Controlled Environment Systems Research Facility
Department of Environmental Biology
Guelph, Ontario CANADA
N1G 2W1 Tel. (519) 824-4120
Fax. (519) 837-0442

## MELiSSA

**Memorandum of Understanding**

**TOS-MCT/2002/3161/In/CL**

# Technical Note 76.2

## Joint Progress Report to the Canadian Space Agency and the European Space Agency MELiSSA Program

## For the Contract Period July 1, 2002 to September 30, 2002

**Version 1**

**Issue: 0**

**Waters G., Dixon, M.A**

**University of Guelph**

# Including a Higher Plant Chamber in the MELiSSA Loop

## Joint Progress Report to the Canadian Space Agency and the European Space Agency MELiSSA Program

For Work Completed Under Canadian Space Agency Contract 9F007-010139/00/ST and European Space Agency MELiSSA Memorandum of Understanding TOS-MCT/2002/3161/In/CL dated January 2002.

For the Period of July 1, 2002 to September , 30, 2002

## Section 1.0 Report Summary

This report presents empirical evidence gathered for the weighted average modeling approach to predicting water use, nutrient uptake and carbon accumulation in staged planting. Two replicates of a full chamber study were completed in which each replicate was stocked with four age classes of beet, separated in age by 10 days. Empirical data were collected for stand water use and net carbon exchange rate (NCER) and compared, using a simple linear regression approach, to modeled values. Model predictions were based on terminal harvest data and water use efficiency data collected in the staged stand since preliminary analyses indicated that data from previous batch planting trials resulted in poor model performance. Using data from the staged stand, model predictions of accumulated water use and carbon gain were good. Results suggest a higher stand relative growth rate of the staged stand as compared to batch planted stand results of previous studies. However, these comparisons are made using replications conducted at separate times and therefore more work is required before concluding that the staged method results in statistically significant increases in yield.

This report also summarizes the experimental design and objectives for a current multiple crop production experiment with beet and lettuce, which follows logically from the results of studies presented here.

## Section 2.0 – Report on Task Set 1.0

### Empirical Modeling of Carbon Gain, Water Use and Nutrient Dynamics Under Staged Planting of Beet in a Closed Environment

### Introduction

Previous work developed the theoretical framework for extending model results for a

batch planted stand to that of the staged (also referred to as staggered) stand. A

particularly important result of that theoretical development was the weighted averaging approach to predicting dynamics from simple growth curves. This section attempts to develop the empirical evidence in support of the theoretical model developed earlier. Empirical support for the model would allow future investigators to quantify a range of mass fluxes (nutrients etc.) from simpler models of stand carbon gain, under staged planting. Such confirmation would also improve the models' application to other studies involving the mass dynamics behaviour of stands consisting of different types of crops (as in integrated planting, see below).

Attempts to model gas dynamics of staged stand in closed environments are limited. Wheeler (1996) developed models of gas exchange for a staged plant stand. Wheeler's models were based on the assumption of staged planting and predicted a stabilization of Net Carbon Exchange Rate (NCER) once full stocking (representation of all age classes) was obtained. Similarly, Barta and Henderson (1998) used staged planting as means to stabilize air revitalization capacity in the Phase III test of the Lunar-Mars Life Support Test Project. Barta and Henderson's design involved the planting of wheat seedlings in all four quarters of their Variable Pressure Growth Chamber, initially, 58 days prior to the integration of human test subjects. At 20 day intervals thereafter, 25% of the growing area was removed and replaced with newly germinated seedlings. Thus, after 60 days of the initial planting the chamber had four crops of wheat each about 20 days apart in age. Barta and Henderson (1998) first developed a predictive model of air revitalization dynamics in the staged production system based on previous results from batch planting with wheat under similar conditions. Their model was based on a weighted average where the relative contribution of each age class to carbon sequestration was calculated from the proportion of the floor area occupied by each age class. Barta and Henderson's (1998) model performed quite well and predicted the observed leveling of air revitalization at one person-equivalent once all ages were represented in the chamber, post 60 days.

Given the success of the previous authors' attempts at modeling carbon dynamics in staged production scenarios, this section uses the same modeling approach. The primary objective of this study was to further develop empirical evidence for that approach and then to extend the modeling effort to additional mass dynamics, particularly crop water and nutrient use.

## Materials and Methods

### Growth Chamber Facilities

For the purposes of model development at the stand level, the same two large sealed environment chambers as described in previous studies were used. The chambers are described in detail in the paper authored by Dixon *et al.* (1997).

### Experimental Design

A total of two independent replications were performed at the full stand level. These replicates were conducted using beet (cv *Beta vulgaris* cv. Detroit Medium Red). Each replicate study was completed in one of the two sealed environment chambers, simultaneously. For each replicate, 11 beet seedlings (*Beta vulgaris* cv. Detroit Medium

Red) were initially placed inside the chamber, following a common 21 day germination period. Following the initial planting, each chamber was planted with an additional 11 plants of 21 days of age at 10 day intervals. Once the chamber was occupied by a total of 44 plants (11 plants of each of four age classes, or 40 days after the initial planting) the following planting of 11 seedlings was accompanied by a harvest of the eleven plants of the most mature age class. This strategy resulted in an even distribution of age classes within the chambers as separated by 10 day planting intervals. Two additional harvests and associated plantings were completed after the initial harvest, for a total harvest of three mature sets of 11 plants.

The study described in this section makes use of two replications with samples of water uptake and Net Carbon Exchange Rate (NCER) taken at defined intervals throughout the study period. These studies are therefore treated as an analogue of a Split-Plot Design with chamber/replication as a main factor and time as a sub-factor. Fixed effect ANOVA models were run prior to any detailed analysis to rule out significance of the main effect using NCER data.

Cultural Conditions

Beet seeds were initially germinated in a research greenhouse at the University of Guelph, using Rockwool (2.5 cm x 2.5 cm) cubes. The plants remained in the cubes for a period of 21 days and until there was sufficient root exposure to facilitate transplant into a deep water hydroponics system. During the germination and true leaf emergence period, seedlings were watered regularly with distilled water and once weekly with a dilute fertilizer solution (20-8-20 ppm N-P-K commercial mix having an EC = 2.5 mS).

Following root exposure and leaf emergence, 11 seedlings were transplanted to a pool with a volume of 220L of hydroponics solution and an area of 2.5 $m^2$. Seedlings, in their rockwool cubes, were placed in small holes cut from styrofoam trays which were designed to float freely within the pools. Planting density was fixed at 17.6 plants $m^{-2}$. Any solution exposed to light was shielded with black plastic film to minimize the growth of algae. The pool was positioned in the center of the chamber growing area on 1.3 cm thick plywood at a distance of 1.5 m from the overhead lights.

As described above, each 10 day interval following the initial planting included the addition of 11 more beet plants of 21 days of age. This procedure continued until the Styrofoam floating trays were fully occupied with 44 plants. At 10 day intervals following full stocking, each additional planting into the chamber was accompanied by a harvest of the most mature age class.

