

# Comparison of photosynthetic rates, transpiration rates, and total conductance of greenhouse-grown tomato plants measured with two open chambers with different ventilation rates

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## Abstract

A real-time monitoring system was developed and applied to monitor the time course of photosynthesis and transpiration in fully-grown tomato plants in a semi-commercial greenhouse. The system was based on an open chamber method in which the ventilator airflow rate is an important parameter affecting the environmental factors in the chamber and physiological response of plants enclosed inside the chamber. So, we assumed that the effects of this parameter on these responses should be evaluated for an agricultural production site. In this study, we investigated differences in the environmental factors in the chamber and physiological response of whole-tomato plants obtained from two chambers (0.5 m [W] × 1.0 m [D] × 2.2 m [H]) implemented with a single ventilator (SV, 0.36 m<sup>3</sup> min<sup>-1</sup>) or double ventilators (DV, 0.72 m<sup>3</sup> min<sup>-1</sup>). The relative humidity and vapor pressure deficit inside the SV chamber were about 10% higher and 0.5 kPa lower than those inside the DV chamber because of the difference in air exchange rates. However, we found no significant effect of airflow rate on net photosynthetic rate, transpiration rate and total conductance of the plants in the SV and DV chambers by analyzing with weighted Deming regression. This simultaneous monitoring method, undertaken in multiple chambers, and weighted Deming regression analysis can be used to check whether measurement conditions are appropriate for on-site monitoring.

**Key words:** Airflow rate, On-site evaluation, Open-bottom chamber, Simultaneous measurement, Weighted Deming regression

## Nomenclature

$P_N$	Net photosynthetic rate	[ $\mu\text{mol chamber}^{-1} \text{s}^{-1}$ ]
$E$	Transpiration rate	[ $\text{mmol chamber}^{-1} \text{s}^{-1}$ ]
$[\text{CO}_2]_{\text{in}}$	CO <sub>2</sub> concentration of the inflow air to the chamber	[ $\mu\text{mol mol}^{-1}$ ]
$[\text{CO}_2]_{\text{out}}$	CO <sub>2</sub> concentration of the outflow air from the chamber	[ $\mu\text{mol mol}^{-1}$ ]
$[\text{H}_2\text{O}]_{\text{in}}$	H <sub>2</sub> O concentration of the inflow air to the chamber	[ $\text{mmol mol}^{-1}$ ]
$[\text{H}_2\text{O}]_{\text{out}}$	H <sub>2</sub> O concentration of the outflow air from the chamber	[ $\text{mmol mol}^{-1}$ ]
$F$	Airflow rate	[ $\text{mol s}^{-1}$ ]
$VPD$	Water vapor pressure deficit	[kPa]
$g_t$	Total conductance to H <sub>2</sub> O	[ $\text{mmol mol}^{-1}$ ]
PPFD	Photosynthetic photon flux density	[ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]

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## Introduction

With the cost reduction of sensors and the spread of information and communication technology in recent years, the measurement of environmental information in commercial greenhouses has become prevalent. As the next step, the practical application of plant diagnostic techniques has begun to attract attention. In the past decade, many plant diagnostic techniques have been developed for commercial greenhouses (Science Council of Japan, 2011; Takayama, 2013) as second-generation Speaking Plant Approach (SPA) (Udink ten Cate *et al.*, 1978; Hashimoto, 1989) techniques. In plant diagnostic techniques, measurement of the photosynthetic rate is of vital importance in monitoring plant physiological status and performance in assimilation. In our previous study (Shimomoto *et al.*, 2020), we developed a real-time photosynthesis and transpiration monitoring system for greenhouse-grown tomato plants. The developed system successfully traced the time courses of net photosynthetic rate (dark respiration rate), transpiration rate, and total conductance under several conditions. It could also capture the stomatal closure and revealed the effect of supplemental



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lighting on the physiological response of the tomato plant.

In the open chamber method, the ventilator airflow rate ( $F$ ) of the air passing through the chamber is the most important parameter to determine the environmental factors in the chamber (Burkart *et al.*, 2007). By changing the  $F$ , numerous parameters including air temperature ( $T$ ), relative humidity ( $RH$ ), vapor pressure deficit ( $VPD$ ),  $CO_2$  concentration ( $[CO_2]$ ), and air current speed can be altered. These changes also affect the photosynthesis (Nilsen *et al.*, 1983; Kitaya *et al.*, 2003; Camejo *et al.*, 2005; Lu *et al.*, 2015) and transpiration (Kitaya *et al.*, 2003; Lu *et al.*, 2015) of the plant inside the chamber. Moreover, it is important to maintain a relatively high  $CO_2$  concentration difference between the inflow air and outflow air of the chamber ( $\Delta[CO_2]$ ) (Munakata, 1970; Miller *et al.*, 1996). Conversely, a sufficient  $F$  is required to avoid increasing  $T$  and  $RH$  inside the chamber during measurements and maintain conditions as close as possible to outside the chamber. The  $F$  should be appropriately balanced to account for these factors and allow for more accurate chamber operation (Ferrari *et al.*, 2016).

