

Estimation of Leaf Area and Light-use Efficiency by Non-destructive Measurements for Growth Modeling and Recommended Leaf Area Index in Greenhouse Tomatoes

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We aimed to monitor greenhouse tomato growth without destructive sampling and investigated an empirical growth model in which dry matter production was obtained as a product of light intercepted by plants and light-use efficiency. The intercepted light values were non-destructively determined on the basis of leaf width and length, and the number of leaves. Light-use efficiency was expressed as a function of daytime CO₂ concentration. Three cultivation experiments were conducted with three tomato cultivars over two years. Significant regression lines and curves, as well as coefficients of the model, were obtained for each cultivar. Using photosynthetic curves of the three cultivars and solar radiation data, we suggest an approach to determine the recommended leaf area index to maximize dry matter production. The developed model has potential to improve yield and labor efficiency in tomato production.

Key Words: CO₂ level, dry matter production, environmental control, light interception, recommended LAI.

Introduction

Crop growth models can assist in pre-season and in-season management decisions on cultural practices, control of greenhouse climate, fertilization, and irrigation (Boote et al., 1996; Marcelis et al., 2009; Spitters et al., 1989). There are several crop growth models for tomato (*Solanum lycopersicum*) production systems in greenhouses (Boote et al., 2012). These models are expected to be used to improve cultivation management technology, labor management, and sales planning. Crop dry matter (DM) predictions can be used to predict fruit fresh weight (Boote et al., 2012). A general approach for predicting fresh weight, comparable to the photosynthesis-based approach used in mechanistic models for DM production, is needed (Heuvelink et al., 2004). In tomato growth models such as TOMSIM, the observed values of the leaf area index (LAI) have been used to predict canopy assimilation (Heuvelink, 1995). Cho et al. (2007) used leaf length and width, SPAD

value, and different combinations of these variables to develop models for predicting the individual leaf area (but not leaf area per plant) in cucumber.

Tomato yield could be determined from total production of DM and its distribution to fruits, and total DM production could be determined from light-use efficiency (LUE) and the fraction of intercepted light (Higashide, 2015; Higashide and Heuvelink, 2009). Kaneko et al. (2015) reported that planting density and seedling stage at transplanting do not affect LUE. Higashide (2018) suggested that DM production and yield could be calculated from cumulative intercepted light and LUE. We propose an outline of a growth model to predict DM production and yield in tomato.

Cumulative intercepted light and LUE are important elements to determine DM production. Light intercepted by the canopy can be obtained as a product of the fraction of light interception and light intensity; the former parameter could be obtained from LAI and the light extinction coefficient in the canopy (Monsi and Saeki, 2005). Thus, to obtain light interception, we must measure the leaf area and light intensity. In previous reports, leaf area was often measured by destructive plant sampling (Heuvelink, 1995; Higashide and Heuvelink, 2009). However, non-destructive measurements are needed to continuously monitor the leaf area.

The high yields of Dutch tomato cultivars are due to

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higher LUE (Higashide and Heuvelink, 2009; van der Ploeg et al., 2007). The yield of Japanese tomato cultivars has not been improved by breeding, although these cultivars differ from each other in LUE values (Higashide et al., 2012). Thus, to predict DM production, LUE should be determined for each cultivar. To improve yield, CO₂ enrichment is often applied in commercial greenhouses; this increases the photosynthetic rate and therefore LUE (Higashide et al., 2015). However, the relationship between LUE and CO₂ concentration needs to be quantified.

In this paper, we assessed the use of non-destructive measurements of leaf length and leaf width to estimate the leaf area, which we then used to determine light interception by tomato plants. We also estimated the effect of CO₂ concentration on LUE. Finally, we recommended a LAI based on light-response curves of photosynthesis and light levels in the canopy. Although optimal LAI may differ depending on solar radiation and also on season, it is difficult for growers to understand these values. Providing a recommended LAI will be helpful for growers to manage this factor.

Materials and Methods

1. Growing conditions

Three cultivation experiments (Exp. 1–3) were conducted over two years under the conditions indicated in Table 1. In all experiments, seeds of Tomato ‘Ringyoku’ (Rn) (NARO, Japan; Matsunaga and Saito, 2017), ‘CF Momotaro York’ (My) (Takii & Co., Ltd., Kyoto, Japan), and ‘Managua’ (Mn) (Rijk Zwaan, De Lier, Bergschenhoek, the Netherlands) were sown in seed trays filled with nursery soil. In Exp. 1 and 2, the cultivars were not grafted; in Exp. 3, seedlings of Rn and Mn were grafted onto ‘Maxifort’ (De Ruiter Seeds, Bergschenhoek, the Netherlands) at 14 days after sowing. The seed trays were placed in a seedling growth chamber (Seedling Terrace; Mitsubishi Chemical Agri Dream Co., Ltd., Tokyo, Japan). The seedlings were fertilized every day with commercial nutrient solution (High-Tempo; Sumitomo Chemical Co., Ltd., Tokyo, Japan) adjusted to an electrical conductivity (EC) of 1.6 dS·m⁻². The seedlings were grown under fluores-

cent lamps at a photosynthetic photon flux density (PPFD) of 350 μmol·m⁻²·s⁻¹, 1000 μmol·mol⁻¹ CO₂, and air temperatures of 25°C (day, 16 h) and 17°C (night, 8 h). The seedlings were transplanted into rockwool cubes (7.5 cm × 7.5 cm × 6.5 cm) and then into six rows of rockwool systems (Table 1) in a greenhouse compartment (9 × 18 m) at the Institute of Vegetable and Floriculture Science, NARO (Tsukuba, Ibaraki, Japan). The spacing was 180 cm between rows in all experiments, 16.7 cm between plants in Exp. 1 and 3, and 25.0 cm between plants in Exp. 2.