Plants were grown under static conditions of 300-600 µmol $m^{-2}$ $s^{-1}$ PPF lighting at stand height (stand height varies depending on age class) as supplied by high pressure sodium (HPS) and metal halide (MH) lamps mounted externally. A 14/10 hr light/dark (06:00 - 20:00) photoperiod was used and coupled to a 26/20 $^{o}$C day/night temperature regime. Atmospheric $CO_2$ concentrations were fixed at 1000 µL $L^{-1}$ $CO_2$ as supplied through an external tank and mass flow controller. Average relative humidity in the chambers over all replications was 73% ± 5% (constant day and night). These conditions were identical to those used in the batch planting studies described in previous studies.

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 mS. The pH of the solution was adjusted to approximately 5.5 with the addition of approximately 40 mL of a 1 M $NaHCO_3$ solution per pool. 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 chamber doors were opened to replace the older solution with a fresh 220 L volume having the same composition as noted above.

At the start of each five day 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 pool and its volume measured. Samples were also taken for HPLC analysis in triplicate. Solution volumes were measured at the start and end of closure periods to allow for the correction of elemental analysis results due to water uptake from the pool. During each five day closure period no amendments were made to the solution composition in any way.

All plant material (i.e. all four age classes) was harvested at the end of the study. Harvested material was pooled by chamber and partitioned, by age class, into edible and non-edible biomass fractions. Leaf area was measured on each of the 11 of the plants belonging to each age class using a Li-Cor 3100 Leaf Area Meter (Lincoln, NE, USA). Fresh weights were determined immediately on all plant material and dry weights were determined following 7 days in a drying oven at 65 $^{\circ}$C.

Carbon Gain Data Collection

The net carbon gain of the developing beet stand was determined using a 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 by injections from an external tank. The volume and duration of $CO_2$ injections were used to estimate day time NCER. 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 NCER estimates over a 24 hour period (in moles C), yielded daily carbon gain (DCG). Daily carbon gain was summed to generate a profile of the accumulated carbon at the end of each of the five day sampling intervals. Water use as determined from the solution sampling and changeover procedure described above were determined over the same five day interval. Water use rates determined over the five day periods were also summed to generate a profile of the accumulated water use.

Data Analysis

*Data Analysis Software*

All data analysis described in this section was completed using the S-Plus statistical software (MathSoft, Data Analysis Products Division, Seattle, WA., 1999) with libraries

derived from Venables and Ripley (1999).

*Growth Models*

Dry weight data collected at the terminal harvest conducted on each of the four age classes was used to generate a growth function for the beet stand. Because four age classes were represented in each chamber, the resulting growth profile is sufficient when data are linearized by a ln transform. The total dry weight was converted to moles of carbon accumulated using a 40% C content in dry tissue (Dutton *et al.*, 1988). The total accumulated carbon (Ac) was then transformed using a natural log (ln) transform to linearize the apparent exponential profile. This was conducted for harvest data collected on each chamber and pooled following confirmation that no significant replication (chamber) effect existed. This assumption was confirmed using a fixed effects ANOVA with replication as a main effect and carbon gain (NCER) as the dependent variable. P-values for the fixed effect term were p=0.25, indicating that no significant effect existed and pooling was justified. Following amalgamation of the data the resulting data matrix contained a column vector of Days In Chamber (DIC) and accumulated carbon at each DIC. The DIC vector corresponded to the number of days each age class remained in the chamber before harvest. The DIC values were either 10, 20, 30 or 40 at harvest.

The resulting data matrix was subjected to a simple linear model of the form:

$$\ln(A_c) = b_1 \cdot DIC + e \qquad [2.1]$$

where $A_c$ was the accumulated stand carbon content (moles) at each of the DIC values, $\varepsilon$ is a vector of random errors and $\beta_1$ is the estimated parameter. Because the growth profile was derived from the ln transform of carbon accumulation data, that parameter also represented the stand Relative Growth Rate (RGR). The quality of model fit was assessed by examining normal residual plots. No significant departures from normality were noted nor was there evidence of heteroscedasticity or non-constant variance.

*Development of Staged Planting Carbon Gain Model*

The growth profile described above was used to develop model based predictions of mixed stand carbon gain. Since data used were derived from biomass determinations at harvest:

$$\int_{t=0}^{a} NCER_{stgd} \cdot dt = \sum_{i=1}^{4} \int_{t=0}^{a} NCER(i) \cdot dt \qquad [2.2]$$

where a is the end point of the accumulation period, the subscript i refers to the age class (1 through 4). Since Equation [2.2] utilizes the integral of NCER, the growth profile generated described by Equation [2.1] may be used to validate the hypothetical model and can therefore be further modified as follows:

$$\int_{t=0}^{a} NCER_{stgd} \cdot dt = \sum_{i=1}^{z} e^{b_1 \cdot (a - 10 \cdot i)}$$

[2.3]

where z=3 if a=30, z=2 if 20=a<30, z=1 if 10=a<20 or z=0 if a<10 and where $b_1$ is the parameter estimated by the regression of Equation [2.1], and a is the days since initial chamber closure (DIC) at which accumulated carbon is to be estimated.

Equation [2.3] is best explained using an example. Assume that the staged planting model aimed at determining the total carbon gain of the mixed age stand at 35 days since the initial chamber closure (DIC). The derived growth curve, providing the measure of integrated NCER (right hand side of Equation [2.2]), is used to determine the total accumulated carbon of the four age classes present in the chamber. The age distribution of the stand at 35 DSC would be 35 days, 25 days, 15 days and 5 days. This amounts to evaluating Equation [2.1] at DIC=15, DIC=25, DIC=35 and DIC=5 or Equation [2.3] at values of a-10·i equal to 35, 25, 15 and 5. This yields a model estimate of mixed stand carbon gain over the four age classes up to 35 DIC. This same procedure was repeated over the duration of the study (days since closure of 0 days to 60 days (final harvest) paying particular attention to the fact that the maximum number of growth stages present in the chamber is 4, and therefore z is constrained to 0=z=3.

*Carbon Gain Model Validation*

The accuracy of the model for carbon accumulation in the staged stand was evaluated using a simple linear regression of modeled carbon gain of the staged stand on that which was observed and determined from empirical DCG estimates. This linear model had the form:

$$A_{c_{model}} = b_0 + b_1 A_{c_{observed}} + e \qquad [2.4]$$

where $A_{cmodel}$ referred to the carbon accumulation of the staged stand as modeled and $A_{cobserved}$ referred to the carbon accumulation of the staged stand as derived from empirical NCER and DCG estimates.

In addition to the linear model applied above, the total modeled carbon gain over the course of the experiment was compared to that obtained, on average, by the two chambers as inferred from integration of empirical NCER data. Further, estimates of model predicted DCG were determined over the period under full stocking (four age classes, DIC=30-60) and compared to the same determined empirically. This was conducted by numerically estimating the DCG from the slope of the modeled $A_c$ profile. For the purposes of estimating the terminal $A_c$ mean of the empirical data, a cubic spline having a model df=6 was applied to Ac as determined from each of the two chamber replicates. This was done because of the need to have a single terminal $A_c$ value from the two chambers for comparison with model predicted $A_c$. The data from the spline fit was only used to estimate mean carbon gain at final harvest. Raw data, not subjected to smoothing, were used for all other comparisons.