However, in the evaluation for the effect of  $F$  on photosynthesis, transpiration and total conductance in tomato plants cultivated inside the chamber, laboratory-based experiments can't reproduce the agricultural production sites, where various parameters interact and the environmental conditions change dynamically. There would be a significant heterogeneous distribution of environmental factors (Kuroyanagi, 2013; Yasutake *et al.*, 2014; Baxevanou *et al.*, 2018; Kimura *et al.*, 2020) and the crop photosynthesis in a single greenhouse (Kimura *et al.*, 2020). Furthermore, our previous report suggested that the photosynthesis functions of the same tomato cultivar grown in different greenhouses varied significantly (Takayama and Nishina, 2010). So, the on-site evaluation to see if the chamber airflow setting is appropriate would be realistic and we need to discuss the practical setting for the on-site measurement. And then, simultaneous measurement of several plants may be a potential solution.

The main objective of this study is on-site evaluation of the effect of the  $F$  on environmental conditions and physiological responses (photosynthesis, transpiration and total conductance) in tomato plants cultivated inside the chamber. To achieve this objective in a semi-commercial greenhouse, we simultaneously

monitored two pairs of tomato plants, cultivated almost homogeneously in two chambers with different airflow rates.

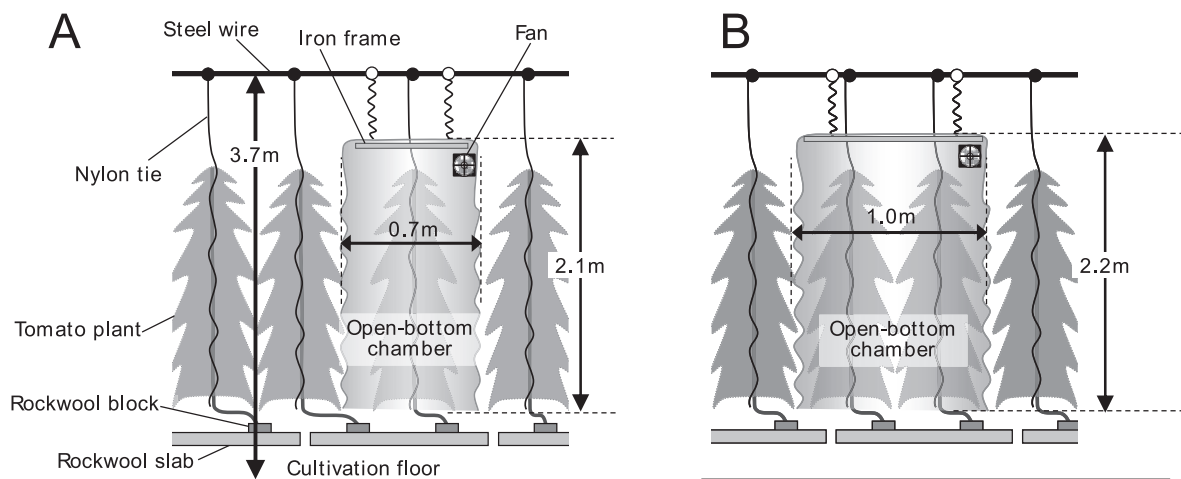
## Materials and methods

### Plant materials and instrumentation of the experimental greenhouse

Tomato (*Lycopersicon esculentum* Mill. 'Taiankitijitsu') plants were transplanted to rockwool cubes (Grodan Delta, GRODAN Group, Roermond, The Netherland, 100 mm [W] × 100 mm [H] × 65 mm [D]) on 18<sup>th</sup> August 2014 in a semi-commercial greenhouse (21 m [N-S] × 19.2 m [E-W] × 5.0 m [H]) oriented in a north-south direction at Ehime University (33°50 N, 132°47 E). The covering material of the greenhouse was glass and the roof ventilation was automatically conducted depending on the temperature inside the greenhouse. Four rockwool cubes were arranged on a rockwool slab (Grotop Expert, GRODAN Group, Roermond, The Netherland, 1.0 m [W] × 0.2 m [H] × 75 mm [D]) at an interval of 0.25 m. The nutrient water (A-type recipe of Otsuka House Solution, Otsuka Chemical Co. Ltd., Japan) was supplied to each cube through a dripper. Each plant was supported by attaching to a nylon tie connected to a steel wire settled above the tomato canopy and managed by routine crop maintenance operations once every week.

