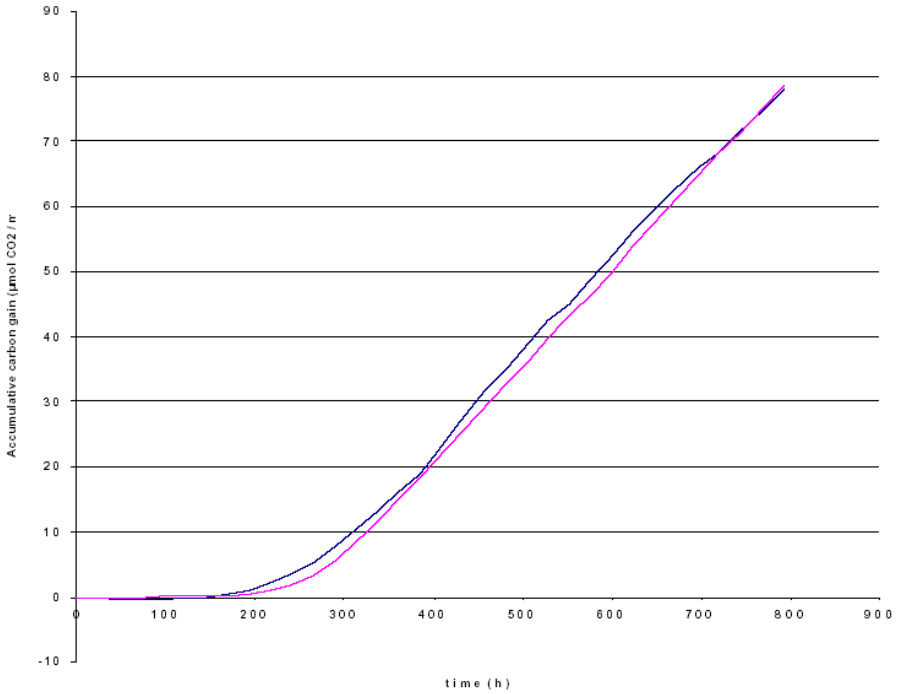

Figure 6.1-1. Comparison between lettuce experimental results (blue) and simulation results (pink) - Accumulated Carbon Gain (mol C)

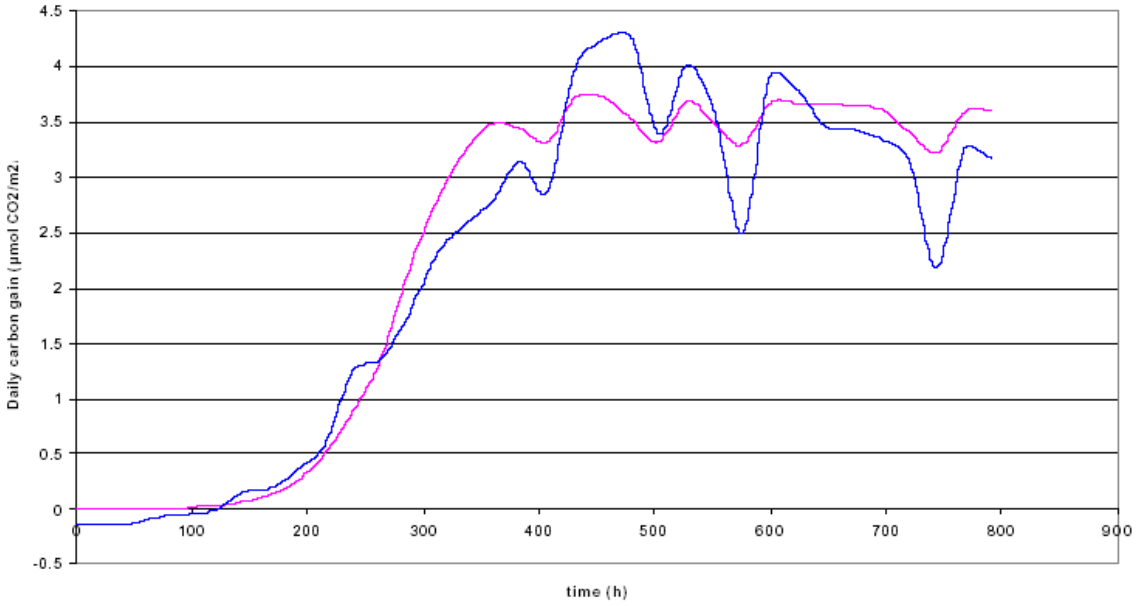

Figure 6.1-2: Comparison between lettuce experimental results (blue) and simulation results (pink) - Daily Carbon Gain (mol C / d)

The table below show the results of the tuning, giving the values for the parameters resulting from the fitting exercise.