In Exp. 1 and 2, the six rows were divided into three blocks from north to south; in each block, plots of the three cultivars were randomly arranged. In each plot, 18 and 16 plants per cultivar (in Exp. 1 and 2, respectively) were planted in double rows. These plants were surrounded by border plants, which were excluded from the analysis. In Exp. 3, Rn (two plots) and My and Mn (one plot each) were used. Each plot contained 64 plants in two double rows and 20 plants in a border row. Individual leaf area was measured in the border plants at 31 days after transplanting (DAT); plants in double rows were used in all other measurements.

Plants were supplied with commercial nutrient solution (OAT House-SA; OAT Agrico Co., Ltd., Tokyo, Japan) that consisted of 17.6 me·L⁻¹ NO₃, 4.4 me·L⁻¹ P, 10.2 me·L⁻¹ K, 8.2 me·L⁻¹ Ca, and 3.0 me·L⁻¹ Mg, adjusted to an EC of 2.6 dS·m⁻¹ in Exp. 1 and 2. In Exp. 3, plants were supplied with nutrient solution (15.8 me·L⁻¹ NO₃, 4.5 me·L⁻¹ P, 9.8 me·L⁻¹ K, 8.8 me·L⁻¹ Ca, 4.6 me·L⁻¹ Mg) adjusted to an EC of 3.4 dS·m⁻¹ until 14 DAT, and then with the same nutrient solution adjusted to an EC of 2.6 dS·m⁻¹. The daily draining volume was 20% of the total volume of the nutrient solution supplied; the drainage was discarded.

According to standard Dutch practice, the plants were trained vertically at a height of 3.1 m and lowered as they grew; old leaves were pruned. All axial buds were removed as they appeared and were excluded from the analysis. Flowers were sprayed with 2-methyl-4-chlorophenoxyacetic acid (Tomato Tone; ISK Biosciences K.K., Tokyo, Japan) to promote fruit set; the number of fruits per truss was not adjusted by pruning.

Table 1. Experimental conditions.

Parameter	Exp. 1	Exp. 2	Exp. 3
Date of sowing (dd/mm/yyyy)	03/06/2015	30/10/2015	Rn, Mn: 16/05/2016 ^z My: 18/05/2016
Date of transplanting (dd/mm/yyyy)	02/07/2015	01/12/2015	13/06/2016
Experiment end date (dd/mm/yyyy)	10/11/2015	26/05/2016	23/03/2017
Experiment duration (days from transplanting)	131	177	283
Planting density (plants·m ⁻²)	3.3	2.7	3.3
Average CO ₂ during daytime (μmol·mol ⁻¹)	400	432	540

^z In Exp. 3, My was not grafted and was sown two days after Rn and Mn, which were grafted onto ‘Maxifort’. Rn, ‘Ringyoku’; My, ‘CF Momotaro York’; Mn, ‘Managua’.

In the greenhouse, the temperature threshold for natural ventilation was set at 25, 27, and 29°C in each season. Heating initiation was set at 14°C in winter. The target daily average temperature was set at 25°C, with cooling at night (20:00–04:00) by a heat pump (Green Package; Nepon Inc., Tokyo, Japan) in Exp. 1 and 3. A fogging system (Tokita, Chiba, Japan) was used during the daytime for humidification, maintaining 70% relative humidity in Exp. 1–3. Air temperature, relative humidity, and CO₂ concentration in the greenhouse were recorded by a Ubiquitous Environmental Control System (UECS, Akisai; Fujitsu Limited, Tokyo, Japan) every five minutes, and solar radiation outside the greenhouse was recorded by another UECS device (Stella Green, Osaka, Japan) every minute.

We harvested all mature fruit from 8–16 plants per cultivar twice a week and measured their fresh weights. Leaf area was measured using a leaf area meter (LI-3100; LI-COR Inc., Lincoln, NE, USA), and the fresh and dry weights of leaves, stems, and all fruit of three plants per cultivar destructively sampled were measured at 40, 74, and 130 DAT in Exp. 1, at 77, 120, 148, 162, and 177 DAT in Exp. 2, and at 248, 263, 269, and 283 DAT in Exp. 3.

2. Estimation of leaf area and light interception

Rate of leaf appearance at n DAT (dNl_n/dt ; leaves per plant per day) was calculated as:

$$dNl_n/dt = a \cdot \ln(T_n) - b \quad \text{Eq. 1.1}$$

where T_n is the daily average temperature in the greenhouse (°C), and a and b are coefficients obtained from the regression analyses in Exp. 1–3.

The number of leaves per plant at n DAT (Nl_n ; leaves per plant) was calculated as:

$$Nl_n = Nl_{n-1} + dNl_{n-1}/dt \quad \text{Eq. 1.2}$$

where Nl_{n-1} is the number of leaves per plant at $n-1$ DAT, dNl_{n-1}/dt is the rate of leaf appearance at $n-1$ DAT (leaves per plant per day).