### Outline of the open-bottom chamber design

Fig. 1 shows a schematic diagram of the open-bottom chamber (B) scaled up (0.5 m [W] × 1.0 m [D] × 2.2 m [H]) compared to our previous prototype (A) (Shimomoto *et al.*, 2020) to enclose two fully-grown tomato plants, giving a higher  $\Delta[CO_2]$ . This method also considers the mutual overlapping of leaves in adjacent tomato plants (Fig. 2) and is closer to natural conditions. Romdhonah *et al.* (2021a, 2021b) and Isoyama *et al.* (2020) also adapted this scale-up and consideration. The bottom of the chamber is open to the greenhouse-air allowing air to enter from outside of the chamber. The chamber was composed of a transparent film supported by thin lightweight steel frames, as described in Shimomoto *et al.* (2020). The sensing unit for measuring  $[CO_2]$  and  $H_2O$  concentration ( $[H_2O]$ ) consisted of a portable photosynthesis system (LI-6400; LI-COR, Lincoln, USA). The LI-6400 has two



**Fig. 1.** Schematic diagram (A: previous version, B: present version) of the photosynthesis and transpiration monitoring system enclosing tomato plants.



Fig. 2. Mutual overlapping of leaves in adjacent tomato plants grown in greenhouses.

sets of CO<sub>2</sub> and H<sub>2</sub>O sensors and can use them at the same time. The one set of CO<sub>2</sub> and H<sub>2</sub>O sensors was exposed to the inflow air coming into the chamber (greenhouse air) sampled by using the air pump of the LI-6400. The another set of CO<sub>2</sub> and H<sub>2</sub>O sensors was exposed to the outflow air from the chamber sampled by using an air pump (EAP-01; AS ONE, Osaka, Japan) with a flow rate of 1000 ml min<sup>-1</sup>.

A ventilator (MB630-B, ORIENTAL MOTOR Co., Ltd., Tokyo, Japan) was equipped at the top of the chamber to exhaust the inside air, maintaining a stable vertical air current in the chamber. The airflow rate of the ventilator ( $F$ ) was 0.36 m<sup>3</sup> min<sup>-1</sup>, which corresponded to 0.33 air changes min<sup>-1</sup>.

#### Calculation of net photosynthetic rate, transpiration rate and total conductance

Net photosynthetic rate ( $P_N$ ) [μmol chamber<sup>-1</sup> s<sup>-1</sup>] and transpiration rate ( $E$ ) [mmol chamber<sup>-1</sup> s<sup>-1</sup>] were calculated using the following equations:

$$P_N = F_{\text{mol}} * ([CO_2]_{\text{in}} - [CO_2]_{\text{out}}) \quad (1)$$

$$E = F_{\text{mol}} * ([H_2O]_{\text{out}} - [H_2O]_{\text{in}}) \quad (2)$$

where  $F_{\text{mol}}$  is the airflow rate [mol s<sup>-1</sup>] of a single ventilator ( $F$  converted into mol s<sup>-1</sup> unit),  $[CO_2]_{\text{in}}$  is the  $[CO_2]$  of inflow air [μmol mol<sup>-1</sup>],  $[CO_2]_{\text{out}}$  is the  $[CO_2]$  of outflow air [μmol

mol<sup>-1</sup>],  $[H_2O]_{\text{in}}$  is the  $[H_2O]$  of inflow air [mmol mol<sup>-1</sup>],  $[H_2O]_{\text{out}}$  is the  $[H_2O]$  of outflow air [mmol mol<sup>-1</sup>]. The effect of the transpiration on the air volume is regarded as negligible since it is less than 1%.

Total conductance ( $g_t$ ) [mmol chamber<sup>-1</sup> s<sup>-1</sup>] assumes that the leaf temperature is equal to the air temperature. Thus, the  $g_t$  is calculated using the following equations:

$$W_s = 611 * 10^{\frac{7.493 * T_{\text{in}}}{237 + T_{\text{in}}}} * \frac{1000}{P} \quad (3)$$

$$W_a = W_s * \frac{RH_{\text{in}}}{100} \quad (4)$$

$$g_t = \frac{E}{W_s - W_a} \quad (5)$$

where  $T_{\text{in}}$  is the air temperature [°C] inside the chamber,  $P$  is atmospheric pressure (assumed to be constant at 1013.25 hPa),  $W_s$  is the saturated water vapor concentration [mmol mol<sup>-1</sup>] inside the chamber,  $RH_{\text{in}}$  is the relative humidity [%] inside the chamber and  $W_a$  is the water vapor concentration [mmol mol<sup>-1</sup>] inside the chamber. The  $g_t$  is composed of stomatal conductance and boundary layer conductance. The boundary layer conductance is assumed to be constant due to the stable air current inside the chamber. Therefore, the dynamic changes in  $g_t$  are regarded as changes in stomatal conductance.