Parameter	Value	Units
C	1000	ppm
O	21	%
l_0	$7.5\ 10^{-4}$	m^2 leaf / m^2
α	$4.5\ 10^{-8}$	kg CO_2 / J
k_p	0.005	No units
c	$5.0\ 10^{-8}$	s^{-1}
k	0.9	No units
m	0.1	No units
rdc	25	s / m
SLA	225	m^2 / g
L_{plant}	95	%
C_{leaf}	40	%
rdo	50	m^2 $kgO_2/kgCO_2/g$
rp	$1.67\ 10^4$	s / m
rx	5	s / m

Table 6.1-1. Lettuce model parameters

The model was also compared to experimental trials with beet. Results are shown in Figure 6.1-3 and Figure 6.1-4. shows the values of the parameters which resulted from fitting the beet model to experimental data. Table 6.1-2 presents estimations of model parameters for fits on beet experimental data.

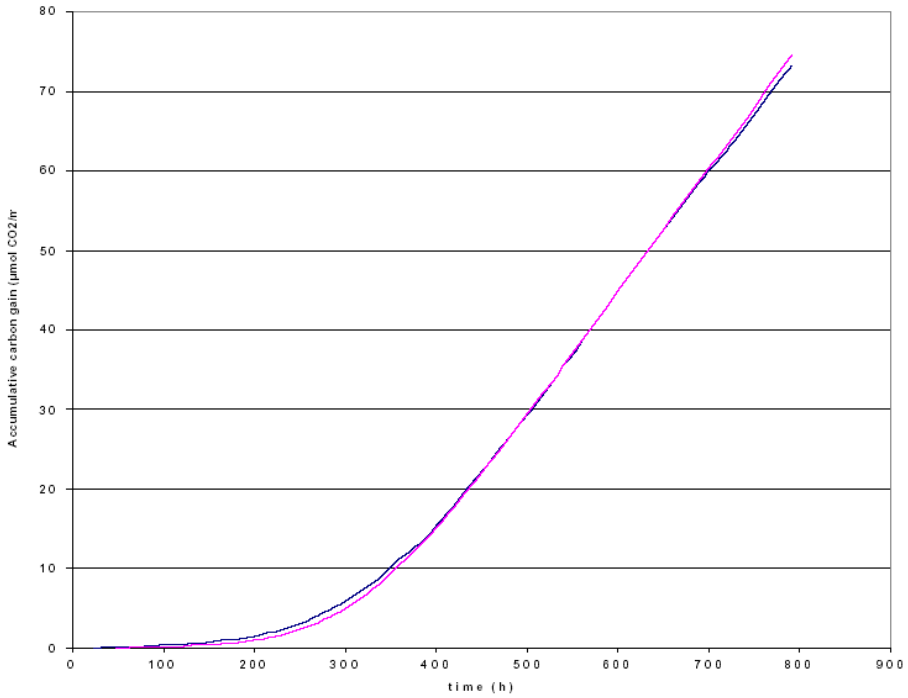

Figure 6.1-3: Comparison of beet experimental results (blue) with simulation results (pink) - Accumulated Carbon Gain (mol C)

