New Discussion on Boysen-Jensen’s Photosynthetic Response Curves Under Plant Canopy and Proposal of Practical Equations for Monitoring and Management of Canopy Photosynthesis

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In this concept paper, a review on the history of mathematical handling of photosynthesis using kinetic equations and the repetition of classical experiments for renewing the discussion on the plant canopy photosynthesis are combined. In the upper half of this article, we reviewed the century-old history of the use of equations in photosynthetic analyses inspired by the mathematical models by A. V. Hill. Then we tried to challenge the 80-year-old mystery of Boysen-Jensen’s plant canopy photosynthesis proposed by Boysen-Jensen in 1932, which induced a series of discussion if the nature of photosynthetic irradiation (PI) in the plant canopy is largely differed from the one in a single leaf. Despite long-lasted belief, we concluded that there would be no mystery in the canopy photosynthesis. We assumed that the apparent lack of saturation in PI-curves in a stand of plant could be attributed to, neither the temporal movements of leaves nor the alteration of the sun’s position, despite the earlier suggestion by Monsi and Saeki who assumed utilization of scattered sun light by plants, but it could be simply an artifact due to the lack of consideration on the positional effect of broad beam-angled artificial light source. Lastly, we revised the photosynthetic modes under layers of leaf canopies by proposing a set of practitioner-friendly mathematical model which could be applicable for estimating the total photosynthesis in the plant canopy structure consisted with layer of inclined leaves. Newly proposed equations can be used for simulation of the photosynthetic capacity in the plant canopy structure simply through measurements of (1) the PI-curve, (2) the state of respiration, and (3) transmittance in a single top-positioned leaf consisting the canopy structure.

Keywords : Boysen-Jensen, canopy photosynthesis, Hill equation, photosynthesis, plant community, science history, simulation

INTRODUCTION

The name of Peter Boysen-Jensen brightly shines in the history of plant biology, especially as one of pioneering researchers studying the actions of plant hormones during phototropic responses as reviewed elsewhere (Pennazio, 2002; Enders and Strader, 2015), although the significance of some key experiments are questioned today (Yamada et al., 2000).

Apart from plant growth regulation by light and/or plant hormones, Boysen-Jensen has two well-acknowledged contributions to photosynthetic studies, namely, a series of study on the photosynthetic assimilation and the study on the plant canopy structure. As reviewed elsewhere (Hirose, 2005), Boysen-Jensen have pointed out that the increase in dry mass reflects the photosynthetic assimilation of carbon dioxide (Boysen-Jensen, 1918; Boysen-Jensen and Müller, 1929a; 1929b), and he also deeply studied the photosynthesis under plant canopy structure in which the leaves of self and non-self origins are layered and compete for light (Boysen-Jensen, 1929; 1932).

The most important factors to be discussed when relating the structural feature of plant canopy and functioning photosynthesis might be the utility of light at different positions within the canopy structure, as photosynthetic rate must be a function of light availability on site. The concept on the competition for light within plant community proposed by Boysen-Jensen is now the basis for understanding the eco-physiological behaviors of plants such as temperature-responsive onset of vegetation growth under competitive inter-species canopy (Dunnett and Grime, 1999).

EQUATIONS FOR PHOTOSYNTHESIS

For assessing the photosynthetic capacities in living lower and higher plants widely covering algae, mosses, ferns, and vascular plants, a graphical representation of photosynthetic performance empirically studied in relation to the intensity of solar irradiance have been developed. Such graphs are now referred to as photosynthesis-irradiance (PI) curves or light response curves, in which two distinct points (of light intensity), namely, the points of compensation and saturation, can be graphically elucidated.

In most green organisms including algae, there is a roughly linear relationship between the velocity of photosynthesis (P) and a given light intensity (J) under relatively low range of J. However, at higher J, most plants and
algae no longer maintains the photosynthetic rate proportional to the increase in \( J \). Therefore, increase in the rate of photosynthesis gradually declines as \( J \) approaches the maximal level at which the plants or algae can perceive without being damaged. Such PI curves must be mathematically reproduced using hyperbolic models.

To date, various mathematical approaches have been made for PI-curve simulation as illustrated in Fig. 1. In 1951, Tamiya at University of Tokyo has proposed an algal photosynthetic equation as follows (Tamiya, 1951):

\[
A = \frac{bI}{1 + aI} - R
\]

which is designed for quantification of photosynthetic assimilation (\( A \)) as function of irradiation (\( I \)), where \( R \) stands for respiratory loss, and \( a \) and \( b \) are constants to be determined. Note that \( b \) represent the slope of photosynthesis under relatively low range light intensity and the ratio of \( b \) over a \((b/a)\) corresponds to the saturated level of photosynthesis.