## Experiments

Using two open-bottom chambers, a single ventilator chamber (SV-C<sub>A</sub>) and double ventilator chamber (DV-C<sub>B</sub>), we simultaneously monitored the changes in  $P_N$ ,  $E$ , and  $g_t$  of different two pairs of tomatoes cultivated almost homogeneously in the same greenhouse. The values of  $P_N$  and  $E$ , measured with the SV-C<sub>A</sub>, were calculated using Eq.1 and Eq.2. The values measured with the DV-C<sub>B</sub> were calculated using the following equations:

$$P_N = 2 * F_{mol} * ([CO_2]_{in} - [CO_2]_{out}) \quad (6)$$

$$E = 2 * F_{mol} * ([H_2O]_{out} - [H_2O]_{in}) \quad (7)$$

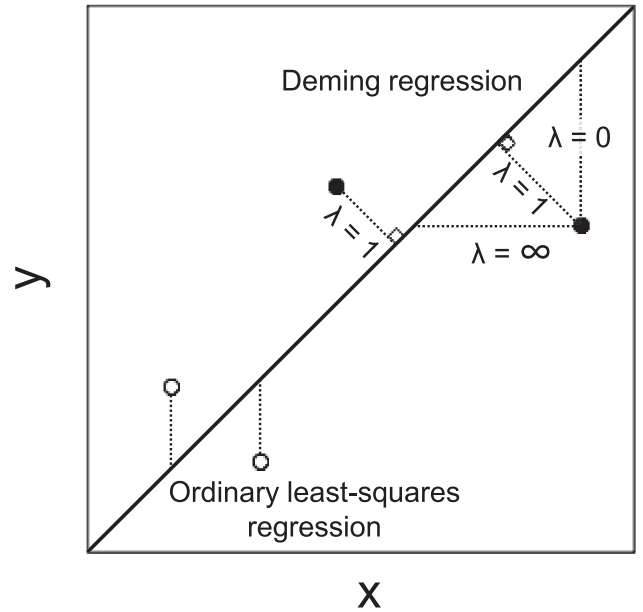
The two pairs of tomatoes measured in the chambers were located in two adjacent cultivation lanes. The measurement was conducted from 6:00 to 18:00 on a typical sunny day (29<sup>th</sup> June 2015).  $T$  and  $RH$  outside and inside two chambers were measured using three data loggers (Thermo Recorder Model TR-76Ui-H, T&D Corporation, Nagano, Japan) with a temperature-humidity sensor (SHA-3151, T&D Corporation, Nagano, Japan). Solar radiation was measured using a line photosynthetic photon flux density ( $PPFD$ ) sensor (SE-SQ31/S/S, Apogee Instruments Inc.) located above the chamber. In total, two LI-6400s were used in this experiment. The one was used for the measurement of  $[CO_2]_{in}$ ,  $[CO_2]_{out}$ ,  $[H_2O]_{in}$ ,  $[H_2O]_{out}$  of SV-C<sub>A</sub>. The another was used for that of DV-C<sub>B</sub>. The data acquisition rate was 5 min and the  $P_N$ ,  $E$  and  $g_t$  data points were calculated using a 15 min moving average.

## Statistical analysis

In this experiment, the dependence of the  $P_N$ ,  $E$  and  $g_t$  values on the type of chamber were analyzed using weighted Deming regression (Linnet, 1990) and correlation tests. Ordinary least-squares regression methods only consider measurement error for parameters on the y axis. In contrast, Deming regression (Deming, 1943) accounts for measurement errors for both parameters (Fig. 3). This regression technique is frequently used for studies comparing experimental methods. Weighted Deming regression is a weighted modification of the Deming method, presented by Linnet (1990) to consider nonconstant (proportional) measurement errors data recorded using different experimental methods. To check for the presence of proportional errors, we used Bland-Altman analysis (Bland and Altman, 1986). Statistical analysis was performed using the XLSTAT statistical software (Addinsoft, NY, USA).