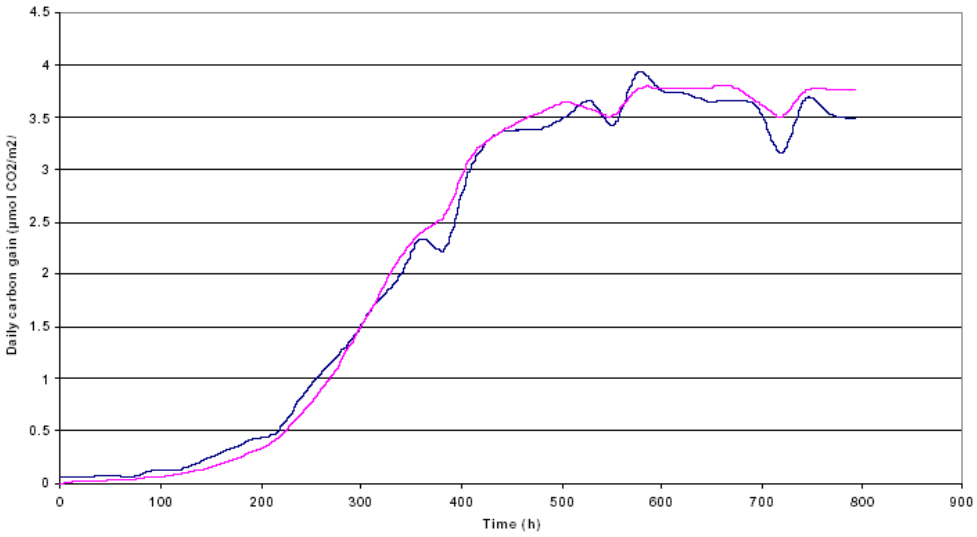

Figure 6.1-4: Comparison of beet experimental results (blue) with simulation results (pink) - Daily Carbon Gain (mol C / d)

Table 6.1-2. Beet model parameters

Parameter	Value	Units
C	1000	ppm
O	21	%
I_0	$5.0\ 10^{-3}$	m^2 leaf / m^2
α	$3.2\ 10^{-8}$	kg CO_2 / J
k_p	0.12	No units
c	$5.5\ 10^{-9}$	s^{-1}
K	0.9	No units
m	0.1	No units
rdc	24	s / m
SLA	110	m^2 / g
L_{plant}	50	%
C_{leaf}	40	%
rdo	50	m^2 $kgO_2/kgCO_2/g$
rp	$1.82\ 10^4$	s / m
rx	3.45	s / m

Despite the fact that the model implemented is at an early stage of development, preliminary results indicate a good performance as shown by the ability to reproduce independently derived experimental results. Several capabilities remain to be added to the model including i) temperature dependence, ii) carbohydrate partitioning models, iii) water uptake, and iv) the ability to simulate staged and integrated canopies.

6.2 Models of Nutrient Uptake by the HPC

Under closure of a hydroponics system it has been found that ion imbalances may result from the indiscriminate control capability afforded by conventional electrical conductivity and pH feedback sensing. Since both commercial greenhouse and advanced life support systems target closure of the hydroponics loop, compensatory nutrient addition to the crop root zone needs to be balanced by uptake. While the design team are also investigating the role of specific ion sensing technologies such as in-line HPLC and ion-specific electrodes, there is the parallel development of predictive models of nutrient uptake that can be integrated into a model and sensor driven control system. An advantage of working in sealed environments is that canopy gas exchange may be readily monitored with conventional gas analysis equipment. This gives rise to opportunity for correlating canopy photosynthetic activity with nutrient uptake. Ideally, mass

dynamics in closed environment system designed for life support could be expressed as a function of a single variable, Net Carbon Exchange Rate.

The theory of steady state nutrition, as proposed by Ingestad and Agren (1988) provides a mechanism by which dynamics in nutrient uptake may be predicted from the carbon exchange of plant canopies. The theory, originally developed for aspen (populus tremuloides), proposes that the relative growth rate (RGR) of plant stands and the relative nutrient uptake rate (RUR) of a given nutrient are equivalent. Ingestad and Agren (1988) explain that the theory of steady state nutrition holds if two conditions are met i) the relative proportions of different plant parts (tuber, roots, flowers etc.), whose mineral concentrations may differ, remains constant during the period of study and ii) the nutrient composition of each different plant part must itself remain constant or the relative proportions of the plant parts adjust to offset any mineral changes. It is very difficult to confirm adherence to steady state nutrition using mineral analysis of plant parts and tissues. First, high numbers of plants must be cultured to generate sufficient biomass for destructive growth analysis and secondly, plant parts must be harvested at regular intervals in order to assess any drift in tissue concentrations as a result of departures in steady state theory.

It can be shown that non-destructive estimations of crop RGR can be determined from NCER as follows:

$$RGR(t) = \frac{NCER(t)}{\int_{t=0}^{t} NCER(t) \cdot dt}$$

Equation 6.2.1

where NCER(t) is an instantaneous estimate of plant Net Carbon Exchange Rate at any age t. Ingestad and Agren's (1988) concept of steady state nutrition states that Relative Nutrient Uptake Rate (RUR) is equivalent to RGR. Under the assumption of steady state nutrition, the ion uptake rate, $U_{\tilde{\eta}}(t)$ may be estimated by non-destructive means as follows:

$$U_{\tilde{\eta}}(t) = \frac{NCER(t)}{\int_{t=0} NCER(t) \cdot dt} \cdot \int_{t=0} U_{\tilde{\eta}}(t) \cdot dt$$

Equation 6.2.2

where $U_{\tilde{\eta}}(t)$ is the instantaneous uptake rate of any ion, $\tilde{\eta}$, at time t.