*Models of Water Use of the Staged Stand*

Models of dynamics in water use led to estimates of Water Use Efficiency of Productivity (WUEPr). WUEPr was determined from empirical determinations of water uptake collected from the 5 day intervals of solution change over. An accumulation profile of water uptake from the hydroponics pools was generated over the course of the experiment and subjected to a linear model of the form:

$$A_{water\ observed} = b_0 + b_1 A_{c_{observed}} + e \qquad [2.5]$$

where $A_{water\ observed}$ is the total water accumulated by the mixed stand, $A_{c\ observed}$ is the total accumulation of carbon by the same stand , $\beta_0$ and $\beta_1$ are the estimated parameters and $\varepsilon$ is a vector of random errors. This model was fit using simple least squares regression by pooling empirical data collected over the two replicates. Again, no significant replication effect was observed at the p=0.05 level.

The intercept of this model form was shown earlier to be equal to the gross evaporation from the pools when no biomass is present (by extrapolation). The reciprocal slope of the model is a form of the Water Use Efficiency of Productivity (WUEPr), often expressed as moles C fixed per mole water utilized.

The relationship between water use and carbon gain was then applied to the model of carbon gain developed for the staged stand as described above. In this case, model predictions for carbon gain by the staged stand were used as independent variables (as in Equation [2.5]) to develop a model of water use by the staged stand.

As for validation of the models of carbon gain, models of water use for the staged stand were subjected to a second linear model with observed uptake as a dependent variable. This model had the form of:

$$A_{water\ model} = b_1 A_{water\ observed} + e \qquad [2.6]$$

where $A_{watermodel}$ were the model predictions of water use and $A_{waterobserved}$ were the empirical determinations. The parameter $\beta_1$ was the parameter to be estimated and the vector $\varepsilon$ was that of random error. An intercept was not included in the final model because it was not significantly different from zero.

*Steady State Nutrition in the Staged Stand*

Models of accumulation in nitrate ($NO_3^-$), ammonium ($NH_4^+$), phosphate ($PO_4^{3-}$) and potassium ($K^+$) were developed in a manner identical to that described in Section 2.0. Accumulated nutrient uptake, as calculated from uptake measurements over the study period, was transformed using an ln function for each of the four ions. Simple least squares regression (SLSR) was used to determine the slope of the ln transformed data (RUR) and confidence intervals were calculated. The null hypothesis (Ho: RGR = RUR), as described was tested by examining overlap in the confidence interval established for the slope of ln transforms in accumulated carbon gain (RGR).

## Results

The results of the terminal harvest of the four age classes within each chamber are presented in Table 2.1. These results are presented on a per plant basis. Primary results to be taken from the table are as follows:

i) the leaf area distribution in column 2 of Table 2.1 corresponds to a maximal leaf area index of the whole stand, at harvest, of 0.9,

ii) there is a slight decrease in inedible:edible biomass ratio over crop maturation,

iii) average total biomass at crop maturity is 33.22 g per plant

The value of the maximal leaf area index of the staged stand is important in the context of interpreting data on staged stand RGR and Water Use Efficiency of Productivity (WUEPr). The leaf area index of the staged stand (0.90) is lower than the leaf area index at harvest of the batch stand. Because in the batch planting experiments a leaf area accumulation profile could not be generated, a linear approximation of leaf area accumulation is used for comparison. At a linear rate of total batch stand leaf area gain of 0.235 $m^2$ per day, it is estimated that the leaf area index of the batch planted stand will intersect that of the staged stand (0.90) no earlier than 10 days after planting in the chamber. This point of intersection is subject to change since the leaf area accumulation profile for the batch stand was assumed to be linear. Regardless of the precise point of intersection however, it is clear that the leaf area index of the staged stand is significantly lower than that of the batch stand throughout most of the study period. Correspondingly, the mean dry weight per plant of the mature age class at terminal harvest in the staged stand is higher than that obtained for the batch planted stand (33.22 versus 24.5 g per plant, respectively)

The results of the simple linear regression performed to generate a growth model are presented in Table 2.2 The relationship between ln(biomass) (dry weight) and the period each age class spent in the chamber before harvest (DIC) was highly significant with a slope of 0.1234. This slope estimate corresponds to the stand relative growth rate (RGR). This estimate of RGR was significantly higher than that obtained for the batch planting trials when compared using the bootstrap quantiles. The RGR of the staged stand was only marginally insignificant when compared to the standard inferential estimates obtained from the batch stands.

Figure 2.1 presents model derived estimates of total carbon content in standing biomass. The model predicts carbon accumulation rates in the stand that parallel the accumulation profiles prior to harvest. This result is consistent with the model form of Equation [2.3]. Constraints on z, as described above, correspond to the periods of harvest well. The plot presented in Figure 2.1 confirms the model's ability to track changes in carbon accumulation following harvest and its subsequent recovery post harvest.

Figure 2.2 presents a plot of empirical estimates of carbon gain as determined from integration of NCER estimates collected in both chambers. The results from both

replications show an exponential growth pattern in accumulated carbon which is consistent with the growth model results presented in Table 2.2. The flattening of the profile for Chamber 1 was likely a result of sensor error in NCER determination. The cubic spline fit (df=6) of the profile is also presented in Figure 2.2 and provides a means to more accurately assess carbon accumulation at terminal harvest. Modeled carbon accumulation are plotted in Figure 2.3 along with the spline fit for comparison purposes. Qualitatively, the model tracks empirical observations quite well, and the marked inflections in the accumulation profile correspond to the declines in standing biomass and growth recovery (release) following harvest (as presented in Figure 2.1). The primary numerical results derived from the plots of Figure 2.2 and 2.3 are presented, along with modeled measures, in Table 2.3. Model predicted carbon gain (terminal point on plot of Figure 2.3) is 41.1 moles. This value has an error of 4.8 % when compared to the observed mean carbon accumulation of 39.2 (as derived from the end point of the spline fit of the empirical data). The mean daily carbon gain during the period of full chamber stocking, as determined from model results, was 1.37 mol C per day. This is not appreciably different from empirical determinations of daily carbon gain of 1.20 mol C per day taken over the same period.

Regression results of predicted versus observed carbon accumulation in the staged stand are presented in Table 2.4. Using data collected or modeled over the entire period of study (corresponding to the full domain of the plots presented in Figures 2.2 and 2.3), a significant relationship between model predictions and observed values exist. The slope estimate presented in Table 2.4 is close to 1 and the magnitude of the intercept, a measure of model bias, is small and only marginally significant at p=0.05. A high magnitude of the coefficient of determination ($r^2$) and a highly significant slope term validates the model's predictive ability.

