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Chamber Design and Performance Measures for Estimating Higher Plant Net Carbon Exchange Rate (NCER)

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Section 1.0 - Introduction

Traditional plant canopy growth analysis involves the destructive harvest of samples of individual plants taken from a full canopy at successive intervals and a determination of the sample dry weights. Biomass accumulation profiles derived from dry weight data are then fitted with models having a defined functional form. Generally, these models have a sole predictor, which is some estimate of plant age (days after planting) or have a number of predictors associated with integrated environment variables (integrated photon flux, degree-days etc.). While these models are predictive in nature the analyst is forced to choose among particular parametric forms which may result in over or under-fitting and poor model performance. This can result in a high degree of collinearity among predictors, especially if a high order polynomial of a single predictor variable is used to model a complex growth profile (e.g. a polynomial in time). Further, sampling from a full canopy induces thinning responses in remaining plants that can obscure growth profiles. Non-destructive techniques have been developed which allow for growth estimation from measures of whole plant or full canopy photosynthetic activity (Dutton et al., 1988). Because these methods are non-intrusive and avoid the need for replicate pairings among successive harvests, they are believed to give better results in growth and eco-physiological modeling studies.

Non-destructive growth analysis techniques use Net Carbon Exchange Rate (NCER) as a predictor of plant growth and response to environment conditions (Dutton *et al.*, 1988). NCER is the amount of carbon gained by the plant as a result of net fixation during photosynthesis less the amount of carbon lost by the plant as a result of net respiration. If NCER is an instantaneous measure, then plant biomass is simply the signed integral of NCER over a given time period divided by the proportion of carbon in plant biomass.

The challenge in using NCER is in the development of chambers which have the capability to measure NCER with sufficient resolution and accuracy. Single leaf cuvettes have been developed and are routinely used for monitoring leaf photosynthesis. Such cuvettes operate on either a differential or compensating principle in which NCER is estimated from differences in carbon dioxide concentration between inlet and outlet air streams (in the case of differential systems) or from the amount of carbon injected into the cuvette in order to keep carbon dioxide concentrations static (in the case of a compensating system). Recently, the University of Guelph has adapted former exposure chambers to operate in a hybrid fashion, including elements from both differential and compensating systems. The University of Guelph has just completed an

intensive period of chamber acceptance and validation studies and has completed preliminary empirical modeling efforts.

Section 2.0 - Open Higher Plant Chamber Design and Operation

2.1 - Overview

A total of 9 glass chambers were re-designed to incorporate mass flow controllers and meters for the automated control of atmospheric CO_2 concentration and the determination of NCER. These chambers were formerly exposure chambers designed to assess plant response to long term exposure to various air pollutants such as ozone. The addition of mass flow controllers, an external CO_2 source and the connection of the chambers' atmosphere to an inlet air source allowed for their operation as hybrid differential/compensating systems.

The chambers are sufficiently sized to house up to five mature plants of medium variety in each chamber and allow for concurrent long term exposure of crops in each chamber to CO_2 enriched atmospheres. Because of difficulty in cooling the chambers during the summer months and the need for a 'blank' or control chamber, only 8 chambers are used at any given time and the 9^{th} is reserved as a control and to reduce cooling system loading.

As flow through chambers, NCER is determined as the difference between CO_2 concentration entering the chamber (inlet concentration) and the concentration of air leaving the chamber (outlet concentration). This technique is described later. Also, each pair of 8 chambers has a distinct air inlet stream. This allows for the addition of CO_2 into the inlet air stream of each pair of chambers to allow for plant exposure to long term CO_2 enrichment. The following sections summarize the major operational and design features of the chambers.