By 1976, two British ecologists, Platt and Jasby have evaluated several candidate photosynthetic equations and selected only single equation by adapting a well-known Michaelis-Menten equation (MME; Michaelis and Menten, 1913) which was originally proposed for enzyme kinetics:

\[
V = \frac{V_{\text{max}} [S]}{K_a + [S]}
\]

By assuming that apparent photosynthesis is a consequence of gross photosynthetic activity \((P, \text{ either } O_2 \text{ evolution or } CO_2 \text{ uptake by plants or algae}) \) and respiration \((R)\), the net photosynthesis \((nP)\) can be expressed as follows:

\[
nP = P - R
\]

Therefore, Platt and Jasby (Platt and Jasby, 1976; Jasby and Platt, 1976) concluded that the modified MME-type equation (from now on which is refer to as Platt-Jasby equation, PJE) nicely reproduces the relationship between the light intensity and photosynthesis of marine algae:

\[
nP = \frac{P_{\text{max}} J}{K_j + J} - R
\]

where the term for gross photosynthesis has identical structure with MME:

\[
P = \frac{P_{\text{max}} J}{K_j + J}
\]

In PJE, \( P \) is the rate of gross photosynthesis, \( P_{\text{max}} \) is the maximal rate of gross photosynthesis, \( J \) is a given light intensity, \( K_j \) is a constant which is the light intensity at which the gross photosynthetic performance remains at half level of \( P_{\text{max}} \), and \( R \) is the rate of respiration. In fact, PJE and Tamiya’s equation mentioned above are equivalent after substitution of Tamiya’s two constants \( a \) and \( b \) with \( K_j \) and \( P_{\text{max}}/K_j \), respectively; thus, confirming that most key photosynthetic equations can be considered as derivatives of MME. The approach by Monsi and Saeki (1953) known as Monsi-Saeki equation (MSE) was made after Tamiya’s model as discussed later, therefore, it can be viewed that MSE is also one of MME derivatives. In the later sections, we trace the contribution of MSE to plant ecological study.

One of biochemically applied photosynthetic equations based on MME model is Farquar’s equation which was designed for describing two opposing biochemical reactions catalyzed by a plant enzyme called Rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase) saturated with its substrate ribulose biphosphate (Farquhar et al., 1980). First of all, assimilation can be expressed as follows:

\[
A = V_c - 0.5V_o - R_o
\]

where \( A \) is the rate of assimilation, \( V_c \) is the leaf area-based velocity of carboxylation \((\mu mol \ m^{-2} \ s^{-1})\), \( V_o \) is the leaf area-based velocity of oxygenation \((\mu mol \ m^{-2} \ s^{-1})\), \( R_o \) is the leaf area-based rate of respiration in the dark \((\mu mol \ m^{-2} \ s^{-1})\), and the coefficient 0.5 indicates the ratio of CO2 release over a single cycle of oxygenation.

Then, overall rate of carboxylation under competition with oxygenation can be written as below:

\[
W_c = \frac{V_{\text{max}}(C - F^*)}{C + K_c\left(1 + \frac{O}{K_o}\right)} - R_o
\]

where \( W_c \) is substrate-saturated rate of carboxylation \((\mu mol \ m^{-2} \ s^{-1})\), \( V_{\text{max}} \) is maximal carboxylation velocity \((\mu mol \ m^{-2} \ s^{-1})\), \( C \) is intercellular partial pressure of CO2 \(\mu bar\), \( F^* \) is CO2 compensation point without dark respiration.
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(μbar), \(K\) is Michaelis constant for \(\text{CO}_2\) (460 μbar), and \(O\) is partial pressure of \(\text{O}_2\) (μbar).

As seen above, even today, MME models (including derived PJE) remain the standards for generation of PI-curves (Marra et al., 1985; Nagasawa et al., 2015). However, we have to view that MME is also a derivative of another well-known biological equation proposed by A. V. Hill (1910) known as Hill’s equation (HE):

\[
y = \frac{y_{\text{max}} x^h}{c^h + x^h}
\]

Interestingly, HE was proposed three years prior to the proposal of MME (Fig. 1).

GRAPHICAL DETERMINATION OF \(P_{\text{max}}\) AND \(K_j\) VALUES FOR PJE

Recently, Nagasawa et al. (2015) have proposed that PI-curves can be generated based on limited number of experimental data points even lacking the data corresponding to saturatingly higher range of light intensities through application of PJE by determining \(P_{\text{max}}\) values and \(K_j\) values from least-sized experiments.

