Variation of Photosynthesis Along a Leaf in *Zea mays* L.

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Abstract

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Maize leaves are characterized by a complex architecture. The maize leaves are curved and this keeps changing with increasing leaf age. Due to the curvature of a maize leaf the amount of light intercepted may vary on different points on the surface of the leaf giving rise to a variation in photosynthesis on different positions along the same leaf. In this study semi-empirical models of photosynthetic light response curve have been used to understand the variation in photosynthesis along the 7th leaf of *Zea mays* P1625HR. The effect of increasing age on photosynthesis of a maize leaf was investigated and modelled. Non-linear regression was used to study the pattern of variation of photosynthesis with increasing leaf age.

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Chapter 1

Introduction

The orientation of a leaf in space or its spatial display can affect the light interception by the leaves. Maize leaves are characterized by a complex curvature which keeps changing over time, with increasing age of the leaf. The architecture of the maize leaf has been studied and various equations have been proposed to model the leaf curvature (Ford et al 2008, Hay et al 2000, Stewart et al 1993 etc.). Due to the curvature in the maize leaf the amount of light intercepted may vary on different points on the surface of the leaf. The light interception studies in maize canopy suggest that the amount of light intercepted by a leaf in the canopy may vary due to its position in the canopy and more importantly the architecture of the leaf (Dwyer and Stewart 1986). As a result the amount of light intercepted by the leaves keeps changing continuously. This may give rise to variation of photosynthesis in different positions on a single leaf in maize. Also the developmental stage of the maize leaf is yet another factor that may give rise to a variation in photosynthesis along a leaf. There is a paucity of studies on whether or not there is a significant variation of photosynthesis along a single leaf in maize.

Question: Is there a significant variation of photosynthesis in 3 different positions along a single leaf in maize?

Here we have used statistical modelling as a tool in order to understand whether or not there is a variation of photosynthesis along a maize leaf. A statistical model describes the relationship between variables in the form of a mathematical equation. The variables in the statistical models are stochastically related and can be analyzed using probability theory. Statistics and statistical models have been used as a tool by plant physiologists and ecophysiologists to get a better insight into complex processes like photosynthesis. Statistical models are well suited to study the variability inherent in natural processes. Empirical and semi-empirical models, with some underlying biochemical and physiological basis, have been proposed to understand complex biological processes like the influence of solar radiation on carbon assimilation (Thornley 1976, Johnson 2010).

Modelling Photosynthetic Light Response

Photosynthetic light response curve is a plot of net photosynthesis over varying light intensities. The light intensity (measured in μmol m^{-2} sec^{-1}) is plotted on x-axis and the net photosynthetic output (represented in μmol m^{-2} sec^{-1}) is plotted on the y-axis. As seen in Figure 1.1 net photosynthesis increases with increasing light intensity. Depending on the biophysical and biochemical factors the net photosynthesis stops increasing and it plateaus at a certain light intensity. The intensity at which this flattening occurs is called the light saturation point and the net photosynthesis is called maximum photosynthesis of A_{max}.

Understanding the relation between solar radiation and carbon assimilation/photosynthetic light response curve is important in predicting carbon fixation in nature because the variation in light environment of a leaf is one of the most important factors affecting photosynthesis (Lachapelle and Shipley 2012). The maximum photosynthetic rate is sometimes beyond the capability of gas exchange analyzers to detect. In order to understand the maximum achievable photosynthesis which is beyond the limit of gas exchanged measurements, it is important to use statistical models to get the estimates of maximum photosynthesis. In this study we use statistical models to answer the biological questions. Classically it is assumed that there is a single correct model or a best model that can be used to obtain the parameter estimates. Then the inferences are based on the fitted model (Burnham 2004). No model can be a perfect fit for a set of biological data. So it is important to pick out the model which fits the data the best. Hence a comparative study of models can give an idea of which model would be a good fit for answering the biological questions. Various empirical and semi-empirical models have been proposed to study and understand photosynthetic light response curves.

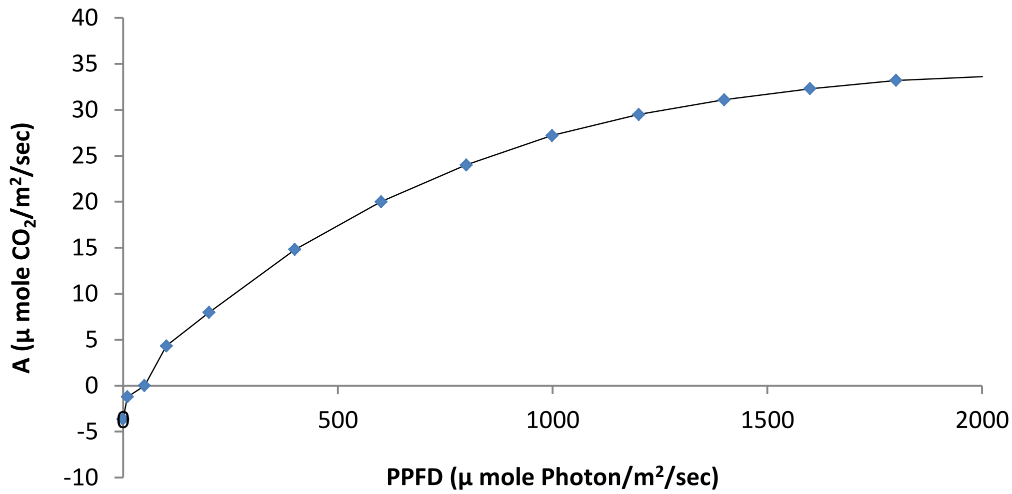

Figure 1.1: Photosynthetic Light Response Curve

The rectangular hyperbola model is one of the earliest models proposed for photosynthetic light response and its parameters. The rectangular hyperbola model commonly called RH model was derived from Michelis Menten equation of enzyme kinetics by Maskell (1928) and later refined by Rabinowich (1951). This was the equation of choice for plant ecophysiologists, modelling photosynthetic light response, in early and middle 20th century. Dwyer and Stewart (1986) used this equation to model the photosynthetic light response in maize leaves. However this traditional model has been described as a poor fit to experimental data (Marshall and Biscoe 1980) and empirically inferior to the equations of photosynthetic light response proposed later (Thornley 1998).

Rabinowich (1951) extended the RH model by adding a quadratic term to it and called the model a non-rectangular hyperbola. Studies on single leaf photosynthesis have more often been done using non-rectangular hyperbola rather than rectangular hyperbola (Stirling et al 1994, Thornley et al 1998, Johnson et al 2003). This model has more parameters and a respiration term added to it. This model has been described in details by Thornley (1976). The NRH model is a more flexible equation and fit a wide range of photosynthetic light response data in C3 and C4 plants.

Another slightly less popular equation used to model photosynthetic light response is an extension of Mitscherlich equation. Potvin et al (1990) described the model as a nonlinear mixed model with 3 parameters. This model is sometimes referred to as the exponential asymptote model or EA model. This model has been used to display the response of single leaves to solar radiation (Aleric and Kirkman 2005 in *Lindera melissifolia*, Potvin et. al. 1990 in *Echinochloa crus-galli*, Peek et. al. 2002 in *Amphicarpa bracteata*, Marino et. al. 2010 in 65 angiosperm species, Lachapelle and Shipley 2012 in 25 herbaceous species).

We investigate single leaf photosynthetic light response in maize using the NRH and EA models. The NRH model has been widely used and has been applied to model single leaf photosynthesis in maize. However the EA model has not been as extensively used as the NRH model. The NRH model has 4 parameters instead of the 3 parameter model of EA. In case of NRH there is a chance that it models random error or noise (overfitting a data set). The chances of overfitting a data set with EA model are lower and therefore EA has better predictive power. Less attention has been paid to modelling single leaf photosynthesis in maize using the EA equation. There are no studies comparing the EA and NRH models in maize.
In this study we compare the accuracy of the EA and NRH equations in modelling photosynthesis in maize.

Question: Does the Non-Rectangular hyperbola or the exponential asymptote model photosynthetic light response with greater accuracy in maize?

The photosynthetic light response curve models have been used to study the effect of various factors like age of plant, nitrogen status of soil/ plant, water status etc. on photosynthesis. Dwyer and Stewart (1986) studied photosynthetic light response in maize leaves as a function of plant age, leaf age and leaf position in the 12th, 13th and 14th leaves. All their photosynthetic measurements were fitted to a rectangular hyperbola model. Their study revealed that after full leaf expansion the light curves flattened to reach asymptote at lower irradiance levels. They also found a strong correlation between photosynthesis at saturation and plant age as well as leaf age.

Moreno-Sotomayor et al (2002) studied the effect of variation in the amount of nitrogen fertilizer on photosynthetic light response in maize leaves 11 and 13. They too found a strong correlation between plant age and photosynthesis.

Stirling et al (1994) found light saturated photosynthesis in maize decreases with increasing age of the 4th leaf. Along with the studies described above, modelling of photosynthetic light response has been done on endospermic leaves or fully expanded leaves at the top of the canopy. Here we seek to answer whether or not the maximum photosynthesis changes significantly with increasing leaf age in the 7th leaf of maize (a non-juvenile leaf in the lower tiers of the canopy).

Question: Does photosynthetic parameter "A_{max}" vary significantly with increasing age of the 7th leaf in maize?

Stirling et al (1994) studied photosynthetic light response as a function of age of the leaf and ambient temperature in corn plants. The study was performed on the unexpanded 4th leaf in maize (a short-lived juvenile leaf). Regression of the maximum photosynthesis over thermal time (starting from the emergence of the plant) showed strong correlation. The maximum photosynthesis showed a triphasic response with increasing thermal time.

Question: What is the pattern of variation of "A_{max}" with increasing leaf age?

Chapter 2

Strategy of Investigation

Many statistical equations have been proposed to calculate and estimate photosynthetic light response curve parameters (see Chapter 1). The two most promising models for maize are the non-rectangular hyperbola model and the exponential asymptote model. The 2 models are described below.