The number of remaining leaves per plant at n DAT (Nrl_n ; leaves per plant) was calculated as:

$$Nrl_n = Nl_n - Npl_n \quad \text{Eq. 1.3}$$

where Nl_n is the number of leaves per plant at n DAT, and Npl_n is the number of pruned leaves per plant until n DAT, respectively.

Individual leaf area (ai ; cm² per leaf) was predicted according to:

$$ai = c \cdot Ll \cdot Lw \quad \text{Eq. 2.1}$$

where Ll is the leaf length (cm per leaf), Lw is the leaf width (cm per leaf), and c is a coefficient obtained from the slope of a linear regression of the observed individual leaf area as a function of the product of leaf length and leaf width measured at 31, 248, 263, 269 and 283 DAT in Exp. 3.

We calculated individual leaf areas by separating at the upper, middle and lower leaves to obtain precise values. Although lower leaves were removed as growing plants, we could choose the appropriate equation for leaf position. Average individual leaf area without upper and lower leaves (Mai ; cm² per leaf) was calculated as:

$$Mai = \sum_{n=Lpl+1}^{Lpu-1} ai / (Nrl - Nlu - Nll) \quad \text{Eq. 2.2}$$

in which Nrl is the number of remaining leaves per plant, Nlu and Nll are the numbers of upper and lower leaves, respectively, Lpu is the leaf position from the highest leaf ($1 \leq Lpu \leq Nlu$), and Lpl is the leaf position from the first true leaf ($1 \leq Lpl \leq Nll$). The number of upper leaves was defined five leaves from the highest leaf over 7 cm. The number of lower leaves in Rn , My , and Mn was defined seven, four, and eight leaves, respectively. The lower leaves in each cultivar were defined as leaves for which the leaf area was significantly lower than the maximum individual leaf area of the plant by multiple comparisons of individual leaf area in each leaf position at 31 DAT in Exp. 3.

Leaf area per plant (Ai_n ; m²) at n DAT was calculated as:

$$Ai_n = (Nrl_n - Nlu - Nll) \cdot Mai + Aiu_n + Ail_n \quad \text{Eq. 2.3}$$

where Nrl_n is the number of leaves per plant remaining at n DAT, and Aiu_n and Ail_n are the upper and lower leaf areas at n DAT (m² per plant), respectively.

Upper leaf area (Aiu ; m² per plant) was calculated as:

$$Aiu = \Sigma((f \cdot e^{g \cdot Lpu}) \cdot Mai) \quad \text{Eq. 2.4}$$

where f and g are coefficients calculated from the exponential curve of the area of each upper leaf per Mai as a function of leaf position at 31 DAT in Exp. 3.

Lower leaf area (Ail ; m² per plant) was calculated as:

$$Ail = \Sigma((h \cdot e^{i \cdot Lpl}) \cdot Mai) \quad \text{Eq. 2.5}$$

where h and i are coefficients calculated from the exponential curve of the area of each lower leaf per Mai as a function of leaf position at 31 DAT in Exp. 3.

In Exp. 3, leaf area per plant at 22–283 DAT was obtained according to Eq. 2.2–2.5; plants at 0–21 DAT had fewer than 16 leaves, and leaf area per plant at early stage (Ai_c ; m²) was calculated as:

$$Ai_c = \Sigma ai \quad \text{Eq. 2.6}$$

To calculate the ai (Eq. 2.1), Ll and Lw of all leaves per plant ($n = 16$ plants for Rn , and $n = 8$ for My and Mn) were measured at 0, 7, and 21 DAT.

Ai_{c_n} of plants with fewer than 16 leaves at n DAT was calculated as:

$$Ai_{c_n} = j \cdot e^{l \cdot Nln} \quad \text{Eq. 2.7}$$

where j and l are coefficients calculated from the exponential curve of the leaf area per plant as a function of the number of leaves at 0, 7, and 21 DAT in Exp. 3.

LAI at n DAT ($LAI_n \text{ m}^2 \cdot \text{m}^{-2}$) was calculated as:

$$LAI_n = A_{i_n} \cdot P_d \quad \text{Eq. 2.8}$$

where P_d is plant density ($\text{plants} \cdot \text{m}^{-2}$). A_{i_n} obtained according to Eq. 2.7 (for plants with fewer than 16 leaves) or Eq. 2.3 (for plants with 16 or more leaves) was used to predict LAI. Plants with fewer than 16 leaves could not be separated by the upper, middle and leaves.

Cumulative intercepted light (IL_n ; $\text{MJ} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$) at n DAT was calculated as:

$$IL_n = (1 - e^{-k \cdot LAI_n}) \cdot 0.55 \cdot 0.5 \cdot Sr_n \quad \text{Eq. 3.1}$$

where k is the light extinction coefficient and Sr_n is solar radiation outside at n DAT ($\text{MJ} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$). The value 0.55 corresponds to the light transmissivity of the greenhouse measured before the experiment. The ratio of photosynthetically active radiation (PAR) to solar radiation was assumed to be 0.5 (Ohtani, 1997). The light extinction coefficient was obtained for each cultivar using the equation of Monsi and Saeki (2005 [1953]) as the slope of a logarithmic regression of PPFD against cumulative LAI at five different heights in the canopy in Exp. 1–3 (0.72 for Rn, 0.99 for My, and 0.77 for Mn; $R^2 = 0.69, 0.75, \text{ and } 0.60$, respectively; $P < 0.01$).