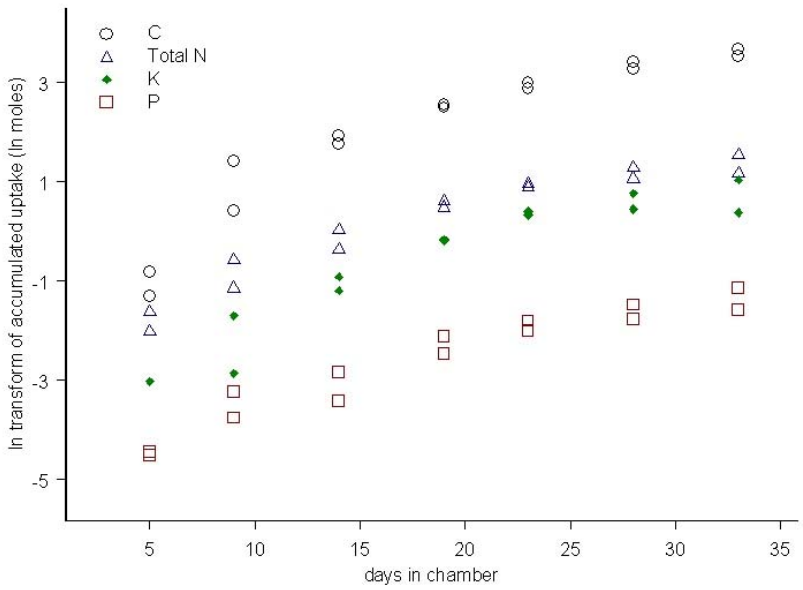

Figure 6.2-1. Patterns of the ln transform of nutrient uptake for beet canopies grown in a sealed environment chamber.

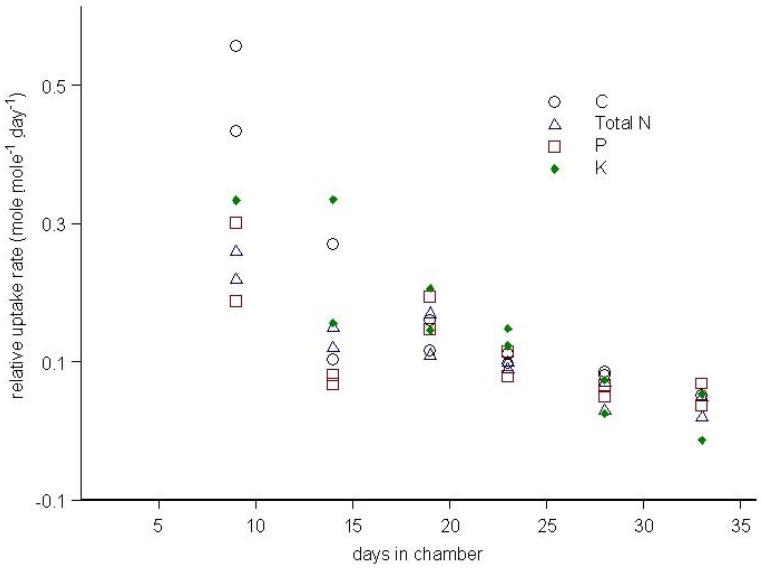

Figure 6.2-2. Relative nutrient and carbon uptake for beet canopies grown in a closed environment