In fact, the gross photosynthetic term in PSE can be rewritten as follows:

\[
P^{-1} = \frac{K_j + J}{P_{\text{max}} J}
\]

\[
P^{-1} = \frac{K_j J^{-1} + 1}{P_{\text{max}}}
\]

By substituting \(P^{-1}\) with \(y\), and \(J^{-1}\) with \(x\):

\[
y = \frac{K_j x + 1}{P_{\text{max}}}
\]

\[
P_{\text{max}} = \frac{K_j x + 1}{y}
\]

\[
K_j = \frac{P_{\text{max}} y - 1}{x}
\]

It is conclusive that \(P_{\text{max}} = y^{-1}\) (where \(x = 0\)) and \(K_j = -x^{-1}\) (where \(y = 0\)). In fact, these conclusions can be graphically withdrawn by double-reciprocal plot known as Lineweaver-Burk plot. Therefore, in order to determine \(P_{\text{max}}\) values and \(K_j\) values, we have shown that Lineweaver-Burk plot can be used, by analogy to enzymatic analyses (Nagasawa et al., 2015). By this way, simulative PI curves can be reproduced based on graphically determined \(P_{\text{max}}\) and \(K_j\). Practically, the least-square method can be applied to the set of double-reciprocal data for PI-curves, namely, \(J^{-1}\) and \(P^{-1}\), to obtain a linear regression:

\[
y = ax + b
\]

The regression resultant from the least-square method tells us that \(P_{\text{max}} = b^{-1}\) and \(K_j = a/b\).

The \(P_{\text{max}}\) and \(K_j\) values can be graphically determined for various light sources of interest, both natural sunlight and vast range of artificial lights including white fluorescence-type light emitting diodes (Nagasawa et al., 2015).

The idea for applying Lineweaver-Burk plot for handling of the non-enzymatic biological phenomena in vivo and/or in planta can be found in earlier works on the interactions between natural auxin, indole-3-acetic acid and synthetic (Kawano et al., 2003) or fungal analogs (Jambois et al., 2004).

From the discussion up to here, we should view that Lineweaver-Burk plot-assisted PJE and related HE-type equations (rather than Tamiya’s equation and MSE) are most useful and practical equations for handling the data from photosynthetic measurements.

GRAPHICAL DETERMINATION OF \(P_{\text{max}}, K_j\) AND HILL’S COEFFICIENT \(\alpha\)

We would like to propose practical procedures for determination of \(P_{\text{max}}, K_j\) and Hill’s coefficient \(\alpha\) for HE-based photosynthetic analysis. \(P_{\text{max}}\) and \(K_j\) can be determined via identical manner for Lineweaver-Burk plot-assisted PJE, since \(P_{\text{max}}\) and \(K_j\) are supposed to be unchanged by definition, even diversified \(\alpha\) were introduced.

For fitting the more or less steeply saturated curves, HE-like equations should be applied and Hill coefficient \(\alpha\) higher or lower than 1 should be applied in order to substitute PJE model with HE model. However, it would become highly difficult to determine the \(K_j\) value based on Lineweaver-Burk plot, in case a number of data points were highly limited. Instead, \(K_j\) can be also determined graphically by considering the size of residual sum of squares (RSS). RSS is the sum of the squares of the difference between the recorded and the simulated values. By definition:

\[
\text{RSS} = \sum_{i=1}^{n} (y_i - f(x_i))^2
\]

where \(y_i\) is the \(i^{th}\) recorded value and \(f(x_i)\) is the \(i^{th}\) simulated value. By minimizing RSS, it is possible to obtain the values closest to the measured values.

Firstly, \(K_j\) values should be randomly chosen and used for preliminary simulation of the PI-curves with Hill-type equation while setting the Hill’s coefficient \(\alpha\) to be 1. Then, RSS can be obtained as the sum of squared difference between the measured values and simulated values. By plotting the RSS against the selected range of \(K_j\), the optimal value for \(K_j\) minimizing RSS can be graphically determined (as the valley bottom of the curve).