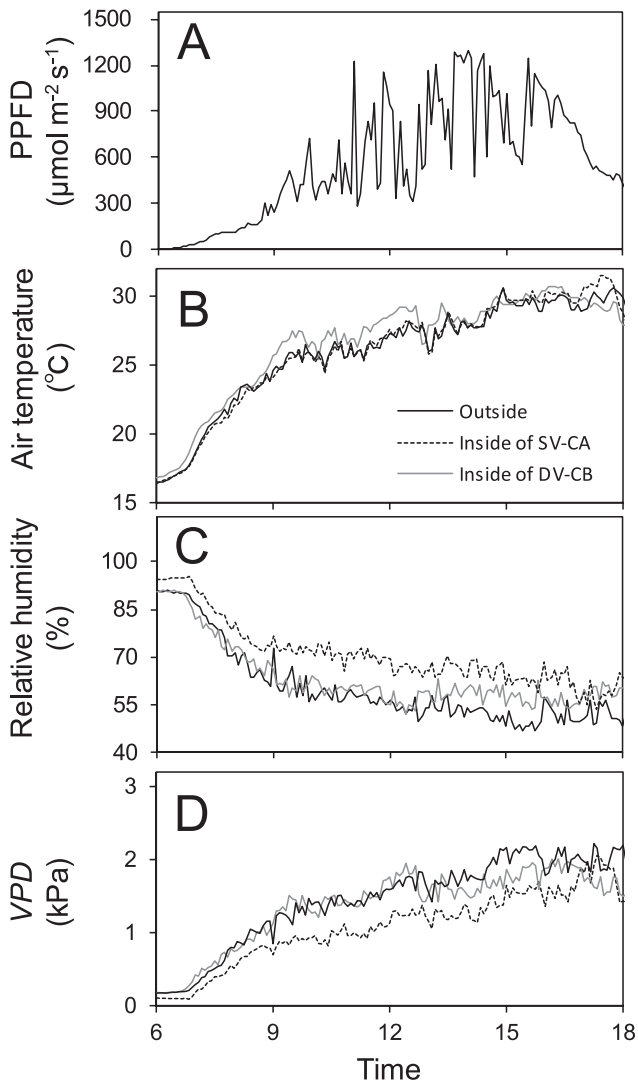
## Results and discussion

Fig. 4 shows the time course of  $PPFD$  (A), and changes in  $T$  (B),  $RH$  (C) and  $VPD$  (D) inside and outside of the chambers on a typical sunny day (29<sup>th</sup> June 2015). The  $PPFD$  steadily increased to  $900 \mu\text{mol m}^{-2} \text{s}^{-1}$  until 12:00 and maintained  $900 \pm 300 \mu\text{mol m}^{-2} \text{s}^{-1}$  until 17:00. The increase in solar radiation caused an increase in  $T$  and decrease in  $RH$  continuing until 18:00. Consequently, the  $VPD$  took values of up to approximately 2 kPa. The values of  $T$ ,  $RH$  and  $VPD$  were maintained from 17:00 to 18:00 regardless of the decrease in  $PPFD$ . This was most likely caused by the setting sun (solar radiation from the horizontal direction). The maximum  $T$  differences between the inside and outside of the SV-C<sub>A</sub> and



**Fig. 3.** The difference between ordinary least-squares regression (○) and Deming regression (●). The former projects the plots onto the line in the vertical to the abscissa direction, and the latter projects the plots onto the line at an angle determined by  $\lambda$  (the angle is  $90^\circ$  when  $\lambda$  is 1). Adapted from Linnet (1998).

DV-C<sub>B</sub> were  $2.0^\circ\text{C}$  and  $2.4^\circ\text{C}$  respectively. According to Ferrari *et al.* (2016) and Burkart *et al.* (2007), the  $T$  difference between the inside and outside of the photosynthesis chamber should be below  $5^\circ\text{C}$  to avoid an increase in evapotranspiration. Garcia *et al.* (1990) calculated the minimum  $F$  in order to limit the  $T$  increase inside the chamber to within  $5^\circ\text{C}$  with no evaporation. In case that the calculation apply to the our chambers, the minimum  $F$  was approximately  $2.5 \text{ m}^3 \text{ min}^{-1}$ . But, in this study, our developed system didn't need such a high  $F$ . Garcia *et al.* (1990) assumed that all  $500 \text{ W m}^{-2}$  of solar radiation was converted to sensible heat in their open chamber and, probably, they seemed to assume that the conversion into sensible heat mainly occurs on the surface of the ground (the basal of the chamber). However, our open-bottom chambers were kept off the ground and were likely almost unaffected by the sensible heat from the surface of the ground exposed to solar radiation. In addition, we could assume that two whole-tomato plants exposed to solar radiation didn't contribute to the  $T$  increase inside the chamber at least, because of transpiration cooling. Burkart *et al.* (2007) referenced the calculation of Garcia *et al.* (1990) for the determination of their  $F$ , and the  $T$  increase inside their chamber was about  $3.5^\circ\text{C}$  under the condition of  $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$   $PPFD$  and  $1 \text{ m}^2 \text{ m}^{-2}$  leaf area index ( $LAI$ ). In our study, we measured typical tomato plants grown in a commercial greenhouse ( $LAI > 1 \text{ m}^2 \text{ m}^{-2}$  at least) and used the open-bottom chambers which the occurrence of sensible heat on the bottom of the chamber would be negligible. Therefore, the  $T$  increase inside our chambers of about  $2^\circ\text{C}$  would be reasonable. And then, it was thought that we can limit the  $T$  increase inside our chamber to within  $5^\circ\text{C}$  if  $F$  is more than  $0.36 \text{ m}^3 \text{ min}^{-1}$ ,  $LAI$  is more than  $1 \text{ m}^2 \text{ m}^{-2}$  and  $PPFD$  is less than  $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ . On the other hand, from 9:00 to 15:00, the averaged  $RH$  and  $VPD$  differences between the outside and inside