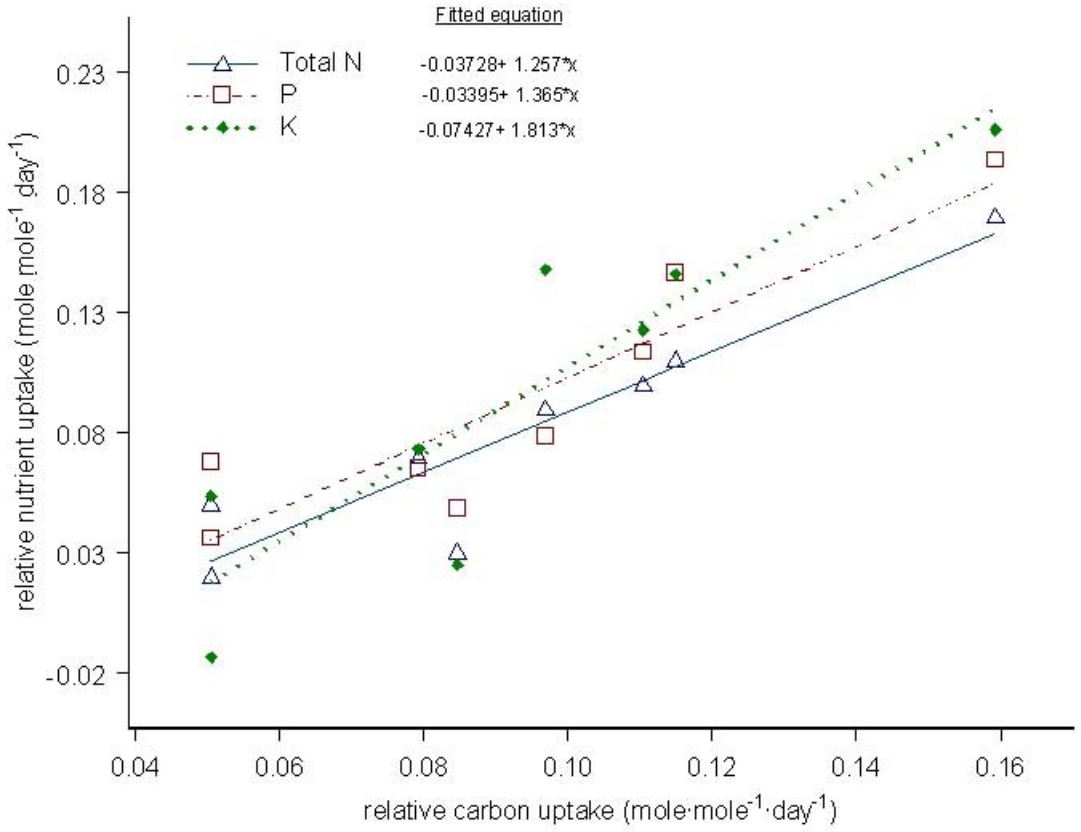

Figure 6.2-3. Reltaionships between relative nutrient uptake rate and relative carbon uptake rate derived from NCER analysis.

Preliminary analysis of the data presented above indicates that congruence between the stand RGR and RUR as postulated in may hold. While there exists for each experiment conducted in 2004 nutrient uptake and gas exchange data much of them remain to be analyzed. Work on the application of steady state nutrition to model driven control of hydroponics solution will continue using NCER as the main predictor and by linking the canopy photosynthesis models described above to ion uptake dynamics.

7. References

RD1. Cloutier G., Dixon M. MELiSSA TN 40.2. "Culture strategies for Higher Plants in Closed Systems" (1997).

RD2. Waters G., Dixon M., MELiSSA TN 53.1 "Chamber Design and Performance Measures for Estimating Higher Plant Net Carbon Exchange Rate (NCER)," (2001).

RD3. Waters G., MELiSSA TN 50.1 and TN 50.2, "Modelling Leafy Vegetable and Root Crop Net Carbon Exchange Rate in Response to CO_2 Concentrations and Light Intensity," (2002).

RD4. Masot, A. Albiol J., Godia F., Waters G., Gidzinski D., Dixon M., MELiSSA TN 75.3 "Higher Plant Chamber Design for the MELiSSA Pilot Plant " (2005).

RD5. Ordonez L., Lasseur Ch., Poughon L., Waters G. "MELiSSA Higher Plant Compartment Modelling using ECOSim Pro," Society of Automotive Engineers (SAE) Technical Paper 2004-01-2435 (2004).

RD6. Favreau M. Ordonez L., Rodriguez A., Waters G. "Application of non-rectangular Hyperbola Model to Lettuce and Beet Crops , "Society of Automotive Engineers (SAE) Technical Paper 2005-01-2823 (2005).

RD7. Thornley J. Johnson I. "Plant and Crop Modeling - A Mathematical Approach to Plant and Crop Physiology," The Blackburn Press ISBN: 1930665059. June 2000.