In order to complete the Hill-type equation for photosynthesis by introducing the graphically determined \(K_j\), optimized \(\alpha\) other than 1 should be introduced through the procedure starting from randomly selected \(\alpha\). Using RSS determined from the difference between the experimentally examined values and newly simulated values, the optimal \(\alpha\) giving the minimal RSS should be graphically determined through plotting RSS against the selected range of \(\alpha\). When necessary, the cycle for determining the temporal \(K_j\) and \(\alpha\) can be repeated for further optimizing the curve fit.
BOYSEN-JENSEN’S CANOPY PI CURVES

PI-curves in most leafy plants are likely saturated under strong light when measured using a horizontally positioned single leaf. However, the work by Boysen-Jensen published in 1932 suggested that the modes of photosynthesis may differ between a single leaf and canopy (Fig. 2A). Boysen-Jensen has reported his observation that the PI-response obtained with a horizontally placed single leaf of Sinapis alba L. showed saturation, and unexpectedly a stand of the same plant (a minimal canopy model) showed only linear increase in PI-response without attaining the saturation.

Monsi and Saeki (1953; 2005), two Japanese ecologists joined the discussion, by assuming that (1) scattered sun light mostly contributes to the canopy photosynthesis, (2) position of the light source moves with time (under sun light), and (3) wind might cause subtle and temporal movement of the leaves. Even today, this view is supported by a number of plant ecologists and field researchers (Anderson, 1966; Diaz-Ambrona et al., 1998; Hirose, 2005; Raabe et al., 2015a; 2015b; Truong et al., 2015).

Fig. 2 Two distinct modes of PI responses in single leaf and an intact plant stand reported by Boysen-Jensen. (A) Typical PI-curve and unusual linear PI response in horizontally placed single leaf and a stand of Sinapis alba L. Image was scanned from original book authored by Boysen-Jensen (1932, available at Okayama University Library, Kurashiki, Japan), and layered with new text and illustrations. (B) The method for attenuating the light intensity described by Boysen-Jensen. Position of light bulb above the plant materials was adjusted.

Note that the report by Monsi and Saeki originally published in German text in 1953 was translated into English and published in 2005 in the special issue of Annals of Botany, reflecting the remaining strong influence of this classical work among the plant ecologists of today. Monsi and Saeki concluded that the canopy effect reported by Boysen-Jensen is likely to happen, since, equation below seem to match with Boysen-Jensen’s data:

\[ P = \frac{b}{Ka} \ln \left( \frac{1+aK_0}{1+aK_0e^{-F}} \right) - rF \]

where \( P \) is productivity of the whole leaf community over a unit group area, and \( a \) and \( b \) are Tamiya’s constants that characterize the PI-curve, \( r \) is dark respiration rate, \( F \) is leaf area index cumulated from top of the canopy, \( K \) is extinction coefficient, \( I \) is the photosynthetic photon flux density (PPFD), \( I_0 \) is the PPFD above the canopy.

As two constants \( a \) and \( b \) are identical as proposed by Tamiya (1951), these constants must be empirically determined or be obtained as regression coefficients. Therefore, it would be more practical, if some simple protocols or procedures to determine these constants from minimal experimental data were proposed.

Some works denied the utility of MSE and Tamiya’s equation, especially in shade plants which show steep increase and saturation in photosynthetic rate at relatively low range of light intensity, thus, hardly expressed with single linear function (Johnson and Thornley, 1984; Terashima and Saeki, 1985). In fact, it could be viewed that the PI-curve for shade plants consists of two lines, namely, an initial steep slope and a flat saturated line.

As mentioned above, Tamiya’s equation and MSE are based on typical hyperbolic models which are equivalent with MME. MME is a specific case of HE-type equations having Hill’s coefficient \( \alpha = 1 \). Here we propose that application of HE-type equations with higher or lower Hill’s coefficient \( \alpha \) other than 1, could be one solution to cover the PI-responses in both shade and sunny plants under the same mathematical structure (Fig. 1). Sunny plants show tendency to possess high \( K_i \) values and low Hill coefficient (\( \alpha < 1 \)), while shade plants show tendency to possess low \( K_i \) values and high Hill coefficient (\( \alpha > 1 \)).

WHY CANOPY EFFECT WAS OBSERVED EVEN IN THE LABORATORY CONDITION?

By reading the original text in “Die Stoffproduktion der Pflanzen” authored by Boysen-Jensen (1932), we noticed that what exactly studied was plant response to artificial light (light bulb). Page 12 of the original German text says ”In Kopenhagen wird eine Lampe von 1500 Watt für Assimilations-untersuchungen benutzt. In dem Falle stafiielt man die Licht-stärke durch die verschieden Entfernung der Lampe von dem Blatt (In Copenhagen, a 1500 Watt lamp was used for assimilation tests. In this case, light-intensity can be controlled only by step-wisely altering the distance between the lamp and the leaves”). Note that there was no description on the use of reflectors to be used in aid of beam angle control.