The Models:
The equation for Non-Rectangular Hyperbola (NRH) was described by Thornley (1976):

$$A = \left\{ \frac{\phi Q + A_{max} - \sqrt{[(\phi Q + A_{max})^2 - 4\phi Q A_{max} \theta]}}{2\theta} \right\} - R$$

where,

ϕ represents the initial slope of the photosynthetic light response curve (PLRC) and is dimensionless

Q represents the PPFD (Photosynthetic photon flux density measured in μ mol photon/m^2/sec

A_{max} represents the upper asymptote of the PLRC , measured in μ mol CO_2/m^2/sec

A is the net photosynthesis

R is dark respiration

θ is the curvature parameter of PLRC and is dimensionless

Figure 2.1 below represents a photosynthetic light response curve fitted to the NRH model

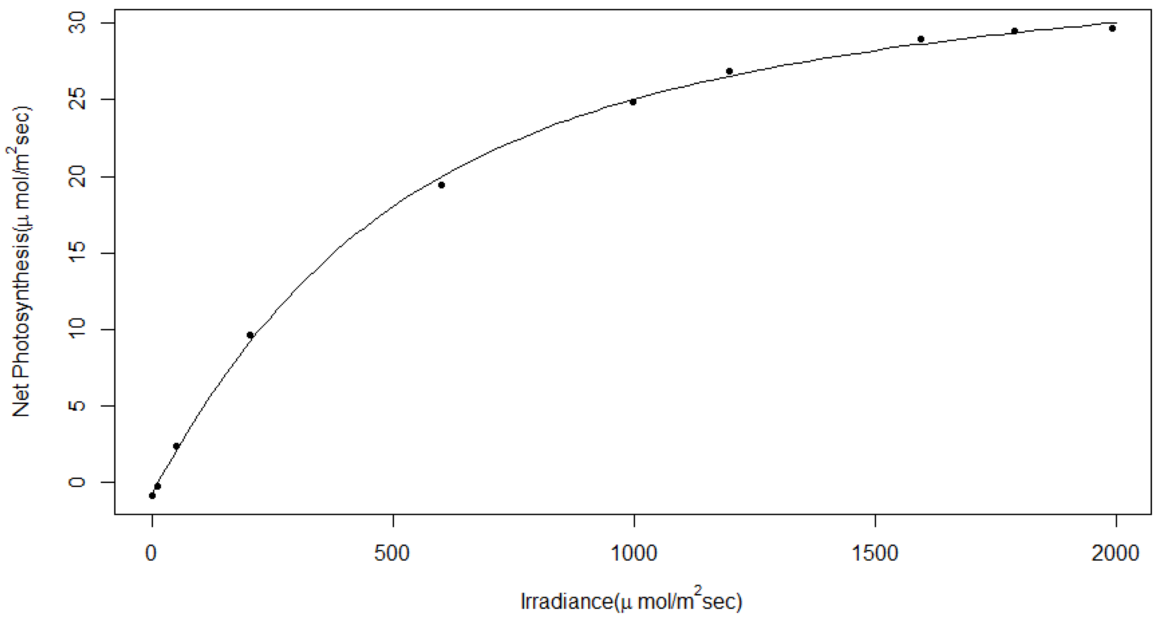

Figure 2.1 Photosynthesis Data fitted with NRH model. Non linear mixed modelling was used to fit the photosynthesis data to the non rectangular hyperbola model.

The parameters of the NRH model corresponding to Figure 2.1 is shown below

Name of Parameter	Estimate
A_{max}	35.279161
θ	0.502889
Φ	0.057336
R	0.821086

Table 2.1. Estimates of Photosynthetic light response curve parameters calculated using NRH model

The equation for Exponential asymptote (EA) was proposed by Potvin et al (1990):

$A = A_{max}[1 - e^{-A_{qe}(PPFD - LCP)}]$

where

A_{max} is the upper asymptote of the PLRC

A_{qe} is the initial slope of the curve

PPFD is photosynthetic photon flux density

LCP is light compensation point where the net carbon gain by photosynthesis equals the loss by respiration

Figure 2.2 below represents a photosynthetic light response curve fitted to the EA model

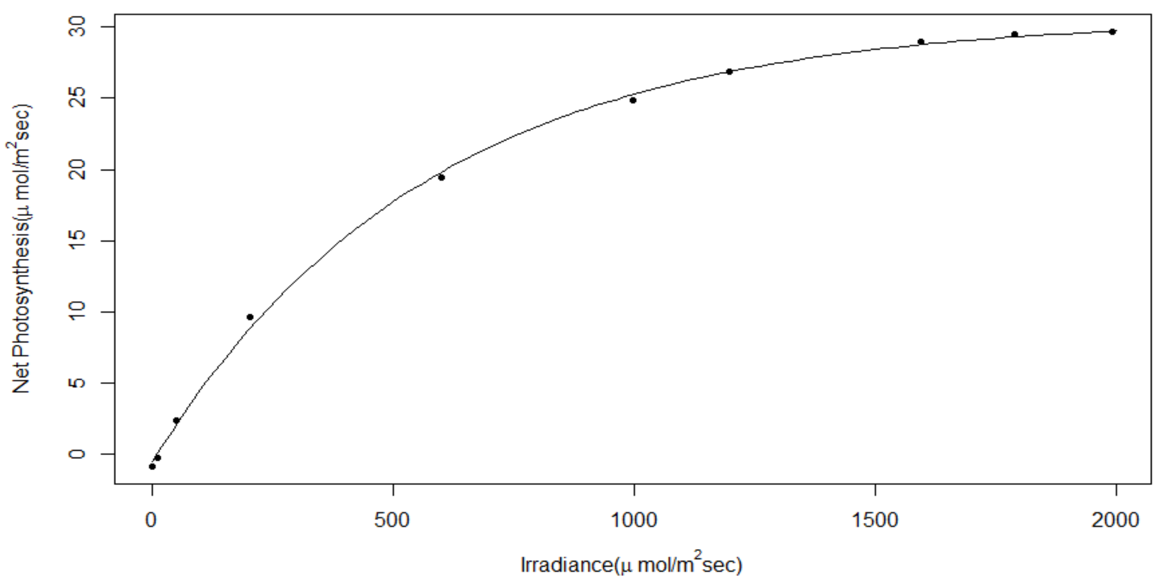

Figure 2.2 Photosynthesis data fitted with EA model. Photosynthesis data from the 4th week of measurement for replicate 7 was obtained and used to fit the EA model using non linear least squares in R studio.

The parameters of the EA model corresponding to Figure 2.2 is shown below

Name of Parameter	Estimate
A_{max}	30.63
A_{qe}	0.001763
LCP	8.929

Table 2.2 Estimates of Photosynthetic light response curve parameters calculated using EA model

Fitting models to the equations:

Net Photosynthesis (Photo) and corresponding irradiance (PARi) outputs extracted from the LICOR 6400xt were used for calculation of PLRC parameters. Table 2.3 shows an example data set of one PLRC from the 4^{th} week of measurement of a replicate. The NRH equation was first used to construct a model that was the best fit for the series of photosynthetic light response data points. The same procedure was next carried out for the EA equation. The model fitting was done by non-linear least square method where the best fitting curve was obtained by minimizing the sum of squared residuals (the residuals are the difference between the observed and calculated value). The non-linear least square calculated the parameters of the model by linear regression and refined the parameters by successive iterations.

Irradiance (μ mol_{photon} m^{-2} s^{-1})	Net Photosynthesis (μ mol_{CO2} m^{-2} s^{-1})
1993.544	29.65706
1788.996	29.4982
1594.97	28.9767
1196.313	26.8419
998.2003	24.8842
599.3741	19.4171
202.4638	9.6186
49.0011	2.3983
10.36135	-0.23425
0	-0.80048

Table 2.3 Net Photosynthesis and corresponding irradiance data points of the 7th replicate (in the 4th week of measurement) extracted from the LICOR 6400xt

Assessing model fit and comparison of models:

In this study NRH and EA models have been compared to understand which model fits the maize photosynthesis data points better and estimates the light response curve parameters more accurately. A very well-known approach for model selection is comparing the Akaike's Information Criterion (AIC) values of the different models (Akaike 1973, Burnham 2004). Another method for model selection is comparing the Residual sum of Squares (RSS) values for models. The 2 methods of model selection have been described below.

Akaike's Information Criterion (AIC)

AIC is a measure of the relative quality of a statistical model. AIC aims to estimate the K-L divergence of a model or the information lost when a model is used to approximate full reality

(Burnham et al 2010) unbiasedly. While comparing two models, the model with the lower AIC value is the better fitting model (Kerby, Shedden 2010).

Residual sum of Squares (RSS)

RSS is a measure of discrepancy between the data and the estimated model. RSS is also the difference between the true value and the predicted value. Hence lower RSS values shows a model is a better fit than other models.

A_{max} data cloud

A_{max} values were used to produce a data cloud of A_{max} over increasing age of the 7th leaf (Figure 2.3).