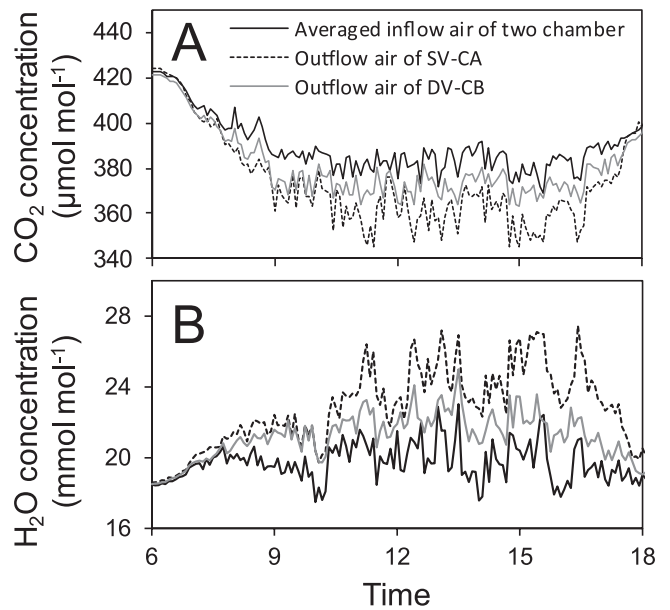


**Fig. 4.** Time course of  $PPFD$  (A), and air temperature (B), relative humidity (C) and  $VPD$  (D) inside of the chamber (SV- $C_A$ : dotted line, DV- $C_B$ : gray line) and outside (black line) on a typical sunny day (29<sup>th</sup> June 2015).

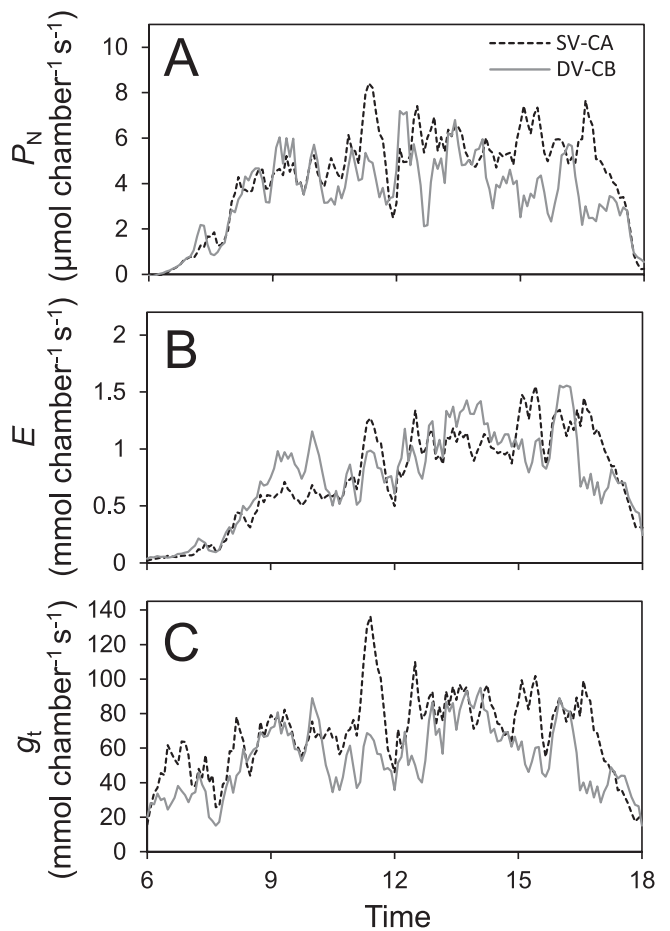
of SV- $C_A$  was 12.8% and  $-0.5$  kPa, and those of the DV- $C_B$  was 3.1% and  $0.0$  kPa. The increases in  $RH$  inside the chambers was due to transpiration of the plants in the chambers. Regarding the  $T$ ,  $RH$  and  $VPD$ , these results implied that the measurement with DV- $C_B$  ( $0.72 \text{ m}^3 \text{ min}^{-1}$ ) was carried out under conditions close to the outside of the chamber even on a typical sunny day.