In fact, Boysen-Jensen’s canopy effect was observed
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through indoor experiments using light bulbs. Therefore, theory focusing on the outdoor conditions (light scattered in the air, wind-dependent motion of leaves, and temporal move of light source position as in the case of sun) proposed by Monsi and Saeki should be hardly applied. The reason why a stand of a weed shows non-saturating linear PI response despite the saturating behavior of a horizontally placed single leaf, must be re-examined.

In order to experimentally compare the PI responses in a single leaf and a stand of a model plant, we repeated the classical experiments using the seedlings of *Gomphrena globosa* as a model material (Fig. 3). Here, we have chosen the experimental set up with a light bulb and the rate of CO\(_2\) uptake by a horizontally placed single leaf or stand with multiple leaves of *G. globosa* sealed in a clear acryl box, were monitored with CO\(_2\)/H\(_2\)O analyzer (LI-840A, LI-COR, Lincoln, NE, USA). If irradiated by a light bulb, due to its broad beam light (360°, even with flat reflector, 180°), the intensity of light at different leaf position significantly differed, even the leaves in different positions never overlapped as illustrated in Fig. 3B.

PI-curves in a single leaf and standing plants were compared under a light bulb (Fig. 3C). Single leaf under light bulb showed saturating PI-curve as expected, while standing plants showed almost linear increase as previously reported by Boysen-Jensen. At first, we thought that the canopy effect showing linear increase in photosynthesis without saturation in a plant stand was observed. Then, we wondered if it is a universally observed phenomenon or not. However, if we consider the effect of leaf position

![Fig. 3 Repetition of the classical Boysen-Jensen’s experiments comparing the PI responses in a single leaf and a stand of a model plant.](image-url)

(A) Experimental set up under a light bulb. Rate of CO\(_2\) uptake by a horizontally placed single leaf or stand with multiple leaves of *Gomphrena globosa* sealed in a clear acryl box, were monitored with CO\(_2\)/H\(_2\)O analyzer. (B) Top view and side view of standing plants showing the model positions of leaves which may be one of key factor determining the photosynthetic status if placed under a wide beam angled light source. (C) Comparison of PI-curves in a single leaf and standing plants under a light bulb. Light intensity above the standing plant was measured at the level of top position leaves. (D) Configuration of model experiments altering the distance between the light bulb and horizontally arranged single leaves. Relationship between light intensity and distance from the light bulb: 6 cm, 170.8 W m\(^{-2}\); 10 cm, 85.4 W m\(^{-2}\) (50.0% decrease); 18 cm, 43.4 W m\(^{-2}\) (74.6% decrease). (E) Effect of distance from the light bulb on the photosynthetic activity on single leaves.
(distance from the light source) within the stand of plant, it is difficult to score the impact of light as it is in the canopy since light intensity under a wide beam-angled light source like a light bulb is drastically lowered even within the height of standing plant. For an instance, the light intensity was lowered by 50% within the distance between the top leaves (6 cm from the light bulb) and leaves below (10 cm from the light bulb) in our model experiments (Fig. 3D), even though there was no overlapping leaves.

On the other hand, in place of conventional light bulb, we examined the effect of the Xenon lamp-based solar simulator (Cell Tester YSS-50, Yamashita Denso, Tokyo, Japan) which was originally designed to evaluate the performance of photovoltaic solar cell modules, by mimicking the solar spectrum, light intensity, and irradiation angle (narrow beam angle). In fact, identical set of experiments were carried out under Xenon-based solar simulator, and only minor positional effect was observed. The light intensity was lowered only by 12% within the distance between the top (6 cm from the lighting surface) and second top leaves (10 cm from the lighting surface) (Fig. 4B).

More importantly, both the single leaves and standing plants showed saturation in photosynthesis (Fig. 4C), suggesting that kinetics with light saturating nature is shared by both the single individual leaves and leaves under canopy structure.

By comparing the normalized PI-curves in a single leaf and standing plants under solar simulator (Fig. 4D), we concluded that a standing plant (with multiple leaves showing partial overlapping) utilizes the light more effectively and more sensitively since Michaelis constant for light ($K_m$) observed in a standing plants ($165 \text{ W m}^{-2}$) was much lower than that observed in a single leaf ($805 \text{ W m}^{-2}$).