3. LUE at different CO_2 levels

LUE was defined as DM production per cumulative intercepted PAR and was calculated in Exp. 2 (148–162 DAT, Duration 1; and 163–177 DAT, Duration 2) and in Exp. 3 (248–263 DAT, Duration 3; and 269–283 DAT, Duration 4). The daytime (08:00–16:00) CO_2 levels in the greenhouse were maintained at $600 \mu\text{mol} \cdot \text{mol}^{-1}$ (Duration 1) and $800 \mu\text{mol} \cdot \text{mol}^{-1}$ (Duration 3), but were not controlled in Durations 2 and 4. Daytime average CO_2 concentrations were 524, 352, 764, and $285 \mu\text{mol} \cdot \text{mol}^{-1}$ in Durations 1, 2, 3, and 4, respectively. LUE in each duration (LUE dn; $\text{g} \cdot \text{MJ}^{-1}$) was calculated as

$$LUE_{dn} = (DM_{\text{end}} - DM_{\text{start}}) / (IL_{\text{end}} - IL_{\text{start}}) \quad \text{Eq. 3.2}$$

where DM_{end} and DM_{start} ($\text{g} \cdot \text{m}^{-2}$) are DM production at the end of each duration ($n = 3$) and average DM production at the start of the duration, respectively, and IL_{end} and IL_{start} ($\text{MJ} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$) are cumulative intercepted PAR at the end of the duration ($n = 3$) and average cumulative intercepted PAR at the start of the duration, respectively.

Daily LUE was obtained as the curvilinear regression of LUE during each duration as a function of daytime CO_2 concentration in each duration.

$$LUE_n = m \cdot \ln(CO_{2n}) - o \quad \text{Eq. 3.3}$$

where m and o are coefficients obtained from the regression analysis in Exp. 2 and 3.

To compare LUE based on Eq. 3.3 with LUE calculated by a conventional method (Higashide and Heuvelink, 2009), the values of LUE based on Eq. 3.3

from all durations were averaged. Conventional LUE was calculated as the slope of a linear regression of total DM as a function of the cumulative intercepted PAR on each sampling date. Total DM was calculated by adding the average dry fruit yield per plant per cultivar and dry weight of removed leaves until the day before the sampling to total DM on the sampling date. Cumulative intercepted PAR was calculated according to Eq. 3.1. Daily LAI was obtained by linear interpolation between sampling dates.

4. Leaf photosynthetic rate and recommend LAI

The individual-leaf photosynthetic rate was measured at 157–167 DAT in Exp. 2 with a portable photosynthesis system (LI-6400; LI-COR) at PPFD of 1500, 1000, 800, 400, 200, 100, 60, 40, 20, 10, and $0 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and $800 \mu\text{mol} \cdot \text{mol}^{-1} CO_2$. The rate was measured between 10:00 and 14:00 in mature leaves (the 6th to 8th leaves from the top of the plant; two plants per plot for each cultivar).

The relation between recommend LAI (i.e., the value that maximizes daily canopy photosynthesis) and solar radiation was obtained as follows. Solar radiation values from June 2016 to March 2017 recorded every minute were averaged every 10 minutes. PAR ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) inside the greenhouse was calculated as

$$PAR = (Sr \cdot 1000 \cdot 4.57) \cdot 0.55 \cdot 0.5 \quad \text{Eq. 4.1}$$

where Sr is solar radiation outside the greenhouse. The coefficient for converting irradiance to photon flux density was 4.57 (Thimijan and Heins, 1983). The value 0.55 corresponds to the light transmissivity of the greenhouse measured before the experiment. The ratio of PAR to solar radiation was assumed to be 0.5 (Ohtani, 1997).

Canopy photosynthesis per 10 min (C_p ; $\mu\text{mol} \cdot \text{m}^{-2} / 10 \text{ min}$) was calculated as:

$$C_p = \left(\left(\left(\left(\varphi \cdot PAR^{(-k \cdot LAI_{pn})} \right) + P_{\text{max}} \right) - \left(\left(\varphi \cdot PAR^{(-k \cdot LAI_{pn})} \right) + P_{\text{max}} \right)^2 - 4 \cdot \varphi \cdot PAR^{(-k \cdot LAI)} \cdot P_{\text{max}} \cdot \theta^{0.5} \right) / (2 \cdot \theta) - r \right) \cdot 600 \quad \text{Eq. 4.2}$$

in which φ , LAI_{pn} , P_{max} , θ , and r are the initial slope, LAI at each position in the plant canopy, maximum photosynthetic rate, convexity, and dark respiration rate of the light-response curve of photosynthesis, respectively, while k is the light extinction coefficient. PAR inside the greenhouse was used.

The C_p values were calculated using LAI from 1.0 to 6.0 with steps of 0.1; $LAI_{p1} - LAI_{p50}$. The sum of the C_p values from 7:00 to 16:00 was defined as daily canopy photosynthesis. Then, daily canopy photosynthesis values at different LAI values were compared, and the recommended LAI at $800 \mu\text{mol} \cdot \text{mol}^{-1} CO_2$ (at which daily canopy photosynthesis was maximized) was determined. We obtained a relationship diagram as

a function of the recommended LAI based on daily solar radiation.