Fig. 5 shows the changes in  $[CO_2]$  (A) and  $[H_2O]$  (B) of inflow air and outflow air of the chambers. From 9:00 to 15:00, the  $[CO_2]_{\text{out}}$  of SV- $C_A$  was maintained at  $350\text{--}370 \mu\text{mol mol}^{-1}$ , in contrast, that of DV- $C_B$  was maintained at  $360\text{--}380 \mu\text{mol mol}^{-1}$ . Therefore the plants enclosed by SV- $C_A$  were exposed to air of lower  $[CO_2]$  and higher  $[H_2O]$  than the plants enclosed by DV- $C_B$  because of the low  $F$ . Also, from 9:00 to 15:00,  $\Delta[CO_2]$ s of SV- $C_A$  and DV- $C_B$  would be kept within a practical range expected in a commercial greenhouse. So,  $F_s$  of both chambers would be within a practical range to the measurement of the photosynthetic rate of greenhouse-grown tomato plants.

Fig. 6 shows the changes in  $P_N$  (A),  $E$  (B) and  $g_t$  (C) measured by SV- $C_A$  and DV- $C_B$ . The diurnal variations of each parameter



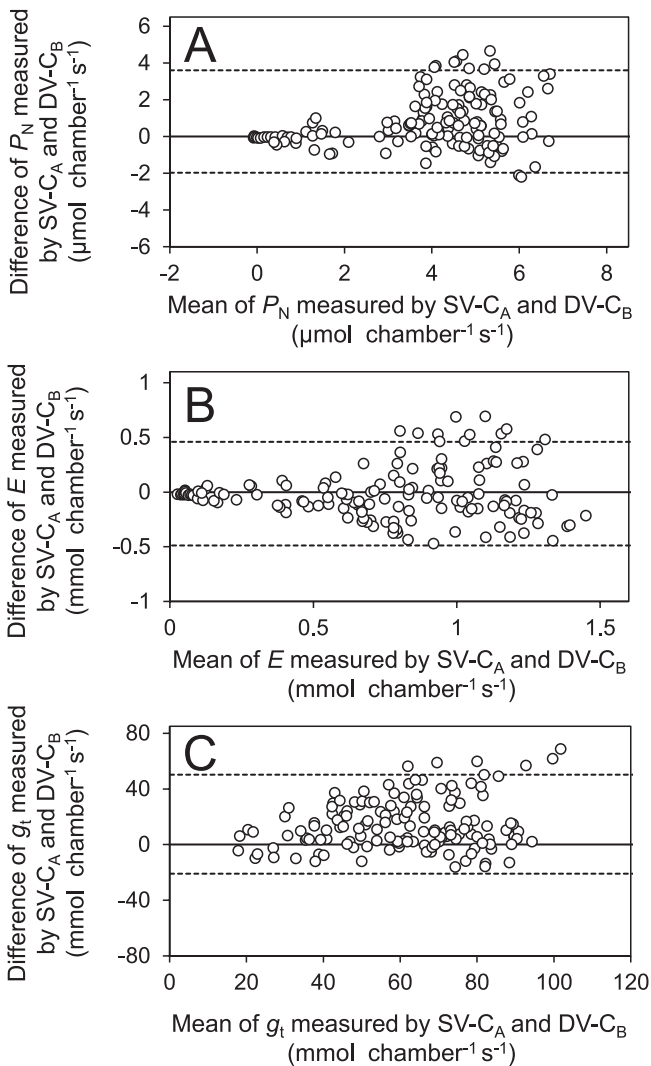
**Fig. 5.** Time course of the  $CO_2$  concentration (A) and  $H_2O$  concentration (B) of inflow air (black line) and outflow air of the chambers (SV- $C_A$ : dotted line, DV- $C_B$ : gray line) on a typical sunny day (29<sup>th</sup> June 2015).