Figure 4E shows the configuration of further experiments comparing overlaid and non-overlaid horizontally arranged leaves (three explants showing single leaves). When three leaves were arranged by avoiding the overlapping configuration, the rate of photosynthesis was shown to

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**Fig. 4** Re-examination of Boysen-Jensen's work on canopy photosynthesis using a solar simulator mimicking the solar spectrum, light intensity, and irradiation angle. PI curve was recorded. (A) Experimental set up under Xenon-based solar simulator. Rate of CO$_2$ uptake by a horizontally placed single leaf or stand with multiple leaves of *Gomphrena globose* sealed in a clear acryl box, were monitored with CO$_2$/H$_2$O analyzer. (B) Top view and side view of standing plants showing the model positions as in Fig. 3, but no positional effect on the photosynthetic status in non-overlapped leaves are expected if the plants were placed under a narrow beam angled light source like a solar simulator. Relationship between light intensity and distance from the light bulb: 6 cm, $549.4 \text{ W m}^{-2}$; 10 cm, $483.2 \text{ W m}^{-2}$ (12.0% decrease); 18 cm, $392.4 \text{ W m}^{-2}$ (28.6% decrease). (C) Comparison of PI-curves in a single leaf and standing plants under a narrow beam angled solar simulator. (D) Comparison of normalized PI-curves in a single leaf and standing plants under solar simulator. (E) Configuration of model experiments comparing overlaid and non-overlaid horizontally arranged leaves. (F) Comparison of photosynthesis by three single-leafed explants with and without overlapping configuration. Red line denotes the level of photosynthesis by a single leaf.
be 3-fold compared to the rate recorded with a single leaf which is proportional to the area occupied by leaves.

In contrast, by overlapping three leaves from different explants differed in height, thus the area occupied by three leaves were equivalent to a single leaf, the rate of photosynthesis per irradiated area was shown to be higher than single leaf suggesting that light was more efficiently utilized for photosynthesis.

As seen above, we need to be aware that there are some technical problems in the PI-curve measurement in tall plants especially under artificial sources of direct light, positioned close to plants, but not under the narrow-angled light sources or scattered sun light in the air. Despite long-lasted belief since the report by Boysen-Jensen, we concluded that there would be no canopy magic in photosynthesis. We assumed that the apparent lack of saturation in PI-curves in a stand of plant in Boysen-Jensen’s indoor experiments could be simply attributed to an artifact due to the lack of consideration on the effect of leaf position in relation to the distance from artificial light source, but it could not be due to the alteration of the sun’s position with time, scattering of sunlight in the air, nor the movements of leaves by wind, despite the suggestion by Monsi and Saeki (1953; 2005).

It is natural to assume that Boysen-Jensen might have assessed the PI-curve based on the light intensity at the top of plants (or at least one level representing the standing plants) without considering the effect of beam angle which drastically alters the photosynthetic response. Why such simple mistake could not be pointed out for over 80 years? One of the likely reason is that MSE proposed in support of Boysen-Jensen’s canopy effect was not practical to most field researchers and thus theoretical and experimental data have not been thoroughly compared until now.

It seems that Boysen-Jensen did not fully appreciate the mathematical work (MSE) by Monsi and Saeki. We can find the opinion by Boysen-Jensen in the letter from Boysen-Jensen addressed to Prof. Monsi which was recently translated from original German text to English (Schortemeyer, 2005), stating that “I happily admit that the agreement of your calculated numbers with those found by me is very nice. However, I would like to recommend being cautious with the mathematical treatment.” He continued, “but one must not forget that the numbers most often used in phytocology are only approximations. The goal of this science is to understand the processes in plant communities.” Finally he concluded that “this understanding can be achieved without mathematics.”

After tracing the history of the studies on the first model of photosynthesis under plant canopy structure initiated by Boysen-Jensen in Copenhagen, which was mathematically supported by Prof. Monsi and his team members at University of Tokyo, we found the necessity to invent a series of field practitioner-friendly equations to be used for handling of experimental data and also for simulation of photosynthesis under canopy.

(A) Gross photosynthesis

| Layered leaves (canopy) |
|-------------------------|
| $P = \frac{P_{\text{max}} \cdot f}{K_f + f}$ |
| $P = \frac{P_{\text{max}} \cdot f \cdot \cos \theta}{K_f \cdot f + f}$ |
| $P = \frac{P_{\text{max}} \cdot f}{K_f \cdot \sec \theta + f}$ |

(B) Net photosynthesis

| Layered leaves (canopy) |
|-------------------------|
| $netP = P - R$ |
| $netP = \frac{P_{\text{max}} \cdot f}{K_f \cdot (1 - \cos \theta)} + f - R$ |
| $netP = \frac{P_{\text{max}} \cdot f}{K_f \cdot \sec \theta (1 - \cos \theta)} + f - R$ |

Fig. 5 Practitioner-friendly equation for canopy photosynthetic analysis. (A) Equations for gross photosynthesis. Note that the use of Hill coefficient $\alpha$ is only optional. (B) Equations for net photosynthesis.