Results

1. Estimation of leaf area and light interception

Leaf appearance rates were significantly correlated with daily temperature in all three cultivars (Fig. 1). Coefficients a and b in Eq. 1.1 are shown in Table 2. Individual leaf area and leaf area per plant in Exp. 3 are

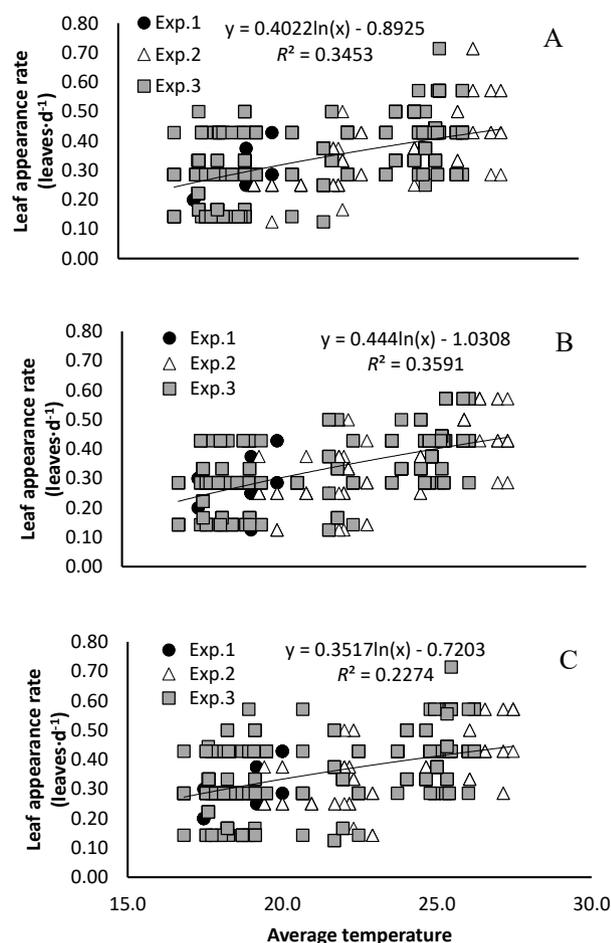


Fig. 1. Daily leaf appearance rate as a function of average air temperature in ‘Ringyoku’ (A), ‘CF Momotaro York’ (B), and ‘Managua’ (C).

shown in Table 3. Observed individual leaf area was significantly correlated with the product of leaf length and leaf width ($n = 40, R^2 = 0.96$ for all 3 cultivars; $P < 0.001$). Coefficient c in Eq. 2.1 is shown in Table 2. The value of average observed individual leaf area without upper and lower leaves (Mai_ob) was significantly larger in Rn and Mn than in My, in which it was larger at 31 DAT than on any other day. For Mai_ob, no interaction was observed between cultivar and DAT (Table 3). Data on Ail_ob, are not shown since the values were available only at 31 DAT.

Upper leaf area (Eq. 2.4) was significantly correlated with the product of the exponent of the upper leaf position and average individual leaf area ($R^2 = 0.94$ for Rn, 0.88 for My, 0.89 for Mn; $P < 0.001$). Coefficients f and g in Eq. 2.4 are shown in Table 2. Lower leaf area (Eq. 2.5) was significantly correlated with the product of the exponent of lower leaf position and average individual leaf area ($R^2 = 0.83$ for Rn, 0.95 for My, and 0.93 for Mn; $P < 0.001$). Coefficients h and i in Eq. 2.5 are shown in Table 2. Leaf area per plant with fewer than 16 leaves; Ai_e_n (Eq. 2.7) was significantly correlated with the number of leaves ($R^2 = 0.97$ for Rn, 0.95 for My, and 0.97 for Mn; $P < 0.001$). Coefficients j and l in Eq. 2.7 are shown in Table 2.

Observed leaf area per plant (Ai_ob) did not differ significantly among the cultivars or among DAT in Mn (Table 3); Ai_ob was significantly smaller at 31 DAT than at 263 DAT in Rn and was significantly smaller at 31 DAT than at 263 and 283 DAT in My.

Observed leaf area per plant and upper leaf area (Aiu_ob) differed significantly among the cultivars (Table 3). In Rn, Aiu_ob was significantly larger at 31 DAT than at 283 DAT. In My, Aiu_ob was significantly larger at 31 DAT than at 263, 269, and 283 DAT. In Mn, Aiu_ob was significantly larger at 31 DAT than at 263 and 283 DAT, and at 248 DAT than at 283 DAT.

2. Light-use efficiencies at different CO₂ levels

In Exp. 2 and 3, LUE was correlated significantly with daytime CO₂ concentration in the greenhouse (Fig. 2; $R^2 = 0.82$ for Rn, 0.84 for My, and 0.69 for Mn; $n = 12, P < 0.001$). Coefficient m and o in Eq. 3.3 are

Table 2. Observed coefficients in the equations.

Cultivar	Coefficient										
	a ^z	b ^z	c ^y	f ^x	g ^x	h ^x	i ^x	j ^w	l ^w	m ^v	o ^v
Rn	0.40	0.89	0.33	0.027	0.65	0.0063	0.96	0.0036	0.37	4.92	-25.43
My	0.44	1.03	0.25	0.034	0.67	0.0109	0.67	0.0023	0.38	4.51	-24.17
Mn	0.35	0.72	0.36	0.025	0.69	0.0151	0.52	0.0044	0.35	4.52	-22.74

^z Obtained from the regression in Exp. 1–3.

^y Obtained from the regression at 31, 248, 263, 269, and 283 days after transplanting in Exp. 3.

^x Obtained from the regression at 31 days after transplanting in Exp. 3.

^w Obtained from the regression at 0, 7, and 11 days after transplanting in Exp. 3.