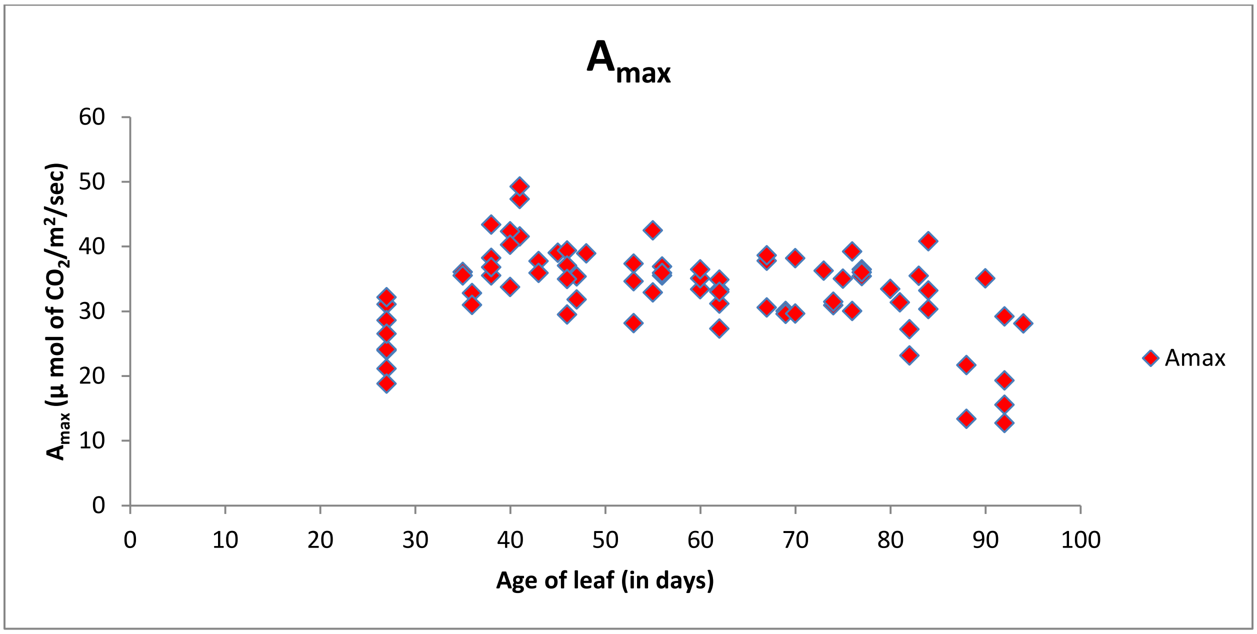

Figure 2.3 Variation of A_{max} with increasing age of 7th leaf

Equations to study the variation of A_{max} with age:

Seven common equations described by Haefner (1996) were fitted on the A_{max} data cloud to understand the pattern of variation of A_{max} with increasing time. AIC and RSS values were used to pick the best fitting model. The equations are shown in Table 2.4

Name of Equation	Equation
Linear	$y = k1x + k0$
Quadratic	$y = k2x^2 + k1x + k0$
Cubic	$y = k3x^3 + k2x^2 + k1x + k0$
Polynomial	$y = k3x^3 + k2x^2 + k1x + k0$
Triangular	$y = \begin{cases} k1 + k2x \; if \; x < k3 \\ k4 - k5x \; if \; x > k3 \end{cases}$
Weibull	$y = k1\; e^{[-\left(\frac{x}{k2}\right)^{k3}]}$
Power	$y = k1\; e^{[-\left(\frac{x}{k2}\right)^{k3}]}$

Table 2.4 Seven equation used to understand the variation of A_{max} with increasing leaf age

Chapter 3

Material and Method

Plant samples and measurements

Zea mays v.P1625HR seeds from Pioneer Hibred were used for the experiment. Seventy two seeds were planted in sunshine soil mix 4 in the UW Botany green house on 20th March 2013 (Figure 3.1)

Figure 3.1 Zea mays v. P1625HR in University of Washington's New Botany Greenhouse before being transplanted to 5.3 gallon pots.

After germination, at two leaf stage the plants were transferred to 5.3 gallon pots. The plants were watered to full saturation every other day. They were also fertigated twice weekly using a combination of 20-10-20 and 17-5-17 cal-mag special NPK fertilizer by Nutriculture. The pots were rotated by 90 degrees every day to reduce any directional bias created by the greenhouse environment. The morphological characteristics were tracked every day.

The seventh leaf of 45 plants emerged on the 26th of April. Nine replicates were randomly chosen from these 45 plants. Three sections were marked on the 7th leaf of each replicate using a sharpie as described below.

Three Sections

The sections are defined as follows:

- Section 1 started at a point 6" proximal from the tip of the leaf 7. The dimension of the section was 3"x2".
- Section 2 started at a point 6" removed from the end of section 1 with 3"x2" dimension.
- Section 3 started at a point 6" removed from end of section 2 with a 3"x2" dimension.

The sections were added to the leaf gradually as the leaf expanded and increased in size.

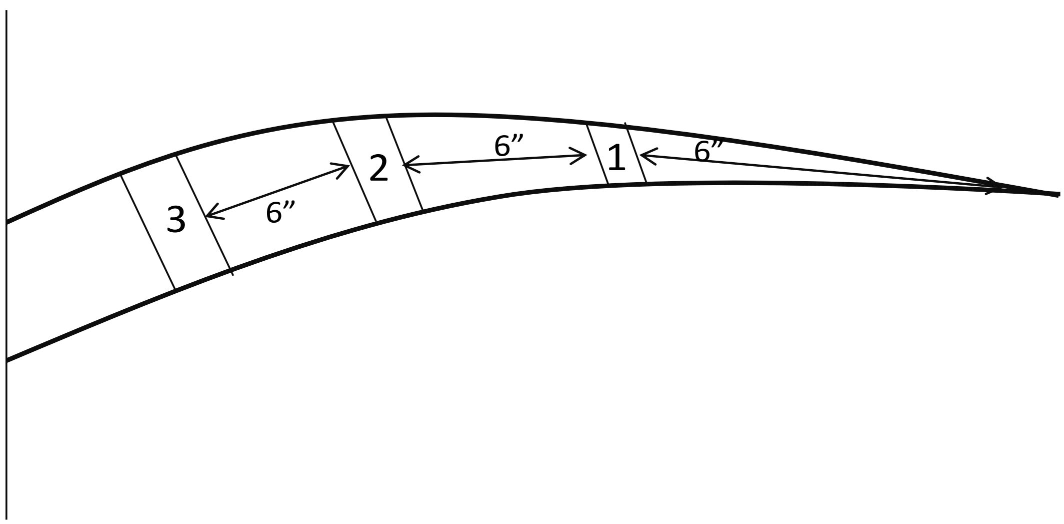

Figure 3.2 The 3 sections of the 7th leaf. As the 7th leaf unfolded from the stem the first section was added followed by the second and the third section. Due to basipetal growth of maize leaves the first section is the oldest. The 3sections were measured repeatedly for 10 consecutive weeks following their emergence.

Measurements:

Light response curves were measured using a LICOR6400xt (Figure 3.3) on all 3 sections (Figure 3.2) of the 7th leaf of each replicate. An automatic program was used to measure these light response curves. For each light response curve, 10 light points were set and the corresponding net photosynthesis was recorded by the LICOR.

These measurements were repeated for each section and replicate every week till the 7th leaf died. Due to the nature of leaf expansion in maize, the first section was measured for 12 weeks, the second section for 11 weeks and the third section for 10 weeks. The measurement on the first section started 11 days after the emergence of the 7th leaf.

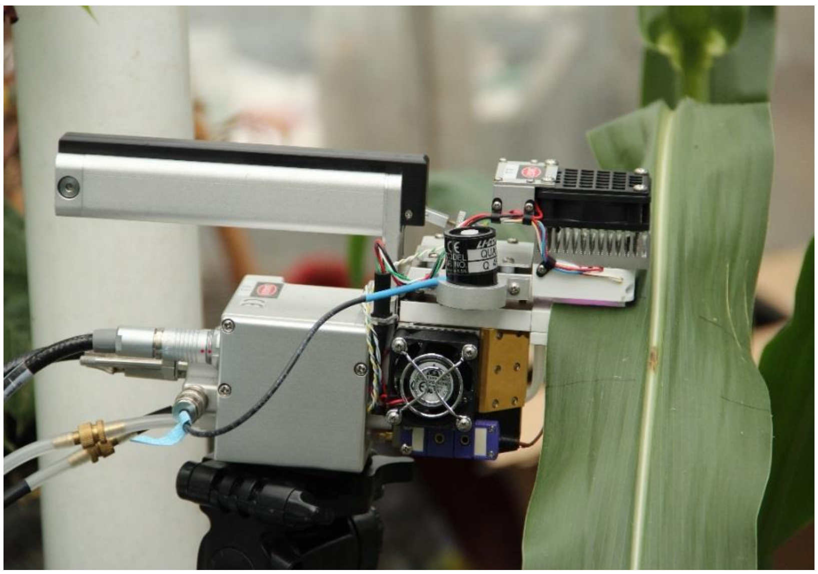

Figure 3.3 Measurement of a Photosynthetic Light Response Curve using LI-6400xt in University of Washington's Greenhouse. An automatic light response curve program was set using 10 light points and the corresponding photosynthetic data was recorded by the LICOR. This study continued for 12 consecutive weeks for the first section. Eleven and ten consecutive weeks for the second and the third section.

Calculation

In all 264 excel sheets (one for each light response curve) were extracted from the LI-6400xt. The net photosynthesis and the irradiance data points were extracted from all the files obtained from the LICOR. R studio version 0.98.495 was used for all the calculations (R codes are included in the appendix). The EA and the NRH models were fitted to 264 sets of light response curve data points using *nls* command in R studio (nonlinear least square method). The parameter estimates obtained from the models were first used to compare the models. The AIC values for all the light response curves for both models were obtained by using the command *AIC* in R. The residual sum of squares were obtained from the last iteration of the *nls* regression with *trace= TRUE*.

The A_{max} data cloud for each section (as described in chapter 2) was obtained by plotting A_{max} values over increasing age of the 7^{th} leaf. 7 equations were fitted to the A_{max} data cloud using *nls* in R to study the nature of variation of A_{max} with increasing age of the 7^{th} leaf.

To understand whether A_{max} varies significantly across the curvature of the 7^{th} leaf an ANOVA was done. The last two weeks of A_{max} values and the last week of A_{max} value was removed from the section1 and section 2 respectively to create a balanced design. A crossed design of ANOVA model with interaction effect was used to test whether A_{max} varied significantly across the curvature of the 7^{th} leaf. The command aov was used to fit the model.