The results of determination of Water Use Efficiency of Productivity (WUEPr) as derived from the regression of observed water accumulation on observed carbon accumulation are presented in Table 2.5. A strong relationship between the two variables was observed as is evidenced by highly significant p-values and a high coefficient of determination ($r^2$). The intercept term provides an estimate of evaporation from pools, while the reciprocal of the slope estimate is the Water Use Efficiency of Productivity (WUEPr). The determined WUEPr of 0.0030 mol C $mol^{-1}$ $H_2O$ is only slightly lower than that obtained under batch production.

A high degree of model accuracy is evidenced by the regression results obtained when modeled accumulated water use was defined as the independent variable and observed accumulated water use as the dependent variable (Table 2.6). The intercept term in the regression was not significant and the slope value was close to unity and highly significant. This result confirms the utility of using predicted accumulation profiles as derived from carbon gain and a fixed WUEPr.

Information relating to the quality of models fit to ln transformed nutrient accumulation data (Figure 2.4) are presented for $NO_3^-$, $NH_4^+$, $PO_4^{3-}$ and $K^+$ in Table 2.7. Also presented are the slope and 95% confidence interval estimates for the ln transformed nutrient and carbon accumulation data collected on the staged beet stand. Examination of the confidence intervals established for the slope estimates (RUR for the case of nutrients and RGR for the case of carbon) indicate no significant difference in RGR and RUR for

the four ions studied. As such, there is no evidence against the null hypothesis and steady state nutrition appears to apply to the staged stand. In general, the quality of model fit was excellent, with coefficients of determination ($r^2$) values ranging from 0.81 for $PO_4^{3-}$ to 0.89 for $NO_3^-$. Total nutrient accumulation over the first 38 days of the experimental period (Figure 2.4) was 2.49 moles for $NO_3^-$, 0.25 moles for $PO_4^{3-}$, 1.01 moles for $NH_4^+$, and 1.50 for $K^+$, when averaged over the results of the two chamber replicates.

## Discussion and Conclusion

One of the findings of this section is the higher RGR of the staged planted standrelative to the batch planted crop. Although the magnitude of statisticalcal significance is drawn into question because of replication through time and the potential for chamber effects, this finding has important repercussions on the validity of batch models and on bioregenerative system design. The theory developed in section 3 for mass dynamics in the staged stand assumed that growth profiles from batch planting trials would be of utility in modeling carbon dynamics in the staged stand. This assumption was partly in error. Preliminary analysis of data presented in this section indicated that use of batch data in modeling the behaviour of the more complex stand resulted in consistent under-estimations of the true carbon dynamic. The significant growth model derived from harvest data collected under staged planting allowed the generation of more relevant growth profiles. Indeed the weighted averaging approach to predicting gas dynamics in the staged stand performed well, albeit with the need for terminal harvest data from that stand. This is in contrast to the findings of Barta and Henderson (1998) who utilized batch data quite adequately in their similar modeling effort with wheat. While the modeling approach was consistent, it is likely that data collected on prior batch planting experiments with wheat adequately predicted the staged planting dynamic because of its stand architecture. Wheat does not have a planar orientation to its leaves, unlike that of beet, and as such, the impacts of staged planting on RGR, as described below, may not be of the same magnitude as for beat. This would allow the successful use of wheat batch data in staged model development.

The increase in the relative growth rate of the staged stand is easy to explain in the context of that stand's lower leaf area index relative to the batch planted stand. Comparatively, as the batch stand develops, its leaf area index quickly exceeds the maximum value obtained by the staged stand. The result is a denser plant stand with decreasing light penetration to lower portions. This is a 'text book' case of the Lambert-Beer Extinction Law (Oke, 1990). Decreased light penetration to the lower stand therefore has the effect of depressing the relative growth rate of the batch planted stand. As such, the staged planted stand with its lower leaf area index has a correspondingly higher relative growth rate. This is also evidenced by the higher yield of the mature age class at terminal harvest, relative to the batch planted counterparts.

The implications of the staged planting regime on the provision of life support requirements is significant. Because the staged planting regime results in higher relative growth rates, the efficiency of carbon sequestration and associated oxygen release during photosynthesis is greater than in the batch planted stands.

The model predictions of stand carbon gain, on the whole, predicted observed values

well. Of particular interest is the correspondence between observed DCG during the period of full stocking (all age classes represented) and model predictions of carbon gain during that same period. The staged planting regime therefore does behave in a manner as predicted, in that there is a stabilization in DCG once all age classes are represented in the chamber. The staged planting regime does, therefore, have the added value of dampening long term growth profiles associated with batch planting. One disappointment of the empirical data however, was that thepredicted declines in standing carbon and associated recovery at harvest were not strongly evident in the observed carbon accumulation profiles. While there is some depression in the magnitude of carbon gain immediately after harvest it is likely that these observations are dampened by chamber leakage and the need to smooth data points collected on harvest days when the chambers had to be opened.

Model predictions of Water Use Efficiency of Productivity (WUEPr) and of accumulated water use also performed well. WUEPr of the staged stand was slightly lower than that of the batch planted stand. It is possible that this may be related to the lower leaf area index of the staged stand and its morphological structure. The staged stand was not uniform and as a result had an irregular stand edge. The behaviour of the microclimate in non-uniform stands in closed environments is not well understood although Oke (1990) provides an excellent summary of the effect of non-uniform boundary layers in field situations. It is possible that a so-called 'clothesline' effect may be at play within the staged stand. Such an effect may result in an accelerated evapotranspiration in regions where the stand is thin or of low leaf area index (Oke, 1990). The absence of a thick boundary layer afforded by a dense plant stand may therefore explain the decline in water use efficiency, associated with rapid transpiration. Further, it is possible that plants in the thinner portions of the stand may have exhibited water stress thereby reducing stomatal aperture and possibly decreasing net carbon gain. It is unknown whether the differences between WUEPr in the staged stand were a result of changes in stand architecture, microclimate or physiological changes or if the observed differences in WUEPr are practically significant. The influence of non-uniform stands on micro-climates within the closed systems may be an attractive area of future research.

Regardless of the mechanisms at play which resulted in slight changes in WUEPr, the water accumulation model based on empirical WUEPr estimations performed very well. Significant correlations were exhibited between predicted water accumulation and that observed in the staged stand. This lends further support to the utility of the weighted averaging approach to modeling the behaviour of water dynamics under staged planting conditions.

Additionally, models of nutrient uptake in relation to RGR as derived from NCER estimates performed well. No significant differences ($p=0.05$) were observed between relative nutrient uptake and RGR for nitrate, phosphate, ammonium and potassium. As such, there is no evidence against the null hypothesis which invokes steady state nutrition.

In summary, the models developed for the staged stand performed as expected with a few notable exceptions. Data collected from batch planted trials may have limited utility in model building for staged stand. This is a result of changes in stand RGR and WUEPr as noted above. While the particular model forms tested performed well, data need to be

collected more intensively on staged stand than is presently being done within life support systems research.