PRACTICAL EQUATIONS

Here, we wish to propose a series of practical equations designed to describe the collective gross photosynthetic response in the plant community such as in the agricultural field, forest and bushes (Fig. 5A), by revising the photosynthetic modes under layers of leaf canopies by proposing a set of practitioner-friendly mathematical model (modified Hill-type equations) which could be applicable for estimating the total photosynthesis in the plant canopy structure consisted with layered and inclined leaves, as preliminarily testified with vegetables such as leafy lettuce. Our newly proposed equations can be used for simulation of the photosynthetic capacity in the plant canopy structure simply through minimal sampling of a single top-positioned leaf consisting the canopy structure, simply to measure the PI-curve (1), the state of dark respiration (2), and transmittance (3).

Collective harvesting of light by layers of leaves

When comparing two PI-curves with normalized $P_{\text{max}}$, the PI-curve in a stand showed better response than a single leaf, at lower light intensity (Fig. 4D). This is a sign that leaf layer collectively captures the light for enhanced photosynthesis.

As pointed by Monsi and Saeki (1953; 2005), transmittance ($T$) of light through a single leaf layer can be expressed according to the definition by Beer-Lambert law as follows:

$$T = \frac{I_0}{I} = e^{-k \cdot L}$$
where \( a \) and \( x \) are absorption coefficient and length of the path within the leaf layer, respectively. For simplification of the model, we assumed that the canopy structure is consisted by uniform leaves. Then, by experimentally determining the value for \( T \) in the top of canopy structure, we can estimate the sum of light available for photosynthesis within the canopy structure in the form of convergent geometric progression as follows:

\[
\sum_{i=1}^{\infty} JT = \lim_{n \to \infty} \sum_{i=1}^{n} JT = -\frac{J}{1-T}
\]

where \( i \) is number of leaves (\( i=0 \) indicate the case of initial light intensity above the leaves), \( J \) is light intensity, and \( T \) is transmittance by a single layer of leaf. Above equation suggests that light is collectively filtered by leaves nevertheless the leaf number, and the yield of photosynthetically active light convergently attains the ultimate level.

By substituting the term for light intensity in PJE, we obtained the following equation:

\[
\sum_{x=1}^{\infty} P_x = \frac{P_{\text{net}} J}{K_j(1-T)+J}
\]

where \( P_x \) denotes the photosynthesis in the \( x^{\text{th}} \) leaf in the canopy. Since the collective light yield rapidly convergent, \( k \) can be replaced with \( \infty \) in a practical sense. This equation can be rewritten as below, in order to express the change due to leaf layering as the change in the term for apparent Michaelis constant:

\[
\sum_{x=1}^{\infty} P_x = \frac{P_{\text{net}} J}{K_j(1-T)+J}
\]

This equation predicts that apparent \( K_j \) value has tendency to be lowered in the canopy (which is supposed to be affected by \( T \)). In support of this view, we observed that, under narrow beam-angled light source, the apparent \( K_j \) value in a stand of plant which is minimal unit of plant canopy, was ca. 1/5 of \( K_j \) recorded in a single leaf (Fig. 4).

When conversion of equation to HE-type model is required, especially in the case of steep increase in photosynthetic rate at relatively lower range of light intensity, Hill’s coefficient \( \alpha \) should be introduced as below:

\[
\sum_{x=1}^{\infty} P_x = \frac{P_{\text{net}} J}{K_j(1-T)^\alpha+J^\alpha}
\]

Effect of leaf angles

For ease of simulation, we propose a simplification of leaf angle composition to be uniform in the canopy consisted of mono-cultured plants. Thus, by defining the representative leaf angle (which affect the angle of incoming light on the surface of leaves), we can modify the PJE or HE-type model as below (note that introduction of Hill coefficient \( \alpha \) is optional):

\[
P = \frac{P_{\text{net}} J^\alpha \cos \theta}{K_j^\alpha + J^\alpha \cos \theta}
\]

In order to express the changes due to leaf angle as the changes in the term for apparent Michaelis constant, the above equation can be rewritten as below:

\[
P = \frac{P_{\text{net}} J^\alpha \cos \theta}{K_j^\alpha + J^\alpha \cos \theta}
\]

This suggests that plant response to the increase in incoming light is less sensitized by inclination of the leaf, thus the apparent \( K_j \) value might show tendency to be higher in the highly inclined leaves such as standing blades of monocots.