The general model for the crossed ANOVA with interaction was:

$$Y_{ijk} = \mu + \tau_i + \beta_j + \gamma_k + (\beta\tau)_{ij} + (\beta\gamma)_{jk} + (\tau\gamma)_{ik} + (\beta\tau\gamma)_{ijk} + \varepsilon_{ijk}$$

Here, $i = 1,\ldots\ldots\ldots\ldots,t$, $j = 1,\ldots\ldots\ldots\ldots,r$, $k = 1,\ldots\ldots\ldots\ldots,g$, τ is the treatment, β and γ are the blocks

And,

$\beta_j \sim N(0,\sigma^2_\beta)$, $\gamma_k \sim N(0,\sigma^2_\gamma)$, $\varepsilon_{ijk} \sim N(0,\sigma^2_\varepsilon)$ are all independent

The ANOVA was done with and without controlling for the difference in age of the sections.

Chapter 4

Comparison of Two Models

Introduction

Most plant ecophysiological studies include the analysis of response curves. These are commonly constructed by plotting a measured variable against a range of factors that affect the variable. The physiological response curves are often non-linear. The Photosynthetic light response curve is one of the most common response curves studied by plant ecophysiologists (Larcher 1995 and Peek 2002).

The photosynthetic light response curve as described earlier (in Chapter 1) shows the relation between the net photosynthesis and the light intercepted by the leaf. Many mathematical models have been proposed to quantify the relation between net photosynthesis and irradiance. These models have been used to produce the best fit for the net photosynthesis data points. The parameters of the best fit model are used for further investigation. The estimated parameters of photosynthesis provide an insight into the photophysiological responses of the leaves (Johnson and Barber 2002). The number of estimated parameters of PLRC and their values depend on the statistical model used.

Hyperbola Models

The rectangular hyperbola model is one of the earliest models proposed by Maskell (1928) and redefined by Rabinowich (1951). Thornley (1976) described this model in detail. The rectangular hyperbola is an extension of the Michaelis Menten equation and has been extensively used to model photosynthetic light response (Dwyer and Stewart 1986, Dwyer et al 1992, Evans et al 1993, Johnson and Barber 2003, Marino et al 2010).

The non-rectangular hyperbola was created by adding a quadratic term to the rectangular hyperbola. This refinement and extension was first proposed by Rabinowich (1951) and later by

Chartier (1970), Acock (1971) and Thornley (1976). The non-rectangular hyperbola was found to be more resilient than the rectangular hyperbola. The non-rectangular hyperbola is a useful equation that has been used to describe the response of leaf photosynthesis to irradiance as well as carbon dioxide response. The generic equation is described as:

$$\Theta y^2 - (\alpha x + y_m) + \alpha x y_m = 0$$

This equation modifies a rectangular hyperbola by adding a quadratic term and the two asymptotes are not perpendicular to each other. The generic equation has two solutions. For biological models the solution with x≥ 0 is used. As described by Thornley (1976) and later Johnson (2010), the nonrectangular hyperbola "is a powerful versatile equation that is easy to work with. The three parameters each control the key aspects of the response: the initial slope, curvature, and asymptote". A respiration term was added by Rabinowich (1951).

$$A = \left\{ \frac{\phi Q + A_{max} - \sqrt{[(\phi Q + A_{max})^\wedge 2 - 4\phi Q A_{max}\theta]}}{2\theta} \right\} - R$$

Equation 4.1 Non Rectangular Hyperbola Model

Equation 4.1shows the form of equation that is used to describe the light response curves for leaf net photosynthesis. This equation with 4 parameters is easy to work with and many authors have used this equation for their research (Thornley 2001, Koyama and Kikuzawa 2010, Moreno-Sotomayor et al 2002, Prieto et al 2010, Ye et al 2007, Thornley 2004, Zufferey 2000, Ogren 1993, Stirling et al 1994, Johnson and Thornley 1984). Figure 4.1 shows a light response curve fitted to the NRH equation. The aspects of the response controlled by the different parameters are also shown.

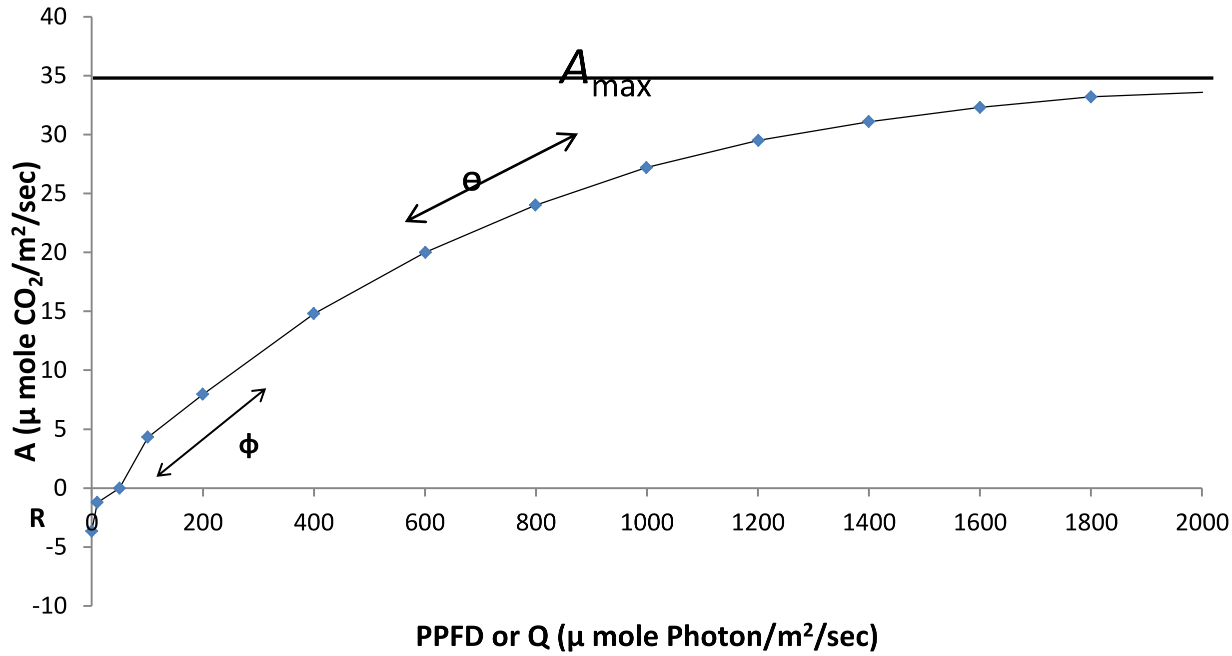

Figure 4.1 Non Rectangular Hyperbola Model parameters.

Exponential Asymptote Model

The modification of Mitscherlich equation (described in chapter2), sometimes referred to as the exponential asymptote equation (EA), has not been used as widely as the NRH equation. Potvin et al (1990) first refined and used the Mitscherlich equation. However the model suggested by Potvin was linear. Peek et al (2002) used the modification of the Mitscherlich equation in a non-linear form in their experiment on two herbaceous legumes with two different light treatments. The EA equation (equation 4.2) parameters are shown in Figure 4.2

$$A = A_{max}[1 - e^{-A_{qe}(PPFD - LCP)}]$$

Equation 4.2 Exponential Asymptote Model

Marino et al. (2010) has described this equation as a "phenomological description of photosynthesis". The 3 parameters corresponding to distinct biological processes make them meaningful and easier to use (Lachapelle and Shipley 2012).

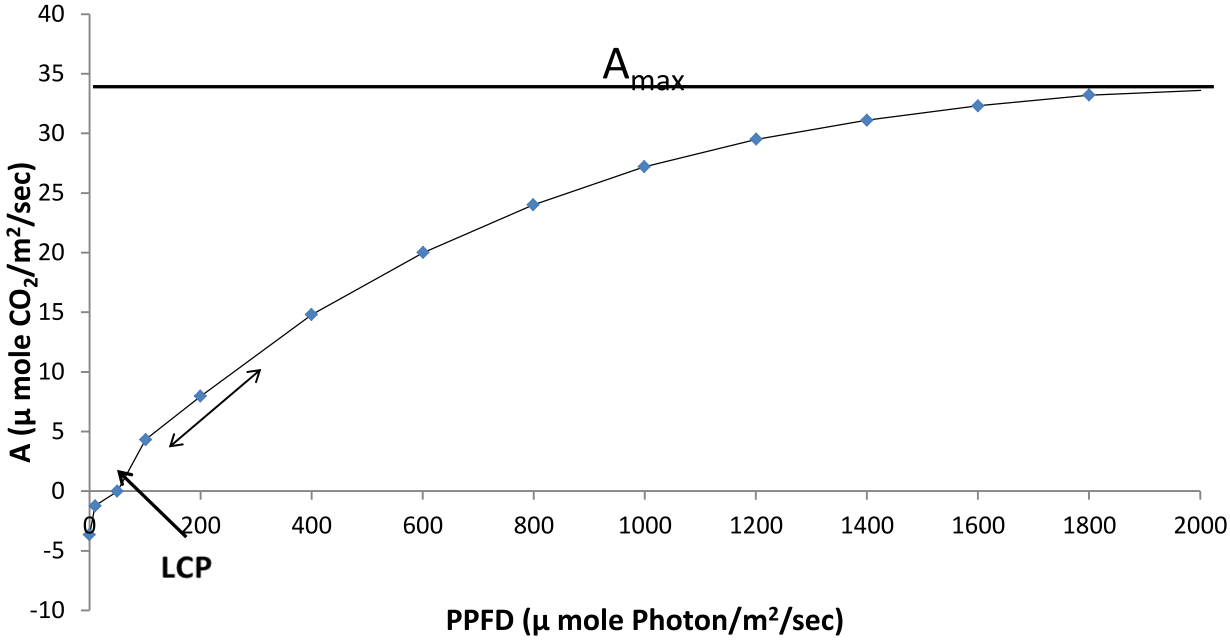

Figure 4.2 Exponential Asymptote Model parameters.