2.2 - Air Handling

Inlet - Chamber inlet air is taken from the University of Guelph supply. The compressor for this air stream is housed in the University's power plant and is delivered to the greenhouses at 30 psi. This air is also used by other researchers using hydroponic cultivation. Air enters a routing manifold and is split four ways. Each route feeds a pair of chambers (although the chambers are not necessarily adjacent). Each route is also connected to a line carrying CO_2 from an external tank. Analogue flow controllers on the CO_2 lines allow for the coarse control of the concentration for enrichment purposes. Typically the chamber CO_2 concentrations are 400, 700, 1000 and 1300 ppm. An additional line of CO_2 feeds the common inlet air line through a mass flow controller. This allows for the common injection of precisely metered CO_2 into the inlet stream to compensate for removal of CO_2 by photosynthesis. This results in variable inlet CO_2 concentrations (mirroring photosynthesis) and static chamber outlet concentrations.

Outlet - Air exits the chamber only after it is well mixed and has passed through the plant canopy. This is ensured by sealing the chamber completely in all areas below the plant canopy. While there is no primary outlet for chamber air out-flow, the positive pressure established means air escapes the chamber through small cracks in areas above the canopy and where inlet air has been thoroughly mixed. Chamber outlet air is sampled on the outlet side of a plenum. Chamber outlet CO_2 concentration is compared to demand concentrations. In compensating mode, a mass flow controller is regulated by the chambers' controller to replenish the CO_2 removed from the chamber atmosphere by photosynthesis. This ensures a static atmospheric CO_2 concentration, suitable, for response studies, during daylight hours.

2.3 - Temperature and Humidity Control

Temperature is measured with a thermocouple and regulated with the use of a radiator and electric heaters. The radiator is fed coolant from a water bath cooled slightly below demand levels using a series of compressors located in an adjacent greenhouse zone. Chambers are heated with electric heaters connected to aluminum plates (to maximize surface area for exposure) positioned adjacent to the chamber air circulation fans.

Pressurized, reverse osmosis water is connected to each chamber. Humidity is measured with wet/dry thermocouples positioned adjacent to the chamber air circulation fan. Humidity is maintained, positively only, by sending a signal to a normally closed solenoid at the water-line / chamber junction.

2.4 - CO_2 and Lighting

CO_2 is controlled to demand concentrations established by the user. The controller uses chamber outlet CO_2 concentration since this air is well mixed within the chamber and therefore represents the true chamber concentration. In compensating mode, a mass flow controller is used to replenish CO_2 lost due to photosynthesis. The controller uses a single chamber outlet concentration to determine the magnitude of the CO_2 concentration error (difference from demand). CO_2 is monitored with an in-line Infrared Gas Analyzer. CO_2 is injected into the inlet air stream common to all chambers (i.e. before it has split at the manifold) via the mass flow controller from an external CO_2 cylinder.

Because of power constraints in the greenhouses, artificial lights could not be installed above each chamber. As such, ambient lighting is used. There is sufficient variability in ambient light intensity throughout the seasons to generate adequate light responses.

2.5 - Nutrient Delivery and Contaminant Detection

Typically, plants are placed in each chamber in 5 L poly-ethylene containers filled with 0.5 x Hoagland's solution. This solution is normally re-placed at 5 day intervals by opening the

chambers and replacing the containers with fresh solution. This is done to ensure that none of the nutrients become deficient. Samples of the atmosphere are collected regularly and analyzed with an off-line GC to ensure that ethylene concentrations are insignificant. Because of the purity of the CO_2 source and the flow through design, ethylene concentrations are routinely below the detection limit.

2.6 - Calculation of NCER

Flow meters placed on the chamber inlet line as well as temperature and CO_2 concentration measurements taken on inlet and outlet air streams allow for the calculation of instantaneous NCER using the following equation:

$$NCER_t = \frac{([CO_2]_{inlet} - [CO_2]_{outlet}) \cdot F}{R \cdot (K - 273) \cdot 22.4}$$

where;

F = Inlet Air Flow Rate (L s^{-1})
R = Gas Constant; 0.0821 (K^{-1} mol^{-1})
K = Absolute Temperature (K)
22.4 = Volume of Gas at STP (L mol^{-1})
[CO2] = Concentration of Inlet or Outlet CO_2 (L CO_2 L^{-1} Air)
NCER = mol CO_2 s^{-1}

The fundamental feature of this technique for NCER analysis is the use of differences in CO_2 concentrations between inlet and outlet air streams. This approach is rare for full canopy chambers and performance measures had to be developed to validate chamber accuracy and efficacy.