^v Obtained from the regression in Exp. 2 and 3.

Rn, ‘Ringyoku’; My, ‘CF Momotaro York’; Mn, ‘Managua’.

Table 3. Observed parameters at different days after transplanting (DAT) in Exp. 3.

Cultivar	DAT	Mai_ob ^z (cm ² /leaf)	ai_ob/ (Ll·Lw) ^y	Ll·Lw (cm ²)	Ai_ob ^x (cm ² /plant)	Aiu_ob ^w (cm ² /plant)
Rn	31	824 a ^v	0.27 cd	2772 a	700 b	1102 ab
	248	793 a	0.33 ab	2430 ab	877 ab	845 abcde
	263	844 a	0.36 A	2134 bc	1257 a	1034 abcd
	269	735 a	0.31 bc	2117 bc	966 ab	617 bcdef
	283	611 a	0.30 bc	1945 bcd	1012 ab	532 def
My	31	588 A	0.25 D	2370 abc	680 B	1066 abc
	248	377 B	0.25 D	1475 d	922 AB	535 cdef
	263	379 B	0.24 D	1544 d	1099 A	507 def
	269	363 B	0.25 D	1427 d	925 AB	408 ef
	283	352 B	0.23 D	1529 d	1042 A	307 f
Mn	31	879 a	0.34 ab	2231 abc	736 a	1169 a
	248	659 a	0.33 ab	1900 bcd	900 a	1096 ab
	263	706 a	0.36 A	1832 cd	1241 a	606 bcdef
	269	702 a	0.34 ab	1952 bcd	1150 a	756 abcdef
	283	650 a	0.33 ab	1870 bcd	1171 a	441 ef
Significance ^u						
Cultivar		***	***	***	NS	***
DAT		***	**	***	***	***
Interaction		NS	**	NS	NS	*

^z Mai_ob, average observed individual leaf area without upper and lower leaves.

^y ai_ob, observed individual leaf area; Ll, leaf length; Lw, leaf width.

^x Ai_ob, observed leaf area per plant.

^w Aiu_ob, observed leaf area per plant and upper leaf area.

^v Values within a column followed by different letters differ significantly for the same cultivar ($P < 0.05$; by Bonferroni's post test in Roman type, or Tukey's multiple-comparison test in italics).

^u NS, no significant difference; asterisks indicate significant differences at $*P < 0.05$, $**P < 0.01$, or $***P < 0.001$ by two-way ANOVA ($n = 3$).

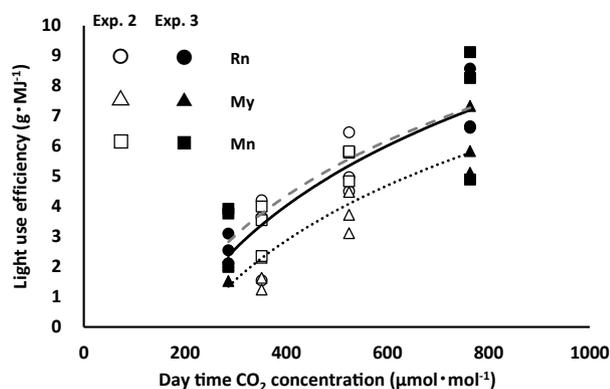


Fig. 2. Light-use efficiency as a function of daytime CO₂ concentration in a greenhouse in Exp. 2 and 3. Rn, 'Ringyoku'; My, 'CF Momotaro York'; Mn, 'Managua'. Open symbols, Exp. 2; closed symbols, Exp. 3. ($R^2 = 0.82$ for Rn, 0.84 for My, 0.69 for Mn; $P < 0.001$; $n = 12$).

shown in Table 2. Based on 95% confidence intervals, no significant difference in these coefficients was detected among the three cultivars. The 95% confidence intervals of coefficient m were 3.258–6.6562 for Rn, 3.102–5.907 for My, and 2.390–6.636 for Mn; those of o were 15.29–35.50 for Rn, 15.54–32.69 for My, and 9.707–35.67 for Mn.

The ratios of the number of harvest days to those of

total experimental days, fraction of DM allocated to fruit at the end of each experiment, average daily LUE (Eq. 3.3), and LUE during the whole experimental term determined by destructive sampling are shown in Table 4. The ratio of the number of harvest days to total experimental days was greater in Exp. 3 than in Exp. 1 and 2. The fraction of DM allocated to fruit did not differ significantly among the cultivars, but was significantly higher in Exp. 3 than in Exp. 1 and 2. There was a significant cultivar \times experiment interaction for the fraction of DM allocated to fruit. There were significant differences in the average daily LUE values among the cultivars and experiments, but no significant cultivar \times experiment interaction. Based on 95% confidence intervals, LUE during the entire Exp. 3 was significantly higher than in Exp. 1 and 2 in each cultivar. In each experiment, there was no significant difference in LUE between Rn and Mn, but LUE values were significantly lower in My than in Rn in Exp. 1, than in Mn in Exp. 2, and than in Rn and Mn in Exp. 3. LUE during the entire experiment was significantly correlated with average daily LUE ($R^2 = 0.85$, $P < 0.01$).

3. Leaf photosynthetic rate and recommended LAI

The photosynthetic rate of individual leaves at different PPFD values is shown in Figure 3A. Regression analysis showed that the photosynthetic rate was signif-

Table 4. Ratio of harvest days to experimental days, fraction of dry matter allocated to fruit, and light-use efficiency (LUE).