Most scientists in the field of plant ecophysiology have focused on refining and using a single model to understand the photosynthetic response to irradiance. It is still not very clear how accurate the models are in calculation of the parameters of photosynthetic light response curves. A statistical comparison of the accuracy of the NRH and EA models in fitting photosynthesis data points has also been slightly overlooked. AIC and RSS (as described in chapter 2 and3) are good statistical tools to compare the accuracy of semi-empirical models. In this chapter we focus on:

1. Using NRH and EA models to fit the photosynthesis data obtained from maize plants
2. Using AIC and RSS to compare the relative accuracy of NRH and EA in calculation/estimation of PLRC parameters.

Methods

As described in Chapter 3, the photosynthesis measurements were taken for 12 consecutive weeks for the first section, 11 weeks for the second section and 10 weeks for the 3rd section on the 7th leaf. EA and NRH equations were used to estimate the PLRC from the first, second and third sections. AIC and RSS values of were obtained for all the weeks for the 3 sections and compared to check which equation (EA or NRH) was a better fit. The measurement and calculation process has been described in detail in chapter 3. A variable TestAIC was defined for each PLRC data set such that if the AIC value of NRH was lower than that of EA for that data set, TestAIC had a value of 0. If the AIC of EA was lower, TestAIC had a value of 1. Another variable TestRSS that compared the RSS values of NRH and EA models was also defined.

Result and Discussion

The ANOVA done on TestAIC and TestRSS showed with a 95% confidence level that the model used was highly significant with a probability value 0.00181. This showed that there was a statistically significant difference in the accuracy of the two models.

In 191 data sets out of 264, the TestAIC and TestRSS values of the EA model were lower than that of NRH. This implies that EA was the more accurate of the two models. In addition TestAIC and TestRSS had the same value for every data set. So both AIC and RSS values show that EA is a better fit than NRH model.

Moreover, the NRH model has 4 parameters unlike 3 parameters in the EA model. Since the EA model has fewer parameters than NRH, the chances of over-fitting data are lower for EA. All the parameters in the EA model correspond to a biological function unlike the parameter theta in the NRH model. Therefore the exponential asymptote model appears to be the more suitable model for photosynthetic light response curves of the shaded leaves in maize.

Chapter5

Is there a significant variation of A_{max} along the 7th leaf?

Introduction

Maize leaves are curved and the curvature keeps changing not only along the length of the leaf but also over time, through various developmental stages of the plant. Various authors have modelled the curvature of the maize leaves. Stewart et al (1993) modelled the maize leaf curvature with a single quadratic equation. Hay et al (2000) studied the bending of maize leaf in their biomechanical analysis of its phenotype. They defined and expressed the curvature of maize leaves as the rate of change of angle θ to a curve ϗ. They found that the curvature of a maize leaf changes over time, so they called it an elastic character. Ford et al (2008) further refined the modeling of maize leaf curvature and used a set of equations to model the curvature. According to them the curvature of a maize leaf cannot be described by a single mathematical function as had been previously proposed by Prevot et al (1991), Stewart et al (1993). However all these studies of modelling the leaf curvature do not provide an insight into the variation of photosynthesis along a single leaf in maize.

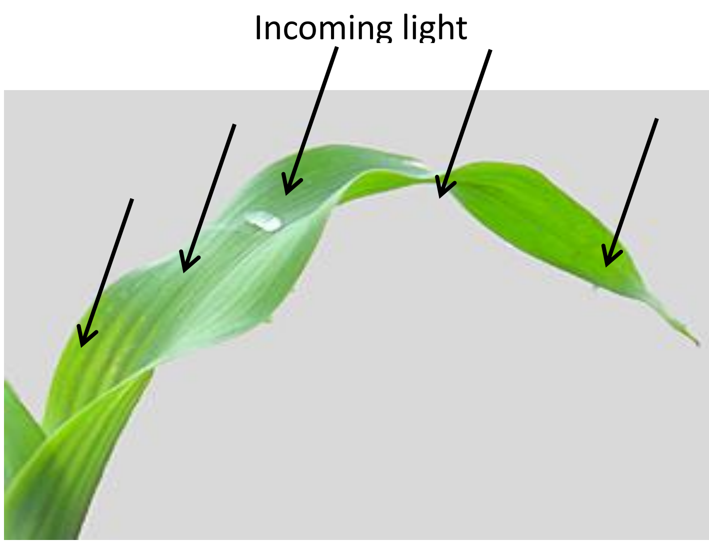

Figure 5.1 Variation of light along the maize leaf. Due to the complex curvature different amounts of light intercepted on the leaf is different on different points. This may give rise to variation in photosynthesis along a maize leaf.

Due to the curvature of a maize leaf, different amount of light may be intercepted at different points on the same leaf (Figure 5.1). Stewart et al. (2003) studied and modelled the interception of light in a corn canopy. They also observed that the change of leaf curvature can influence the amount of light intercepted by the leaves. The variation in amount of light intercepted in turn contributes to the variation in photosynthesis.

Johnson and Thornley (1984) recognized the influence of light attenuation on photosynthesis. They incorporated the effect of diurnal variation into models of photosynthesis (Thornley and Johnson 2000). It was established that the photosynthetic potential of a leaf is influenced by the environment in which it is grown and principally the light environment (Kull 2002). Charles – Edwards (1981) held that the light saturated leaf photosynthesis for leaves within the canopy was proportional to the attenuation of light at that position in the canopy.

These results indicate that photosynthesis may vary in different positions along a single leaf due to the variation in light interception. All the studies on maize leaf photosynthetic parameter so far have focused on measuring a single spot on a specific leaf iteratively. There are no studies to show whether or not there is any variation along a maize leaf.

However, the difference in light attenuation may not be the only source of variation in photosynthesis along the leaf. Since the entire leaf does not emerge at the same time (the basipetal growth of maize leaves), different sections along the leaf have different ages. So we would have to control for the difference in section ages to identify any variation in photosynthesis caused by variation in light attenuation.

In this chapter we focus on the following questions:

- Is there a significant difference in the A_{max} parameter of a PLRC in 3 different positions along the 7th leaf of maize?
- When controlled for the age of the sections, does A_{max} vary significantly along the 7th leaf of maize?

Method

The A_{max} values calculated from the EA equation (Chapter 2, 3 and 4) have been used here for further calculations. An ANOVA was done to check the variation of A_{max} along the 7th leaf as a function of increasing leaf age (starting from the day of emergence of the 7th leaf). Here the age of the sections is not clearly demarcated (described in Chapter 2). We control for the difference in age between the different sections for the second ANOVA (described in chapter2). The age of each section was measured in weeks starting from its emergence. The first section appeared one week prior to the second section and two weeks prior to the third section. Hence there are more weeks of measurements in the first two sections than the third section and this gives rise to an unbalanced design. The last 2 weeks of measurement for the 1st section and the last week of measurement for the second section were removed to create a balanced design.

Results and Discussion

Figure 5.2 shows the A_{max} for all three sections when the leaf age was around 40 days. The data for all eight replicates are plotted for each section. The age of the leaf was measured starting from the first day of emergence of the 7th leaf. We can see from the trend line through the A_{max} values that there is a distinct variation in A_{max} among the 3 sections.

We performed an ANOVA to check if the variation in A_{max} as a function of leaf age over the three sections is statistically significant. The ANOVA results showed that A_{max} varies significantly among the 3 sections as a function of increasing leaf age.

Figure 5.2 reflects the ANOVA result that if ages of the sections are not controlled for, there is a significant variation in A_{max} among the 3 sections.

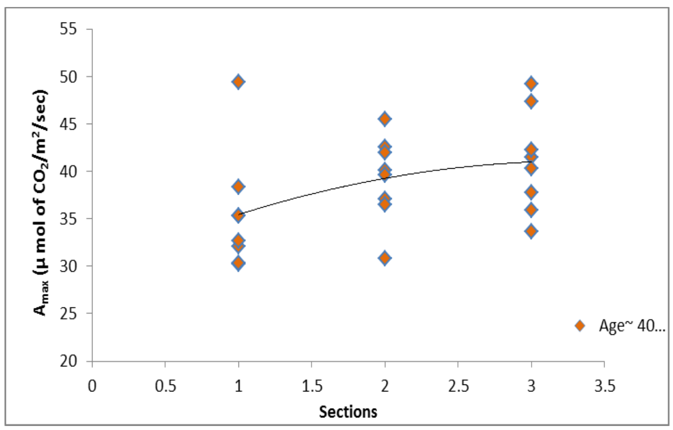

Figure 5.2 Variation in A_{max} when age of section is not controlled. A two way ANOVA among the 3 sections show that A_{max} varies significantly along the 7th leaf of maize when ages of the 3 sections are not controlled for.

Figure 5.3 below shows the A_{max} for all replicates when the age of each section was around 5 weeks. The age of each section was measured from the first day of that section's emergence (not the leaf's). A trendline through the A_{max} values reflects that there is hardly any variation in A_{max} among the three sections when we control for the age of the sections.

A second ANOVA was performed to check if the variation in A_{max} as a function of section age over the three sections is statistically significant. The second ANOVA showed that A_{max} does not vary significantly among the 3 sections when it is controlled for age of the sections. However there is a significant interaction between the age of the sections and the 3 sections.