2.7 - Chamber Performance Evaluation

Over the last year, a number of validation trials have been performed to ensure the chambers are accurately measuring carbon gain. In the past, chamber validation studies have been conducted using a series of short term closures. NCER data from the experiments would be integrated and correlated with small biomass gains obtained over the 14 days or so under closure. In the case of validating the flow through chambers, Beet, Lettuce and Kale plants were placed under closure for the duration of their grow-out period. This meant a larger set of NCER was integrated and correlated with larger biomass gain. This approach was believed to give a better assessment since even small chamber errors in estimating NCER would be amplified as a result of a wider

integration interval.

A series of experiments were conducted in the eight functional chambers using either beet, lettuce and kale. Plants were started from seed in Rockwool cubes and placed in an adjacent greenhouse until sufficient root exposure and leaf area were developed to warrant transfer into the chambers. Four plants were placed in each chamber using the nutrient delivery technique described above. NCER was logged at 15 minute intervals over the course of the grow-out period. NCER data were integrated and converted to carbon gain.. The mass of seedlings was insignificant so carbon gain was correlated with total plant dry weight in each chamber at the end of the grow-out period.

Results indicate good performance of the chambers. Regression analysis of actual dry weight as the dependent variable and chamber estimated whole plant carbon gain as the independent variable indicates that chamber data are highly significant ($p<<0.01$) predictors of true biomass gain. Correlations for analyses were better than 0.90 and the slope was not significantly different from 1 (at $p=0.01$). This indicates that the chambers have little if any bias in predicting whole plant carbon gain. Calibration curves will be generated for every future experiment conducted in the chambers to further establish the utility of the chambers in non-destructively estimating carbon gain. It is important to note that NCER determinations in these chambers is at the whole plant level and does not provide information on carbon partitioning unless destructive harvest is done. Further, NCER is dependent upon the culture conditions of the plant. Therefore, if the plants were grown in stress conditions total plant carbon gain may be subject to change. While this value would be reflected in integrated NCER estimates, changes in partitioning are not. Fortunately it is possible to develop NCER responses to varying environmental conditions (see below, i.e. light and CO_2) and it is therefore possible to quantify the effects of both transient and long term stresses on whole plant carbon gain using this technique.

An additional means to assess chamber performance is to develop light and CO_2 response curves for candidate crops and to determine if the response profile has a shape typical of what has been reported in the literature. Classical plant responses to CO_2 and light take the form of a rectangular hyperbola. This type of curve is characterized by a linear response to increasing light/CO_2 levels at low to moderate magnitudes followed by an asymptotic response at higher or saturating levels. Each growth stage has a unique curve, with such curves shifting to the right (higher saturation levels) as the canopy matures and the leaf area index increases. In theory then, a series of light or CO_2 curves collected throughout crop development will exhibit the shape of the rectangular hyperbola, but with the point of saturation shifting further and further to the right as the crop ages. The result is a three dimensional surface with a profile (cross section) taken at any crop age similar in shape to the rectangular hyperbola. It is important to note that this model is used extensively in describing crops response to environment conditions in forthcoming TNs. The reader with more interest in response curve formulations for single growth stages/ages is referred to Iqbal *et al* (1997) who provides an excellent review of reponse funstions in photosynthesis studies.

To assess this data were collected in six sealed of the chambers located using Lettuce (*Lactuca sativa* cv. Bella-green). Each of the six chambers were stocked with 4 lettuce plants from the seedling stage (21 DAP) to harvestable stage (65 DAP). Net-Carbon Dioxide Exchange data were recorded in each of the six chambers at 15-minute intervals over the course of crop development. This allowed for the generation of a response profile relating net-photosynthesis during day-light hours (Pn) to ambient light intensity at each observation and crop age (DAP 40 to 65 only).