Combining the effects of leaf angle \( \theta \) and transmittance \( T \)

Taken together, we understood that two major factors determining the characteristics of canopy structure, namely, the leaf angle effect and collective use of light through the layer of leaves, drastically alter the size of apparent \( K_j \) values. Therefore, we propose to marge two distinct series of equations into one unified equation as follows:

\[
\sum_{x=1}^{\infty} P_x = \frac{P_{\text{net}} J^\alpha}{(K_j \sec \theta (1-T \cos \theta))^{\alpha}+J^\alpha}
\]

To consider the impact of incoming light angle on the inclined leaves, \( T \) was multiplied with \( \cos \theta \) which is function of leaf angle.

Up to here, equation covering the gross photosynthesis has been discussed. To complete the overall reactions in the canopy, we need to consider the increase in respiration which might show the increase proportional to the increase in the mass of tissues as follows (Fig. 5B):

\[
\sum_{x=1}^{\infty} R_x = \frac{P_{\text{net}} J^\alpha}{(K_j \sec \theta (1-T \cos \theta))^{\alpha}+J^\alpha} \sum_{x=1}^{\infty} R_x
\]

In the canopy, overlapping by leaves may result in compromised consequence between the saturating photosynthetic increase by geometric progression with apparent limit (convergent value) and linear increase in the total respiration proportional to the size of canopy (number of leaves). This indicate that capacity for the number of leaves to be maintained under the canopy structure is limited and compensation point in the canopy is the likely measure.

CONCLUSION

In the present study, we traced the century-old history of the use of equations in photosynthetic analyses inspired by the mathematical models by A. V. Hill (1910). Then, we tried to challenge the mystery of plant canopy photosynthesis reported by Boysen-Jensen (1932). Accordingly, PI-curves in Sinapis alba L. is likely saturated under strong light when measured using a horizontally positioned single leaf, but a stand of the same plant (a minimal canopy model) unexpectedly showed linear increase in PI-response without attaining the saturation. This milestone study has induced a series of discussion if the nature of PI-response in the plant canopy is largely differed from the one in a single leaf, and thus hardly attaining the level of saturation, possibly indicating the importance of leaf angles and the
degree of leaf overlapping in the plant stands or plant communities. This view was theoretically supported by Japanese researchers, Monsi and Saeki (1953).

As we analyzed the original text by Boysen-Jensen, we noticed that the light source employed in the original work in 1932 was a position-altered light bulb, with which the light intensity could be adjusted based on the the distance between the light bulb and the leaves or standing plants. Here, we repeated the classical experiments using the seedlings of *Gomphrena globosa* as a model material. We compared the rate of CO₂ uptake by a horizontally placed single leaf or stand with multiple leaves of *G. globosa* sealed in a clear acryl box, under two distinct light sources, namely a light bulb and a solar simulator.

Now, we need to point out some technical problems in the PI-curve measurement in tall plants under broad beam-angled artificial light sources positioned closed to plants. Our data obtained after model repetitions using a light bulb were almost identical with the original observation by Boysen-Jensen, only if we do not consider the effect of leaf position (distance from the light source) within the stand of plant. On the other hand, same set of experiments were carried out under Xenon-based solar simulator, mimicking the solar spectrum, light intensity, and irradiation angle. However, no positional effect was observed and both the single leaves and standing plants showed saturation in photosynthesis.

Despite long-lasted belief, we concluded that there exists no canopy magic in photosynthesis. We assumed that the apparent lack of saturation in PI-curves in a stand of plant could be attributed to neither utilization of scattered sun light by plants, the temporal movements of leaves, nor the changes in the position of sun, despite the earlier suggestion by Monsi and Saeki (1953). It could be simply an artifact due to the lack of consideration on the problems associated with a broad beam-angled light source.

Lastly, we revised the photosynthetic modes under layers of leaf canopies by proposing a set of practitioner-friendly mathematical model (modified Hill-type equations) which could be applicable for estimating the total photosynthesis in the plant canopy structure consisted with inclined leaves. Our newly proposed equations can be used for simulation of the photosynthetic capacity in the plant canopy structure simply through minimal sampling of a single top-positioned leaf to testify the PI-curve (1), dark respiration (2), and transmittance (3).

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