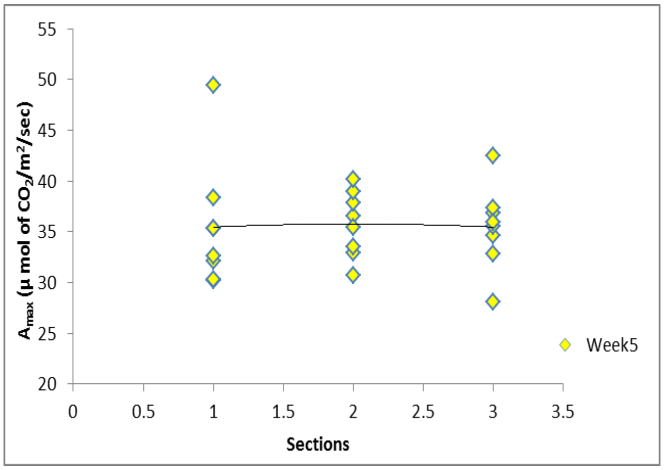

Figure 5.3 Variation of Amax value when age of sections are controlled. A second 2 way ANOVA was done to check the variation of A_{max} among 3 sections. With age controlled for A_{max} appeared to be not significant among the 3 sections on the 7th leaf.

The results of the two ANOVAs show that when there is no clear distinction in section age, A_{max} varies significantly over the curvature of a leaf. When we control for the age of the sections the A_{max} does not vary significantly among the 3 sections. However there is a significant interaction between section age and sections. Hence it is clear that the variation in A_{max} along the curvature of a leaf is due to increasing age of the sections.

Chapter 6

Variation of A_{max} with Leaf Age

Introduction

Multiple studies have shown that photosynthetic rate changes with the age of the plant. Thiagarajah et al. (1979), in their experiment on photosynthetic light response on maize leaves, observed a decrease in CO_2 assimilation rate with increasing age of the plant and the leaf. The 11th and the 13th leaves showed a sharp decrease in photosynthesis post silking, 42 days after germination. Moreno-Sotomayor (2002) observed a strong correlation between maximum photosynthesis and increasing age of the maize leaves. Dwyer and Stewart (1986) also observed a strong negative correlation between increasing leaf age and maximum photosynthesis in maize. Stirling et al (1994) found that the net photosynthesis of the 4th leaf in maize (integrated over the whole leaf) shows a triphasic response with increasing thermal leaf age (in degree days). They found an initial increase followed by a small plateau and a linear decrease in the photosynthesis of whole leaf represented over leaf age. Kitajima et al (2002) used 2 tropical tree species for photosynthesis and age experiment. They found that photosynthesis of the total leaf has a negative correlation with increasing age of the leaf. They however showed a linear decline in photosynthesis with increasing leaf age. Suriyagoda et al. (2010) found a triphasic response of whole leaf photosynthesis with increasing leaf age in three perennial legumes.

Even though there was evidence of an increase in photosynthesis with leaf age if measured on young leaves (Stirling et al 2002, Suriyagoda et al 2010) , most studies started their measurement on leaves after full leaf expansion (Dwyer and Stewart 1986 in maize, Kitajima et al 2002, Heschel et al 2004 in *Impatiens capensis*, Fletcher et al 2008 in maize). Moreover in maize most of the measurements have been taken on leaves at the top of the canopy (Thiagarajah 1979 leaves 11 and 13, Moreno-Sotomayor et al 2002 leaves 13 and 17, Dwyer and Stewart 1986 leaves 12, 13 and 14) or on juvenile leaves (Stirling et al. leaf 4). The leaves on the top of the canopy emerge later and the endospermic leaves die very early compared to the rest of the leaves. In either case the measurements were not taken for long. Also it is not clear whether PLRC in non-endospermic leaves at the lower layer of the maize canopy have a similar response to leaf age. Therefore in this study we concentrate on studying variation of photosynthesis with leaf age on a shaded non juvenile leaf in maize.

In this chapter we focus on answering

1. Does A_{max} vary significantly with increasing leaf age for shaded non juvenile leaf (7^{th} leaf)?
2. What is the pattern of variation of A_{max} with age?

Method

A_{max} for all the 3 sections were plotted against increasing age of the 7^{th} leaf. The age of the leaf was measured in days from the emergence of the 7^{th} leaf. A set of seven equations (linear, quadratic, cubic, polynomial, triangular, Weibull and power) (Haefner 1996) were fitted to the A_{max} data cloud using the non-linear least square method (described in chapters 2 and 3). The parameter estimates from the nonlinear regression was used to understand whether A_{max} varied significantly with time. The best fitting equation for all the 3 section was chosen from the set of 7 equations using AIC values.

Result and Discussion

All the regression models show that the A_{max} varies significantly with increasing age of the 7^{th} leaf.

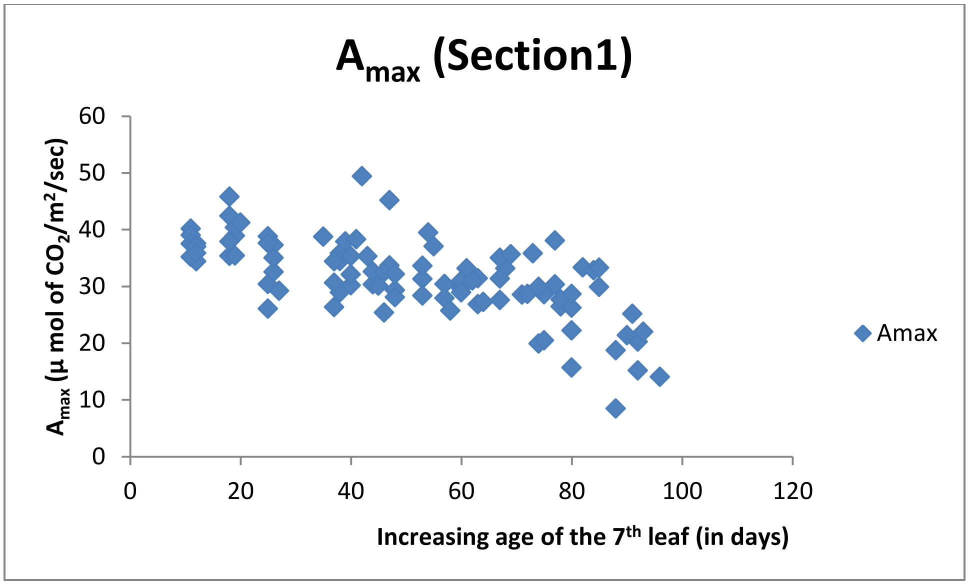

Figure 6.1 Distribution of A_{max} for the first section over increasing age of the 7th leaf. The A_{max} values estimated from the exponential asymptote model was used to plot over increasing age of the 7th leaf for each of the 3 sections for all the weeks and all 8 replicates.

The A_{max} values for the first section (Figure 6.1) starts around 30-40 µ mols m^{-2} sec^{-1}. The distribution starts from a relatively high value. This is followed by a plateau in the A_{max} and a near linear decline starting from around 75-80 days after emergence of the 7th leaf. There is a peak close to 40 days.

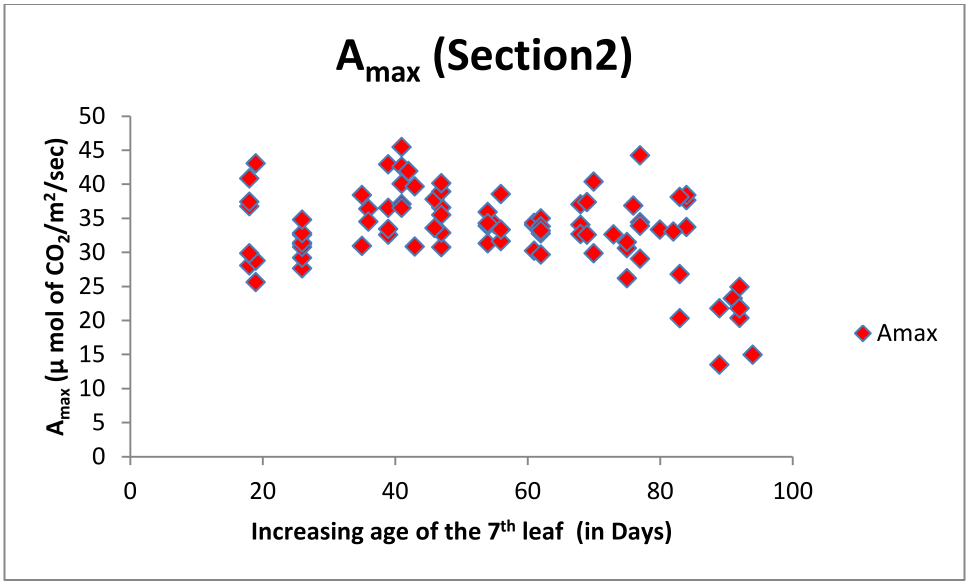

Figure 6.2 Distribution of A_{max} for the second section over increasing age of the 7th leaf. The A_{max} values estimated from the exponential asymptote model was used to plot over increasing age of the 7th leaf for each of the 3 sections for all the weeks and all 8 replicates.

The second section shows a triphasic response (Figure 6.2) of A_{max} plotted over increasing age of leaf. There is an initial rise in the A_{max} values followed by a plateau and a decline starting between 75-80 days after the emergence of the 7th leaf. There is a peak close to day 40.