	Cultivar	Harvest day/ Experimental days ^z	Fraction of dry matter allocated to fruit ^y	Average daily LUE ^x	LUE during experiment ^w
Exp. 1	Rn	0.57 (75/131)	0.33±0.04	3.93±0.82	4.09±0.43
	My	0.63 (82/131)	0.33±0.05	2.79±0.76	3.54±0.43
	Mn	0.60 (78/131)	0.35±0.03	4.27±0.76	3.72±0.39
Exp. 2	Rn	0.52 (92/177)	0.38±0.06	4.11±1.69	4.73±1.31
	My	0.55 (98/177)	0.34±0.04	2.95±1.55	4.12±0.83
	Mn	0.45 (79/177)	0.34±0.01	4.43±1.56	5.12±1.78
Exp. 3	Rn	0.80 (226/283)	0.53±0.02	5.26±1.51	7.29±0.77
	My	0.80 (226/283)	0.62±0.04	4.00±1.39	5.38±0.46
	Mn	0.75 (212/283)	0.48±0.02	5.49±1.39	7.23±0.31
Significance ^v	Cultivar	—	NS	***	—
	Experiment	—	***	***	—
	Interaction	—	**	NS	—

^z Experimental days is the number of days from the transplanting date to the end of the experiment.

^y Fraction of dry matter allocated to fruit was obtained as a quotient of the total dry weight of fruit divided by the total dry matter above ground at the end of the experiment. Average±SD (n=3).

^x Average daily LUE based on Eq. 3.3 and CO₂. Average±SD (n=132 in Exp. 1, 177 in Exp. 2, and 286 in Exp. 3).

^w LUE during each experiment was calculated as the slope of a linear regression of TDM as a function of the integrated interception of solar radiation on each sampling date. Slope±95% confidence intervals.

^v NS, not significant; asterisks indicate significant differences at ***P*<0.01 or ****P*<0.001 by two-way ANOVA.

icantly correlated with PPFD in all cultivars ($R^2 > 0.99$). The maximum photosynthetic rate extrapolated from the light–photosynthesis curves was 37.2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for Rn, 38.4 for My, and 41.9 for Mn. The initial slope of the curve was 0.08 for Rn and Mn, and 0.09 for My. The convexity of the curve was 0.14 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for Rn, 0.15 for My, and 0.10 for Mn. Respiration rate was 3.0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for Rn, 1.6 for My, and 1.8 for Mn.

The recommended LAI at 800 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂ was significantly correlated with daily solar radiation in all cultivars (Fig. 3B; $R^2 = 0.99$, $P < 0.001$). A logarithmic regression curve of recommend LAI vs. solar radiation was obtained for each cultivar. There were significant differences in the regression curves and coefficients among the three cultivars. The slope of the logarithmic regression (95% confidence interval) was 1.4271 (1.408–1.447) for Rn, 0.9968 (0.9848–1.009) for My, and 1.2834 (1.270–1.297) for Mn; the intercept of the logarithmic regression was 0.8054 (0.7611–0.8497) for Rn, 1.6927 (1.666–1.720) for My, and 1.6072 (1.577–1.637) for Mn.

Discussion

Simulation by using growth models may support crop production in a greenhouse, including crop management, environmental control, labor planning and management, and sales (Boote et al., 1996; Heuvelink, 1995; Heuvelink et al., 2004; Higashide, 2018; Marcellis et al., 2009). In the present study, we modeled tomato DM production without destructive sampling. DM production was calculated from light intercepted by plants and LUE (Heuvelink, 1995; Higashide and Heuvelink,

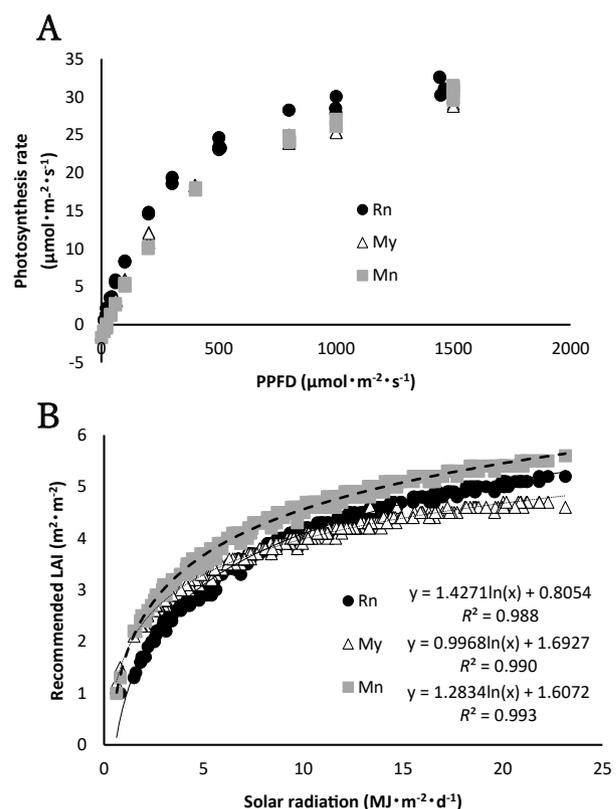


Fig. 3. Light-response curves of photosynthesis as a function of photosynthetic photon flux density (PPFD) (A) and recommend leaf area index (LAI) as a function of daily cumulative solar radiation (B) from June 2016 to March 2017. Rn, ‘Ringyoku’; My, ‘CF Momotaro York’; Mn, ‘Managua’.