# Section 3.0: Determination of Various Parameters in Multi-cropping of Beet and Lettuce – Report on Task Set 1.0

## 3.1 Study Objective

The purpose of this current experiment is to evaluate the effect of multiple crops with rotational planting on the net carbon exchange rate (NCER), evapotranspiration, and nutrient uptake dynamics within a sealed environment. Two of the three MELiSSA candidate crops, beet and lettuce, will be continuously grown with a ten day staged planting interval. This will result in a plant canopy with all representative stages of physiological growth within a common atmosphere. Concurrent experiments on volatile organic compound (VOC) composition and accumulation will take place over the course of this study.

The objectives of this study are as follows:

1. Monitor $CO_2$ and $O_2$ gas exchanges
2. Monitor ethylene evolution
3. Monitor evapotranspiration
4. Monitor nutrient uptake
5. Evaluate various harvest parameters
6. Implement concurrent integrated canopy light curve experiments
7. Monitor TVOC s by GC/PID
8. Monitor VOCs by GC/MS

## 3.2 Study Test Parameters

$CO_2$, $O_2$ and evapotranspiration will be recorded at 3 minute intervals by the Lander control system. Ethylene will be monitored on a daily basis using an in-line gas chromatograph and automated sampling system. Crop wet and dry weights of edible andinedible biomass and other growth parameters (TBD) will be determined at each harvest interval. Nutrient uptake analysis by HPLC will be performed at the beginning and end of each nutrient solution cycle. Total VOCs will be monitored by GC/PID on a daily basis, and VOC characterization by GC/MS will be performed at the end of each closure period (prior to chamber opening for harvest/planting).

## 3.3 Study Period

The study period refers to the time between initiation and completion of analysis. The study schedule may be changed during this period depending on schedules, priorities, system failures, and earlier results.

Estimated Starting Date: September 30, 2002

Estimated Completion Date: January 8, 2003

## 3.4 Study Site

The study will be carried out at:

Controlled Environment Systems
SEC2
University of Guelph
Guelph, Ontario

## 3.5 Study Procedures

Planting: Seeds (lettuce - cv. Grand Rapids [185C] 45 days; and beets - cv. Detroit Dark Red Medium Top [34] 63 days; both from Stokes Seeds) will be sown directly into individual pre-rinsed (de-ionized water) rockwool blocks (12 blocks of beets and 12 blocks of lettuce per chamber per planting interval). The depression in each block will be half filled with sand, followed by three seeds and additional sand. After sowing, the doors will be sealed and the experiment begun. At each harvest/planting interval, the 10-day-old plants will be thinned to 1 plant per block. Planting will be performed as per the attached planting/havesting schedule.

Watering: Plants will be watered using a recirculating NFT irrigation system consisting of a 160 litre in-chamber reservoir and 10 stainless steel growing trays. Nutrient solution will be changed every five days.

Harvesting. Lettuce and beets will be harvested according to the planting and harvesting schedule (attached). Lettuce will be harvested 40 days after planting, and beets 60 days after planting.

Detailed procedures which are carried out from time to time will be recorded in the SEC2 experiment log book. A copy of the log book will be included with the data from this study at the end of the experimental period. Prior to beginning this experiment, the SEC2 Startup SOP (CES-01-002) should be read and followed.

## 3.6 Sampling Schedule

The majority of sampling is done automatically. Ethylene will be analyzed daily with four subsamples in each analysis. TVOC samples will be collected on a daily basis for each chamber, and VOC characterizations will take place at the end of each closure interval. Sampling of hydroponic solution will be performed at the beginning and end of each nutrient cycle (5 days).

## 3.7 Analytical Methods

Analysis of ethylene and VOC characterizations will follow SOP's CES-02-002 and CES-02-006 respectively. Nutrient solution composition will analyzed according to SOP CES-02-007.

## 3.8 Reporting

Upon completion of analysis a report will be issued to document the analytical results and present appropriate information necessary for the review of the data.

## 3.9 Amendments

Alterations of this protocol may be made as needed.. Any changes must be documented in the form of a protocol amendment. All protocol amendments will be included as raw data in the final report.

# Section 4.0 : Report on Milestones 2.0 – 5.0

## Milestone 2.1: Integration of Steady State Models for All MELiSSA Compartments including the HPC

This milestone is being handled in conjunction with milestone 1.4. Some data were reported upon in TNs 53.2 and 53.3, particularly with regard to nutrient uptake and supply, water use and efficiency and carbon balance. No new data are available at the time of this report. Data will be made available at the completion of the current experiment outlined in Milestone 1.3

## Milestones 3.1 – 3.3: Sizing of the HPC and the Development of Cultural and Atmospheric Management Strategies

These milestones are also on-going and progressing in tandem with integrated canopy trials outline above (Milestone 1.3). Data should be made available on schedule.

## Milestones 4.1- 5.1: Development of Control Algorithms of the HPC, Design of the HPC Compartment and Interface with Other Compartments

At the time of this report these milestones have not been commenced, with exception of, to our understanding, Milestone 4.1. Results of simulated process modeling will be made available at the ESA ESA-MELiSSA annual meeting to be held in Claremont-Ferrand, FR. in May, 2003. All other milestones will be started pending the completion and/or sufficient receipt of data from previous milestones.

## Section 5.0: Citations

Barta D. and Henderson K. Performance of wheat for air revitalization and food production during the lunar-mars life support test project phase III test. Society of Automtoive Engineers Technical Paper Series. 1998; 981704.

Dixon M.A.; Grodzinski B.; Cote R., and Stasiak M. Sealed environment chamber for canopy light interception and trace hydrocarbon analyses. Advances in Space Research. 1997; 24(3):271-280.

Dutton R.G.; Jiao J.K.; Tsujita M.J., and Grodzinski B. Whole plant $CO_2$ exchange measurements for non-destructive estimation of growth. Plant Physiology. 1988; 86:355-358.

Oke T.R. Boundary layer climates. 2nd ed. New York: Routledge; 1990; pp. 134, 158-167.

Venables W.N. and Ripley B.D. Modern applied statistics with S-Plus. New York: Springer; 1999.