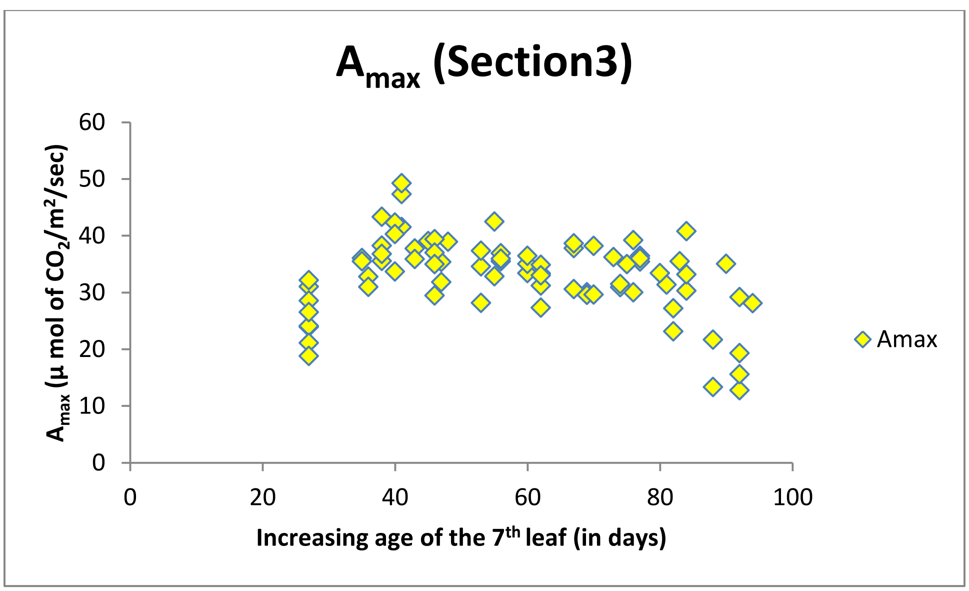

Figure 6.3 Distribution of A_{max} for the third section over increasing age of 7th leaf. The A_{max} values estimated from the exponential asymptote model was used to plot over increasing age of the 7th leaf for each of the 3 sections for all the weeks and all 8 replicates.

The plot of the A_{max} against increasing age of the 7th leaf (Figure 6.3) for the third section shows a typical triphasic response with a peak close to the 40th days after the emergence of the 7th leaf. There is a gradual increase in A_{max} followed by a plateau and a sharp decrease around the 80th day.

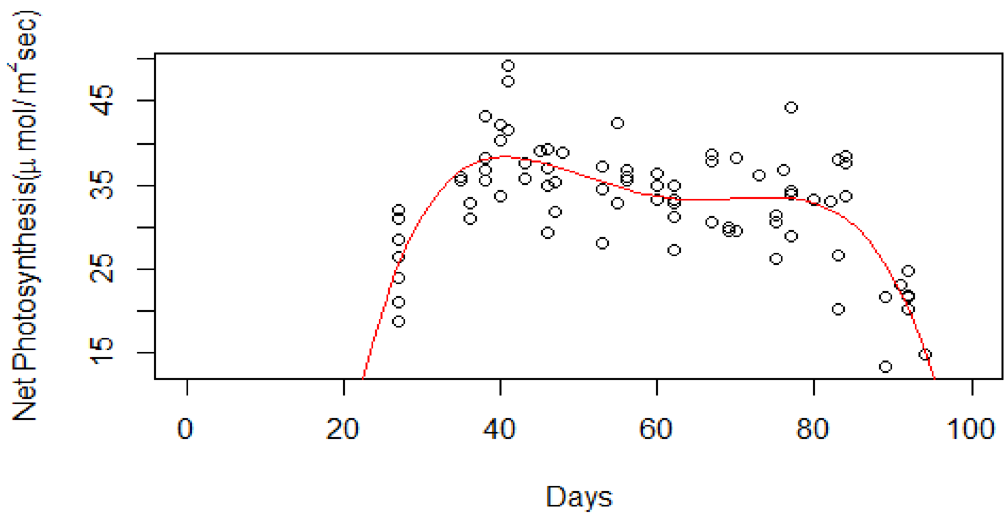

Figure 6.4 Polynomial model fitted on A_{max} data cloud of the third section. A triphasic response of the A_{max} data cloud for the third section. AIC and RSS values show the polynomial model (power 4) is the best fit for the A_{max} data cloud for third section.

third section (Figure 6.4). A residual plot (Figure 6.5) of the polynomial model shows the residuals distributed uniformly. This shows that the model is a good fit.

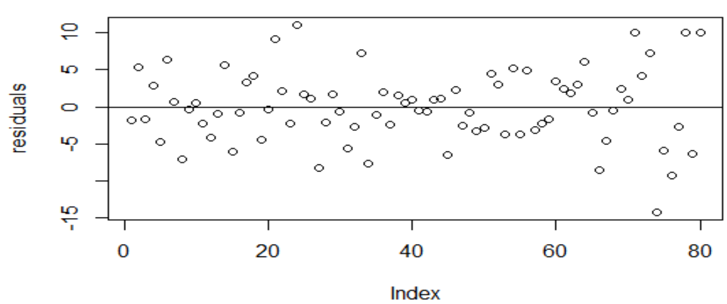

Figure 6.5 Residual plot for the polynomial model fitted in the A_{max} data cloud for the third section

According to the AIC value the cubic equation (Figure 6.6) is a good fit for the second sections A_{max} data. However the RSS value shows that the Weibull model (Figure 6.7) is the best fit for the second section.

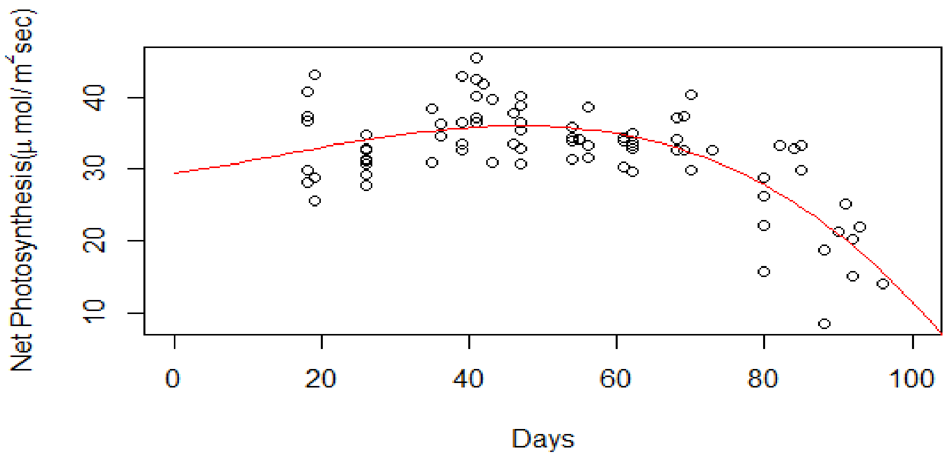

Figure 6.6 Cubic model fitted on the A_{max} data cloud of the second section. The A_{max} data cloud for the second section also shows a triphasic response, although less pronounced than the distribution of the third section. According to AIC value the cubic model was the best fit fot the A_{max} distribution of the second section.

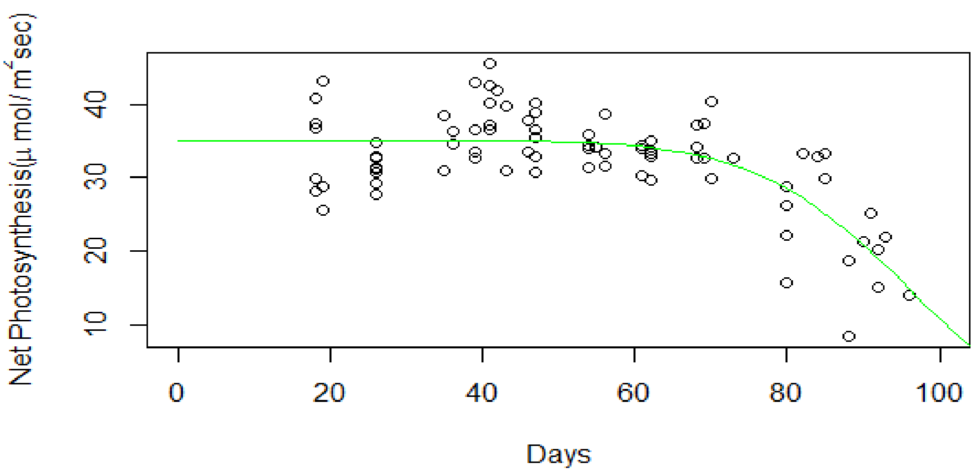

Figure 6.7 Weibull model fitted on the A_{max} data cloud of the second section.

The A_{max} data cloud for the first section does not show any initial rise in the A_{max} values. The distribution starts from a high value and gradually the A_{max} values decline. The triangular equation (Figure 6.8) is the best fit for the first section and clearly models the decline of A_{max} around 80th day.

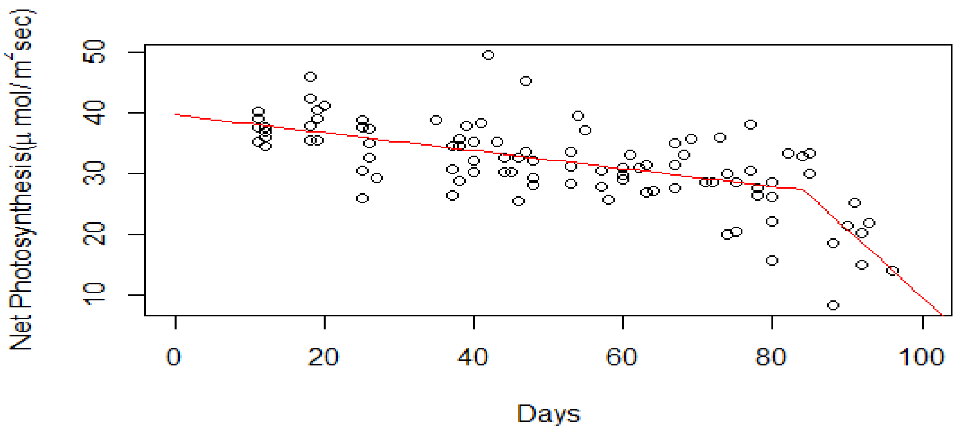

Figure 6.8 Triangular model fitted to the A_{max} data cloud of the first section. The A_{max} distribution for the first section shows a diphasic distribution unlike the third and the second sections triphasic distribution. The Triangular model is the best fit for the A_{max} distribution of the first section.

The age of the first section at the time of its first measurement was higher than the age of the other sections at the time of their first measurements. This is probably accounts for the absence of an initial increase in photosynthesis for section 1.