A rectangular parabola (non-linear, parametric) model was applied to the data using the *nls* function of S-Plus (Data Analysis Products, 2000). This model is similar to the model presented by Iqbal *et al*, (1996) but allows for dynamic maximum gross photosynthesis and dark respiration rates in relation to crop age. This model has the form;

$$Pn = \frac{\alpha \mathrm{I}(\beta_o + \beta_1 DAP)}{\alpha \mathrm{I} + (\beta_o + \beta_1 DAP)} + (\beta_2 + \beta_3 DAP)$$

where α is a non-linear least squares estimate of photosynthetic efficiency, b_0 b_1 b_2 and b_3 are parameter estimates of the βs, I is the incident photosynthetic photon flux at canopy height, and DAP is days after planting. The surface response resulting from the non-linear least squares fit is presented in Figure 2. The model fit the data well with a residual-sum of mean-squared error of 0.460 with df=2597 and model df=4. All parameters were significant at the p=0.05 with the exception of b_2. Parameter estimates were a=0.057, b_0 =-10.28, b_1 = 0.289, b_2 = 0.064 and b_3 = -0.226.

The excellent performance of the rectangular hyperbola model (which is commonly used to describe crop response to light) over the duration of the crop grow out period illustrates the chambers' utility in generating such curves.

Section 3.0 - Chamber Design for MELiSSA Loop Integration

While the chambers described above would be similar in operation to a chamber designed for the test-bed significant differences would exist in terms of construction materials, degree of atmospheric closure, plant production area required, thermal control requirements and design and

lighting sources. The University of Guelph is willing to participate in the design of the test-bed chamber.

Section 4.0 - Future Work

Performance evaluation of the chambers indicate that they are suitable for continuing studies. Such studies will establish crop NCER response to light and long term CO_2 exposure. Response models are of direct use to dynamic simulations of the HPC within the MELiSSA loop. Work will continue on developing these dynamic models using both the smaller flow through chambers described here and the full canopy sealed chambers.

Section 5.0 – Acknowledgments and References

The authors would like to thank the contributions of Dr. Youbin Zheng and Ron Dutton for their assistance in the flow-through chamber development and design.

References

Dutton, R., Jiao, J., Tsujita, J.,Grodzinski, B. (1988). Whole Plant CO_2 exchange Measurements for Nondestructive Estimation of Plant Growth, Plant Physiol. 86: 355-358.

Iqbal, R., Rao, A.R., Rasul, E., Wahid, A., (1996). Mathematical Models and Response Functions in Photosynthesis: An Exponential Model, in: Handbook of Photosynthesis ed. M. Pessaraki, Marcel Dekker, Inc. New York.

Larcher, W. (1995). Carbon Utilization and Dry Matter Production, pp. 97-99, in: Physiological Plant Ecology, Springer, Paris.

Salisbury, F., Ross, C. (1992). Photosynthesis: Environmental and Agricultural Aspects, pp. 257-261. In: Plant Physiology 4^{th} Ed. Wadsworth Inc. Belmont California.