Table 2.1. Harvest data from staged planting trials with beet. DIC at Harvest refers to the number of days in the chamber before harvest. Beet Root Biomass refers to the biomass of the enlarged beet hypocotyls. Inedible Biomass refers to root biomass. All values are expressed on a dry weight (dwb) per plant basis and are averages taken over two chambers. Bracketed values indicate lower and upper 95% confidence interval bounds for a sample size n=22 (11 plants per chamber).

| DIC at Harvest | Leaf Area ($cm^2$ $plant^{-1}$) | Shoot Biomass (g dw $plant^{-1}$) | Beet Root Biomass (g dw $plant^{-1}$) | Edible Biomass (g dw $plant^{-1}$) | Inedible Biomass (g dw $plant^{-1}$) | Inedible: Edible | Total Biomass (g dw $plant^{-1}$) |
|---|---|---|---|---|---|---|---|
| 10 | 34 [26, 42] | 0.29 [0.23, 0.35] | 0.04 [0.03, 0.05] | 0.33 [0.26, 0.40] | 0.08 [0.06, 0.10] | 0.23 [0.18, 0.39] | 0.41 [0.33, 0.49] |
| 20 | 133 [87, 180] | 1.24 [0.82, 1.61] | 0.25 [0.17, 0.33] | 1.46 [0.99, 1.93] | 0.25 [0.16,0.34] | 0.18 [0.13, 0.21] | 1.71 [1.16, 2.25] |
| 30 | 693 [532, 855] | 7.5 [5.69, 9.30] | 4.18 [3.01, 5.34] | 11.67 [8.78, 14.55] | 1.14 [0.82, 1.45] | 0.11 [0.09, 0.13] | 12.81 [9.64, 15.98] |
| 40 | 1186 [908, 1463] | 15 [11.94, 18.23] | 16.3 [12.30, 20.39] | 31.42 [24.54, 38.31] | 1.79 [1.38, 2.21] | 0.07 [0.04, 0.09] | 33.22 [25.98, 40.47] |

Table 2.2. Results of simple linear regression models of biomass accumulation under staged planting. Model form is $\ln(\text{Biomass})=b_1(\text{DIC})$, df(error) = 7. The lower and upper 95% confidence intervals for the slope estimate are listed as 95% LCL and 95% UCL respectively. The slope estimate in this context of ln transformed data is also the canopy relative growth rate (RGR). DIC refers to the number of days in the chamber.

| Parameter | Value | 95 % LCL | 95 % UCL | t-value | P-value | $r^2$ |
|---|---|---|---|---|---|---|
| Slope ($b_1$) | 0.1234 | 0.1151 | 0.1317 | 2104.6 | 0.00 | 0.89 |

Table 2.3. Staged model predictions and observed values for canopy carbon gain. Total Carbon Gain refers to the total moles of carbon accumulated over the duration of the study (3 harvests). Mean daily carbon gain (DCG) refers to either the mean observed DCG in the two chambers following full planting (4 age classes represented) of the chamber or the model estimated DCG for the same period. The upper and lower 95% confidence intervals are also presented (95% LCL and 95% UCL respectively).

| Parameter | Model Prediction | Observed Value |
|---|---|---|
| Total Canopy Carbon Gain (mol) | 41.1 | 39.2 |
| Model Accuracy (%) | 95.2 % | |
| Mean DCG after full planting (mol C $day^{-1}$) | 1.37 | 1.20 |

Table 2.4. Regression results of predicted versus observed carbon accumulation under staged planting. Model form is $Ac_{model}=b_0 + b_1 Ac_{observed}$. Where Ac refers to carbon accumulation in moles as modeled or observed. In this analysis df(error) = 102. The lower and upper 95% confidence intervals for the slope estimate are listed as 95% LCL and 95% UCL respectively.

| Parameter | Value | 95 % LCL | 95 % UCL | t-value | P-value | $r^2$ |
|---|---|---|---|---|---|---|
| Intercept | -0.866 | -1.62 | -0.112 | -2.23 | 0.025 | 0.95 |
| Slope ($b_1$) | 0.9409 | 0.900 | 0.982 | 45.57 | 0.000 | – |

Table 2.5. Regression results of water use efficiency of productivity ($WUE_{Pr}$) under staged planting. Model form is $A_{water\ observed}=b_0 + b_1 Ac_{observed}$. Where Ac refers to carbon accumulation in moles as observed and $A_{water\ observed}$ refers to the total moles of water lost from pools. In this analysis df(error) = 21. The lower and upper 95% confidence intervals for the estimates are listed as 95% LCL and 95% UCL respectively.

| Parameter | Value | 95 % LCL | 95 % UCL | t-value | P-value | $r^2$ |
|---|---|---|---|---|---|---|
| Intercept | 1973.33 | 1293.77 | 2652.89 | 6.04 | 0.000 | 0.95 |
| Slope ($b_1$) - $WUE_{Pr}$ (mol $H_2O$ mol $^{-1}C$) | 338.48 | 304.18 | 372.78 | 20.53 | 0.000 | – |
| Slope ($b_1$) - $WUE_{Pr}$ (mol C mol $^{-1}H_2O$) | 0.0030 | 0.0033 | 0.0027 | – | – | – |

Table 2.6. Regression results of $A_{water\ observed}$ and $A_{water\ modeled}$. Model form is $A_{water\ observed} = b_1 A_{water\ modeled}$. Where A refers to water accumulation from pools as modeled or observed. In this analysis df(error) = 22. The lower and upper 95% confidence intervals for the estimates are listed as 95% LCL and 95% UCL respectively.

| Parameter | Value | 95 % LCL | 95 % UCL | t-value | P-value | $r^2$ |
|---|---|---|---|---|---|---|
| Slope ($b_1$) | 1.04 | 0.951 | 1.12 | 25.26 | 0.000 | 0.91 |

Table 2.7. Relative growth rate (RGR) and relative uptake rate (RUR) estimates and inference statistics for staged stand beet experiments. The term $b_1$ refers to the ln-transformed model slope. SLSR refers to those estimates obtained from Simple Least Squares Regression (SLSR). LCL and UCL refer to the Lower and Upper 95% confidence limits, respectively, as obtained from standard inferential techniques. RUR can be interpreted as RGR for the case of carbon. In this analysis, df=14 for nutrient data and df= 103 for carbon data.

| Ion | SLSR RUR ($b_1$) | LCL | UCL | Mean Squared Error | $r^2$ |
|---|---|---|---|---|---|
| $NO_3^-$ | 0.1014 | 0.0807 | 0.1221 | 0.4267 | 0.87 |
| $NH_4^+$ | 0.1013 | 0.0800 | 0.1226 | 0.4371 | 0.88 |
| $PO_4^{3-}$ | 0.1094 | 0.0794 | 0.1394 | 0.6169 | 0.81 |
| $K^+$ | 0.1181 | 0.0935 | 0.1369 | 0.5108 | 0.87 |
| $\mathbf{C^{4+}}$ | 0.1243 | 0.1151 | 0.1317 | 0.6627 | 0.89 |

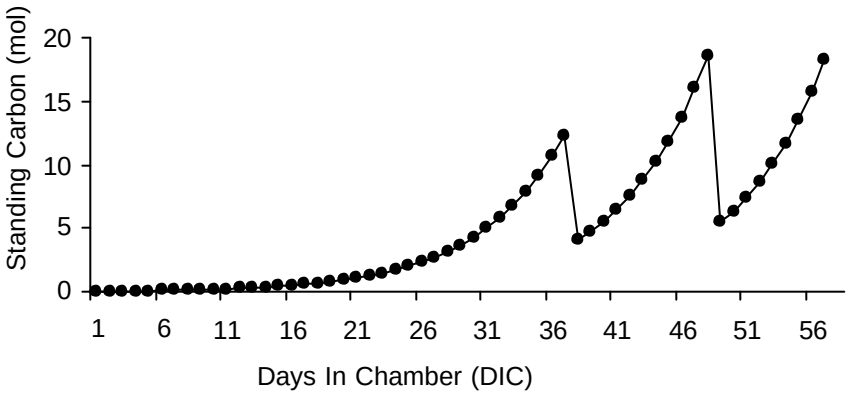


Figure 2.1. Model predicted canopy carbon content. Sharp decreases in carbon content are associated with periods of harvest.