2009; Higashide et al., 2015; Kaneko et al., 2015). Although LAI and PAR are required to estimate the intercepted light, there are only a few reports of sensors and other methods for continuously and automatically measuring LAI (Ohishi, 2016). Here, we estimated LAI from the number of leaves and the individual leaf area, which was measured non-destructively from leaf width and length (Eq. 2.1). This method has been reported in cucumbers (Ahn et al., 2015; Cho et al., 2007) and is well known in tomatoes (Hiei et al., 2018; Higashide and Heuvelink, 2009; Higashide et al., 2015). In the present study, observed individual leaf area was significantly correlated with leaf width and length (Table 3). Thus, we could estimate individual leaf area using Eq. 2.1. Since the products of leaf width and length significantly differed among cultivars and DAT (Table 3), we needed to measure leaf width and length in each cultivar. To estimate individual leaf area, we needed to obtain coefficient c by destructive sampling for each cultivar. Coefficient c in Eq. 2.1 also differed significantly among the cultivars. Coefficient c differed significantly among DAT in Rn, but not in My or Mn.

In commercial tomato production, the number of leaves is easy to count and it is controlled by leaf picking. To facilitate management of the number of remaining leaves, we expressed the rate of leaf appearance as a function of temperature (Eq. 1.1; Fig. 1). There was a significant correlation between leaf appearance rate and daily temperature in each cultivar (Fig. 1), similar to other crops such as rice, wheat, and maize (Tollenaar et al., 1979; White et al., 2012; Yin et al., 1996). Thus, the leaf appearance rate in each tomato cultivar can be calculated using Eq. 1.1 from the daily average temperature in the greenhouse. Because inflorescences appear after every three leaves in tomatoes, the inflorescence appearance rate can also be calculated using Eq. 1.1 from the daily average temperature.

Many growth models require a photosynthetic parameter measured in an individual leaf (Heuvelink, 1995; Heuvelink et al., 2004; Vanthoor et al., 2011a, b, c). Although we measured the photosynthetic rate for individual leaves (Fig. 3A), it could be difficult to obtain photosynthetic parameter values for each cultivar in commercial tomato production. DM production was obtained as the product of intercepted light and LUE without the need for photosynthetic parameters (Higashide, 2015; Kaneko et al., 2015). We considered using this approach for each cultivar for commercial production. Elevated CO_2 increases LUE in tomatoes (Aikman, 1996; de Koning, 1997; Higashide et al., 2015; Thongbai et al., 2010). In the present study, LUE was significantly correlated with the daytime CO_2 concentration (Fig. 2); therefore, daily LUE was calculated as a function of daytime CO_2 concentration (Eq. 3.3). In this equation, the difference in LUE among cultivars is expressed by the coefficients m and o . Most of the average daily LUE values estimated using Eq. 3.3 were

smaller than experimentally determined LUE values averaged over all experiments (Table 4). The daily LUE by Eq. 3.3 at high CO_2 concentrations may be a higher value even under a low PAR condition. However, DM production may be low at low PAR. Although LUE over all experiments accounted for this difference in DM production per day, the average daily LUE values by Eq. 3.3 were not accounted for. This may have caused the difference between the two LUE values. However, they were significantly correlated ($R^2 = 0.85$, $P < 0.01$). Thus, DM production could be calculated as the product of daily LUE and intercepted light.

Dry weight yield could be calculated as the product of total DM and fraction of DM distributed to fruit. This fraction differed among experiments (Table 4), probably because of the differences in the ratio of the number of harvest days to that of experimental days.

DM production may be decreased by both low LAI (because of a decrease in light intercepted by plants) and high LAI (as the respiration rate exceeds the photosynthetic rate in lower leaves). In potato, optimal LAI was estimated on the basis of the relation between the net assimilation rate and LAI for different values of daily cumulative solar radiation (Taguchi et al., 1969). We obtained a light-response curve of photosynthesis for each cultivar (Fig. 3A) and investigated the relation between DM production per plant and solar radiation; we found a significant correlation between the recommended LAI at $800 \mu\text{mol}\cdot\text{mol}^{-1} \text{CO}_2$ and daily cumulative solar radiation indicating that the canopy photosynthetic rate was maximal (Fig. 3B). Based on this figure, the recommended LAI for each cultivar can be obtained at a certain daily solar radiation; seasonal and local values of daily solar radiation could be obtained from past meteorological data. Then, growers can obtain a strategy to manage LAI by leaf picking and increasing extra-stems. To maximize DM production in greenhouse tomatoes, growers could manage LAI appropriately on the basis of solar radiation and the function shown in Figure 3B. Since the photosynthetic curve differs depending on the CO_2 concentration, the recommended LAI would differ. Even if at the same solar radiation, the recommended LAI at a lower CO_2 concentration would be lower than that in Figure 3B. However, providing the recommended LAI would help to improve yield.

We concluded that individual leaf area and LAI, and therefore light intercepted by plants, could be estimated by the model described here. By using this model, LUE can be estimated as a function of daytime CO_2 . In this study, we were able to monitor DM production without destructive sampling because it was determined by the product of intercepted light and LUE. The recommended LAI to maximize DM production depends on solar radiation and could be determined on the basis of the photosynthetic curve for each cultivar. The developed model has good potential to improve yield and labor ef-

efficiency in tomato production in Japan. We will validate the model and improve yields in the near future.

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