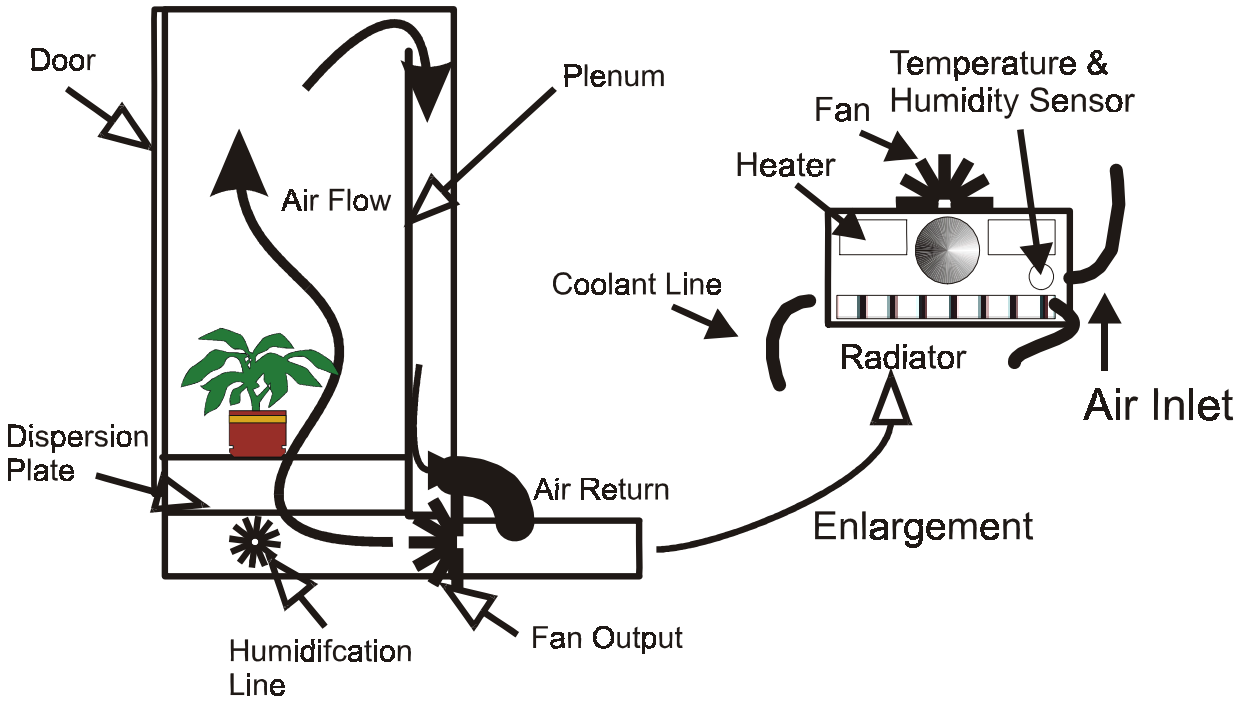

Figure 1. Chamber schematic of one of the flow through chambers illustrating major functional components and air handling.

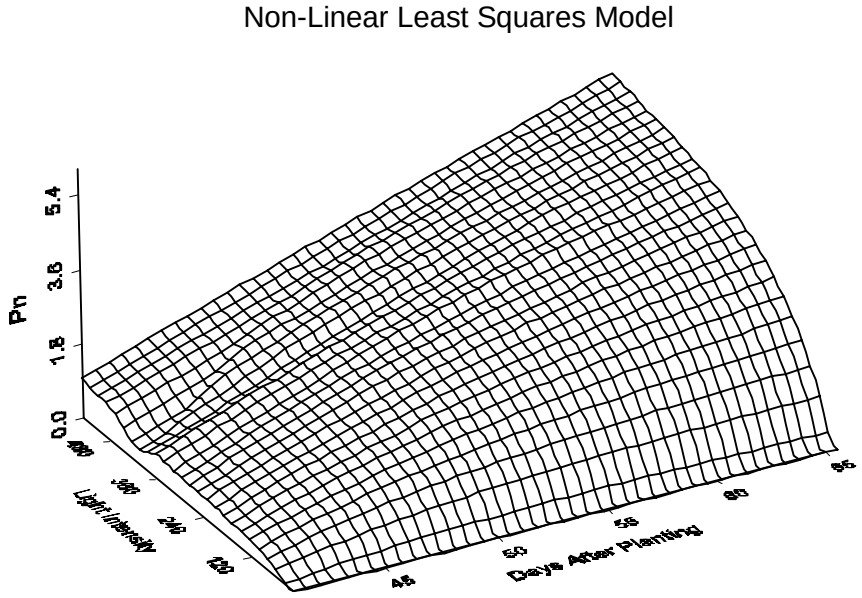

Figure 2. Response surface developed using a non-linear rectangular hyperbola model. This surface indicates that chambers are generating acceptable data.