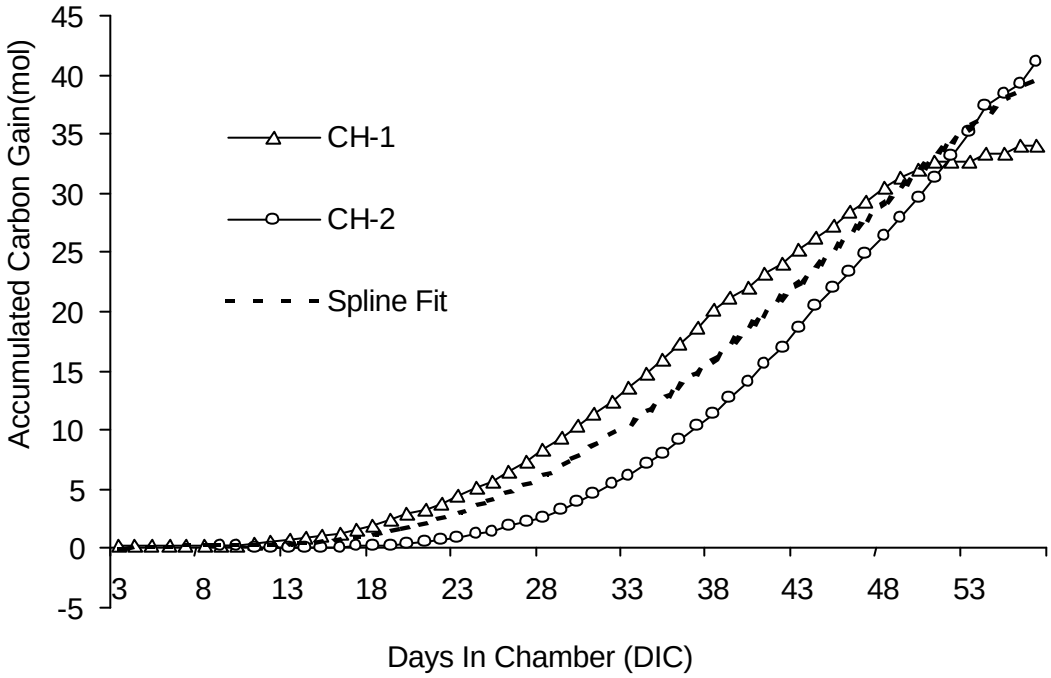


Figure 2.2. Observed accumulated carbon gain. Observed carbon gain as derived from integration NCER and Daily Carbon Gain (DCG) are presented for two chambers under staged planting (CH-1 and CH-2). The spline fit is a result of a cubic spline smoothing algorithm having df=6 for the modeled data.

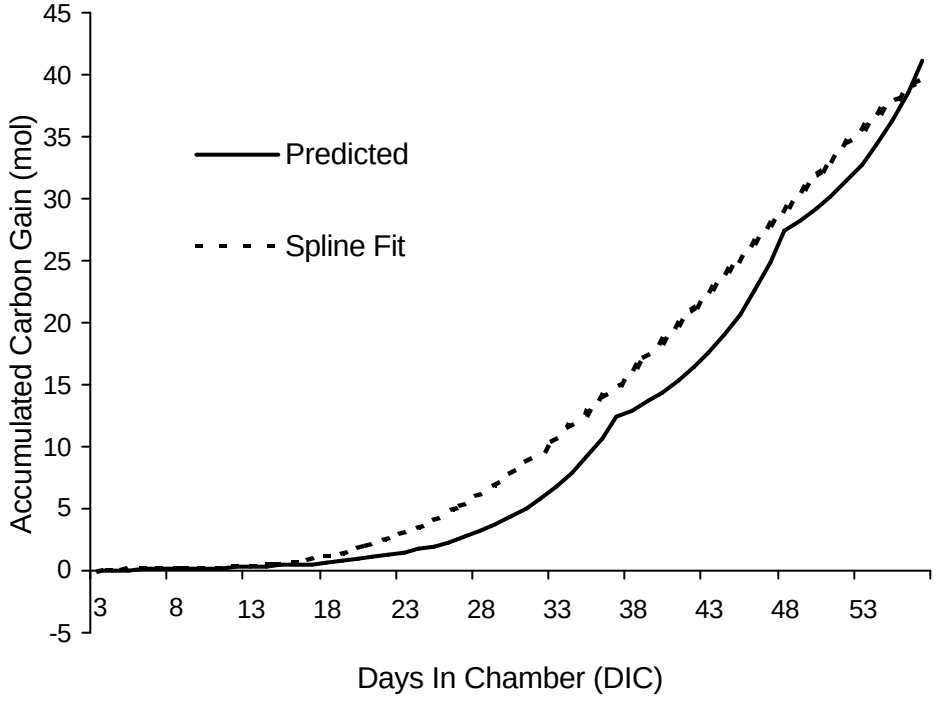


Figure 2.3. Accumulated carbon gain as modeled or as observed. The spline fit is a result of a cubic spline smoothing algorithm applied to observed data. Inflections in the modeled curve are a result of harvest of the stage canopy and its associated recovery.