Conclusion

Comparison of two models

The Exponential Asymptote model proved to be a better fit than Non Rectangular Hyperbola model for the Photosynthetic Light Response Curves of the 7^{th} leaf in maize. Both Akaike's Information Criterion and Residual Sum of Squares show that the EA model was a better fit for most PLRC parameters in the data set. Statistical analysis of the AIC and RSS values from both models showed that within a 95% confidence level EA fitted better than NRH model ($p \leq 0.00181$). Additionally the ANOVA showed that there were no statistically significant interactions between the model used and the age of the leaf or the section of the leaf. If either one model had been better for younger stages of the leaf and the other for older it would have shown up as statistically significant in the interaction between the model used and the age of the leaf. Therefore EA is better for PLRC than NRH for all sections and ages of the 7^{th} leaf in maize. The statistical comparison of EA and NRH on multiple PLRC datasets has not been demonstrated before.

The NRH model has 4 parameters namely $A_{max,}$ ϕ, θ and R unlike 3 parameters namely A_{max}, A_{qe} and LCP in the EA model. The additional parameter in NRH makes it more susceptible to overfitting (Thornley 2002, Johnson et al 2010 and Marino et al 2010). Marino et al (2010) and Lachapelle et al (2012) also reported that NRH showed strong covariance between parameter estimates. Moreover all the parameters in the EA model correspond to a biological function unlike the parameter theta in the NRH model. These along with the results of the statistical analysis show that the exponential asymptote model appears to be the more suitable model for photosynthetic light response curves of the shaded non juvenile leaves in maize.

Variation of Photosynthesis of 3 different positions on a leaf

There have been no results showing whether or not photosynthesis varies along a maize leaf. The complex curvature of a maize leaf leads to varying amounts of incident light along the leaf. This along with results showing that the light intercepted by the leaves is principally responsible for any variation in photosynthesis (Johnson and Thornley 1984) leads to the expectation that photosynthesis may vary significantly in different sections of the same leaf. This expectation is further bolstered by the results from previous light penetration experiments (Terashima and Hikosaka 1995, Thornley and Johnson 2000, Kull et al. 2002). The basipetal growth pattern of maize leaf (Freeling 1992) means that all the sections of the leaf are not the same age. Since age of a leaf has also been shown to be a contributing factor in variation of photosynthesis (Thiagarajah et al 1979, Dwyer and Stewart 1986, Stirling et al 1994, Suriyagoda et al 2010) the differing ages of the sections can act as a confounding factor.

A preliminary ANOVA did indeed show that A_{max} varies significantly among the 3 sections as a function of increasing leaf age. However after controlling for the different ages of the 3 sections on a leaf, an ANOVA showed that A_{max} does not vary significantly among the three sections. In the ANOVA the variation of A_{max} within sections is more than the variation between sections. Hence it is clear that any difference in photosynthesis among the sections is largely due to the difference in age between the three sections. Any variation in photosynthesis due to difference in light intercepted by the sections is not statistically significant after accounting for their difference in age. This agrees with Stirling et al(1994) who stated that the assumption that variation of light intercepted on the leaf causes all the variation in photosynthesis is an oversimplification. We found that the different ages of the sections of a leaf is the most significant cause of variation in photosynthesis along the length of a maize leaf.

Age

Our experiment on leaf age showed that the photosynthetic parameter A_{max} changes significantly with increasing age of the leaf. In our study we not only found a strong correlation of A_{max} with leaf age but also a definite pattern of variation of A_{max}. Seven equations were fitted to A_{max} data cloud for all the three sections. A model validation was done by three methods to show the good fit of a models. AIC and RSS values showed that polynomial model was a good fit for the third section while Weibull was the best fitting model for the second section and triangular for the first. The residual plots for all the three sections showed the models to be a good fit. A_{max} increases gradually with age followed by a plateau and a sharp linear decline. This has been called a triphasic response to age by Stirling et al (1994) and Suriyagoda et al. (2010). The triphasic response is clearly seen in the second and the third section. The first section does not show the triphasic response clearly because the age of the first section was higher than the other two sections when measurements were started. As a result the first part of the triphasic response (when A_{max} increases with age) was not measured for the first section.

The triphasic response seen in the seventh leaf (shaded non-juvenile leaf) of maize is similar to the triphasic response reported by Stirling et al (1994) in the juvenile leaves of maize and Suriyagoda et al. (2010) in two other C3 plants. The decline in Amax in the last phase of the triphasic response also agrees with Thiagarajah et al (1979), Dwyer and Stewart (1986), Moreno-Sotomayor et al (2002) who noticed a negative correlation of photosynthesis and leaf or plant age. Therefore we can conclude that A_{max} shows a triphasic response to age in a shaded non-juvenile leaf of maize.

Appendix 1

Nitrogen

Nitrogen is important for crop productivity. Up to 75% of the reduced nitrogen in cereals is located in the mesophyll cells, mainly as Rubisco and is involved in the photosynthetic process (Evans 1989). It has been suggested that there is a vertical gradient of nitrogen in plants similar to a gradient of PPFD penetration into a canopy (Hirose and Werger 1991). The prior studies on variation in nitrogen content in plants focused principally on the vertical partitioning. Drouet and Bonhomme (1999) found a horizontal gradient of nitrogen in corn leaves 6-10 which keeps changing with the amount of light intercepted by the leaves. However it is not clear from their study how nitrogen content would change along the curvature of a shaded non juvenile maize leaf with increasing age of the leaf.

Material and method

Leaf squares measuring 6 cm^2 were cut out of the three sections for nitrogen content measurement. 8 replicates were used and samples were collected on week 1,2,3,8 and 10. The leaf squares were dried at 75°C for 72 hours. Leaf nitrogen content was measured using a NC analyzer.

Statistics

The nitrogen content for each section was plotted over increasing leaf age and was fitted with a polynomial model. An ANOVA was done to check the variation of nitrogen content with age and section of the 7th leaf.

Result and Discussion

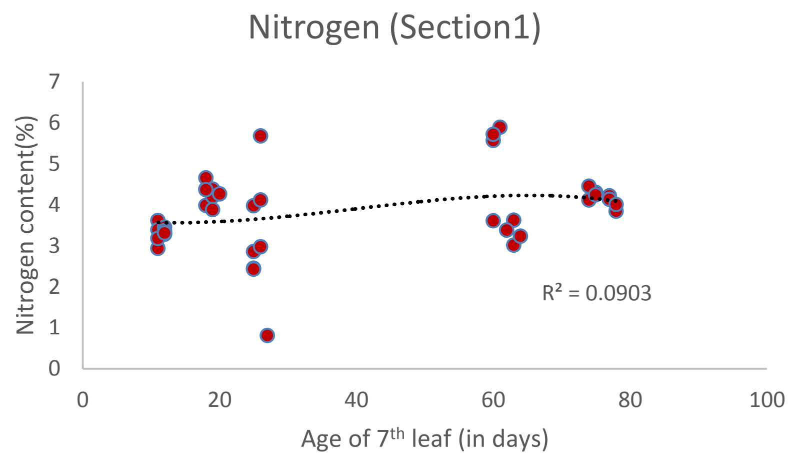

Figure A1.1. Nitrogen content of section 1

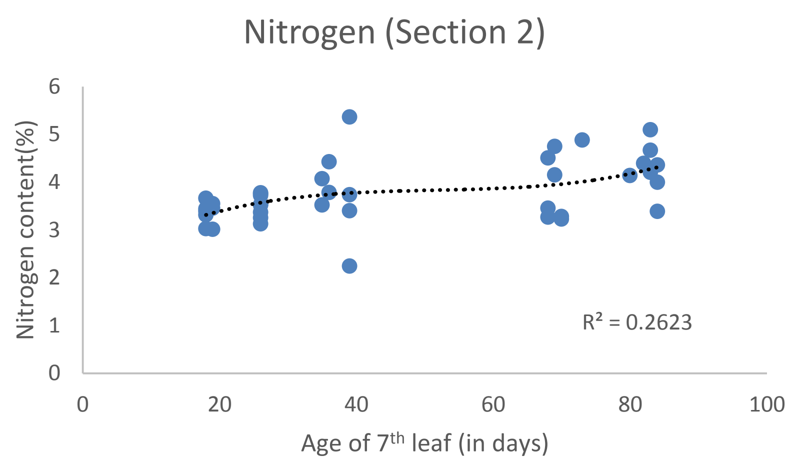

Figure A1.2. Nitrogen content of section2

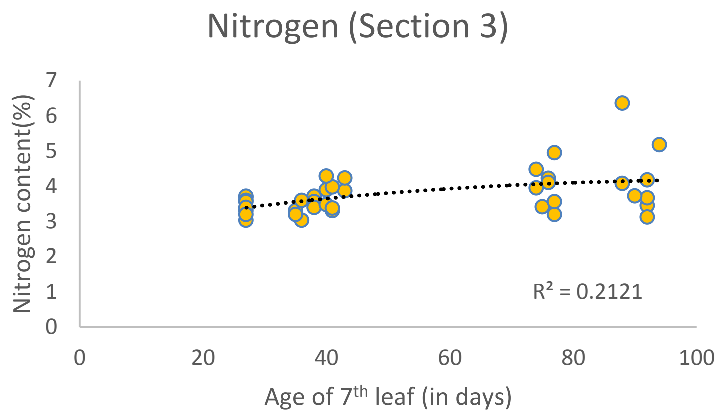

Figure A1.3. Nitrogen content of Section 3

A two way ANOVA showed that nitrogen content varies significantly with increasing leaf age ($p < 2.23 \times 10^{-5}$). This result is in agreement with the findings of Hikosaka et al (1994) and Milroy (2000) where they found a strong correlation of leaf age with nitrogen content of leaves.

Drouet and Bonhomme (1999) found a gradient of nitrogen along the maize leaf. In our experiment a two way ANOVA showed that there was a significant variation of nitrogen among the 3 sections ($p < 1.46 \times 10^{-06}$). However there was no significant correlation between the nitrogen content and A_{max} of the 3 sections ($p < 0.576$).

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