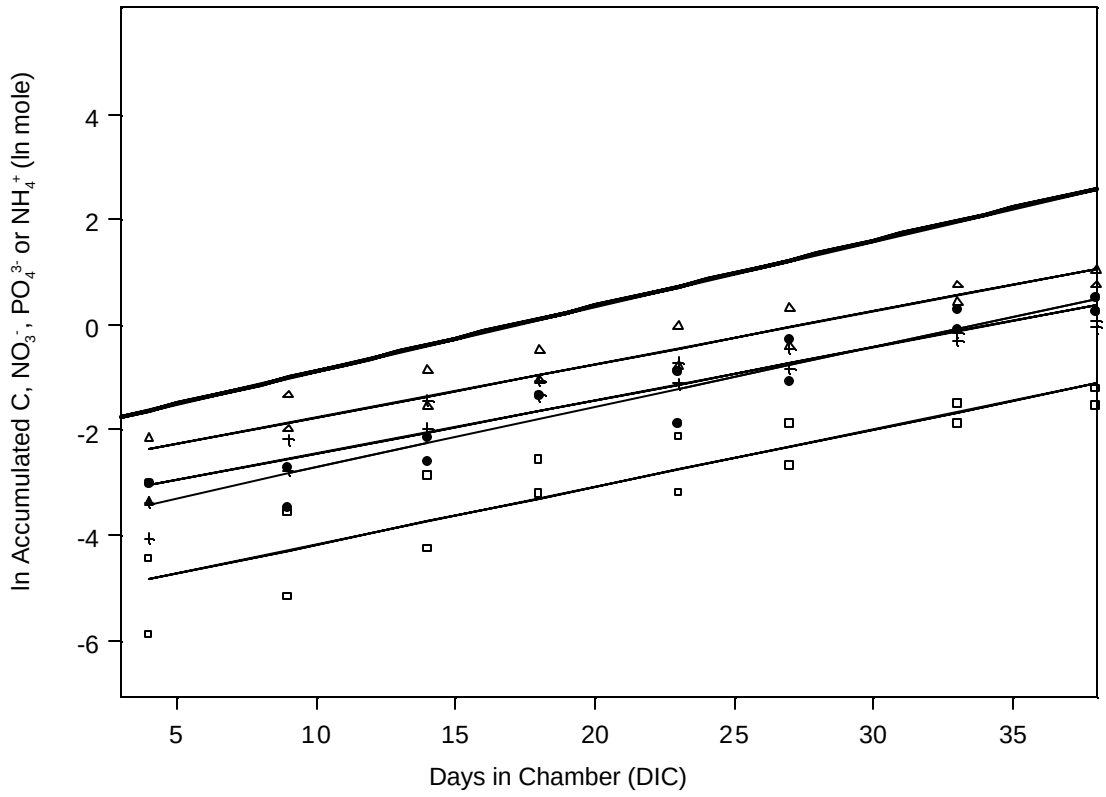


Figure 2.4. Plot of observed and model fitted ln transform of accumulated carbon (thick solid line, fitted values), nitrate (open triangle), phosphate (open square), ammonium (plus) and potassium (solid circle) for all replications of the beet staged stand study. Solid lines indicate the fitted model values.

## Appendix 1 – Summary of Milestones

1. **Steady State and Dynamic Modeling of the Higher Plant Chamber (HPC)** – The purpose of this objective is to collect data relevant to the dynamic and static modeling of the HPC, including harvest yield and partitioning, crop response (NCER, transpiration, nutrient uptake) to environment conditions (light and $CO_2$) and the degradation of inedible biomass in the fermentative compartment. The development of empirical models from the resulting data set will then be used to assess steady state of the loop including the HPC. (UoG, CF, EPAS)

2. **Integration of Steady State Models for All MELiSSA Compartments including the HPC** – This objective aims at assessing steady state of the MELiSSA loop including the HPC, with respect to $CO_2$, $O_2$, water, major nutrients (including those materials from the degradation of inedible biomass in the fermentative compartment). (CF, UoG)

3. **Sizing of the HPC and the Development of Cultural and Atmospheric Management Strategies** – From the data collected for various crops, particularly with respect to crop NCER responses to environment variables, management strategies for the stabilization of long and short term gas and water exchange dynamics will be established. Cultural management strategies for the production of candidate crops in a common atmosphere will also be established based on the same data (UoG, CF)**.**

4. **Development of Control Algorithms of the HPC –** The Higher Plant Compartment has to be elaborated and tested on a simulator before being transferred into the controller of the pilot process, as it has been done in the current MELISSA project for other compartments (ADERSA, Guelph)

5. **Design of the HPC Compartment and Interface with Other Compartments –** An HPC will be designed based on the results of the steady state and dynamic simulations, with particular emphasis on its interface of other compartments (UAB, EPAS, ADERSA, CF, UoG)

# Appendix 2 – Summary of Schedule and Milestones

| Deliverable | Forecasted Completion |
|---|---|
| 0.0 Kick-Off meeting, appointment of PDF | Nov., 2001 |
| 1.1 Development of dynamic carbon exchange models for monocultures<br>1.2 Assess degradation efficiency of inedible biomass in compartment I<br>1.3 Development of carbon exchange models for the integrated canopy<br>1.4 Development of dynamic and steady state models for the HPC | Mar., 2002<br>Dec., 2002<br>Mar., 2003<br>Oct., 2003 |
| 2.1 Assessment of system level mass balance with respect to water, nutrients, gases and biomass | Jan., 2004 |
| 3.1 Development of models for atmospheric management of integrated canopies under staggered planting and photoperiod offset<br>3.2 Validation of models of mass dynamic for integrated canopies under staggered planting and photoperiod offset<br>***3.3 Determination of the HPC size required for interfacing with the MELiSSA loop*** | Mar., 2004<br>Jun., 2004<br>Oct., 2004 |
| 4.1 Software of the simulated process written with Simulink® and Matlab® advanced languages<br>4.2 Model Based Predictive Control software written in C language<br>4.3 Specifications of the sensors and actuators<br>4.4 Implementation of the control in a PC by means of a DLL (Dynamic Link Library) directly built from the C language software, without any transcription of the C software into another automated language | Oct., 2002<br>Jun., 2003<br>Oct., 2003<br>May, 2004 |
| 5.1 Design of the Higher Plant Chamber for loop integration based on results of previous studies | Dec., 2004 |
| Annual Report and Annual Review<br>Annual Report and Annual Review<br>Annual Report and Annual Review<br>Final Report | April, 2002<br>April, 2003<br>April, 2004<br>Mar. 31, 2005 |

## Appendix 3 – Project Management Timeline

| **Public Works and Government Service Canada** | **Contract Plan and Report Form** |
|---|---|

**Contract No.** 9F007-010139/00/ST
**Requisition No.** 9F007-010139
**File No.** 009ST.9F007-010139
**Contractor:** University of Guelph, Controlled Environment Systems Research Facility

| **Task Description** | | **Task Duration** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 11/01 – 03/02 | | 04/02 – 09/02 | | 10/02 – 03/03 | | 04/03 – 09/03 | | 10/03 – 03/0 | |
| 1.1 Note 1 | | | | | | | | | | |
| 1.2 | | | | | | | | | | |
| 1.3 | | | | | | | | | | |
| 1.4 | | | | | | | | | | |
| 2.1 | | | | | | | | | | |
| 3.1 | | | | | | | | | | |
| 3.2 | | | | | | | | | | |
| 3.3 | | | | | | | | | | |
| 4.1 | | | | | | | | | | |
| 4.2 | | | | | | | | | | |
| 4.3 | | | | | | | | | | |
| 4.4 | | | | | | | | | | |
| 5.1 | | | | | | | | | | |
| Annual Report & Review | | | | | | | | | | |
| Annual Report & Review | | | | | | | | | | |
| Annual Report & Review | | | | | | | | | | |
| Final Report | | | | | | | | | | |
| Original Estimate | | | | | | | | | | |
| Completed | | | | | | | | | | |
| In Progress | | | | | | | | | | |
| **Joint Activity** | | | | | | | | | | |
| ADERSA/Guelph | | | | | | | | | | |
| EPAS/Guelph | | | | | | | | | | |
| Clermont-Ferrand/Guelph | | | | | | | | | | |
| UAB/Guelph Activity* | | | | | | | | | | |

Note 1. Task 1.1 is reported upon in ESA TNs 50.1 and 50.2. Since these annual report under this contract, the first of the joint reports filed to ESA 31, 2002. An addendum to the original TNs 50.1 and 50.1 is currently und in the progress and reporting section for Milestone 1.1