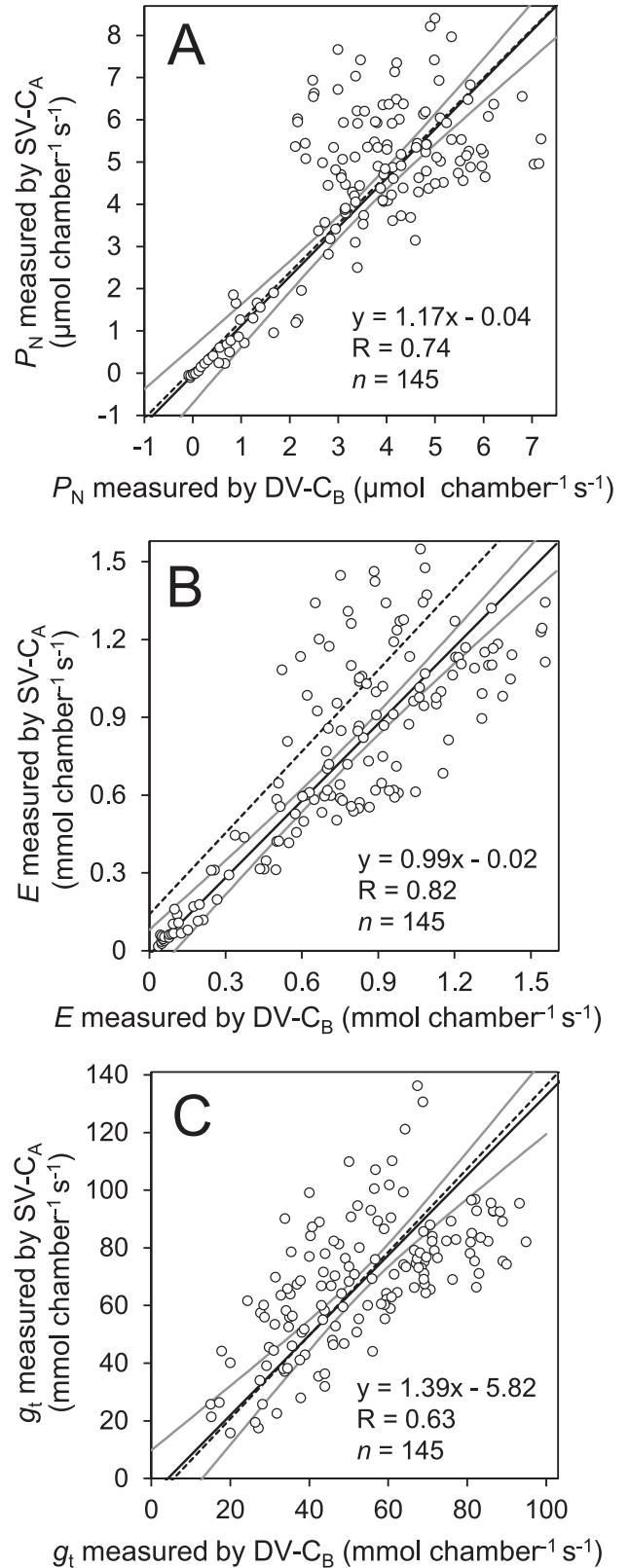
**Fig. 6.** Time course of the  $P_N$  (A),  $E$  (B) and  $g_t$  (C) measured by SV- $C_A$  (dotted line) and DV- $C_B$  (gray line) on a typical sunny day (29<sup>th</sup> June 2015).

measured in the two chambers were almost the same. All parameters measured by the two chambers increased gradually from 6:00 to 10:00, and then fluctuated intensely within a certain range from 11:00 to 17:00. The higher values of  $P_N$ ,  $E$  and  $g_t$  measured by SV-C<sub>A</sub> were recorded at specific times (just before 12:00, just after 15:00, and just before 17:00). However, the higher measured value continuously alternated between the two chambers, and the precise relationship was not clear.

Fig. 7 shows the relationship of  $P_N$  (A),  $E$  (B) and  $g_t$  (C) of the tomato plants enclosed in the two chambers determined using Bland-Altman analysis. This analysis method plots the difference of parameters measured by two methods against the average level to check for the existence of proportional errors or bias (the mean difference between the two methods). In all parameters, these plots suggested proportional errors. Thus, the relationships were analyzed with weighted Deming regression (Fig. 8). This increase in the error at higher values is most likely owed to intense fluctuations in solar radiation and ventilation of the greenhouse during the daytime (from 11:00 to 17:00). The ratio between the squared analytical standard deviations (variance) for the two



**Fig. 7.** Bland-Altman analysis plots of  $P_N$  (A),  $E$  (B) and  $g_t$  (C) measured in the SV-C<sub>A</sub> and DV-C<sub>B</sub> on a typical sunny day (29<sup>th</sup> June 2015). The dotted lines represent a 95% confidence interval.



**Fig. 8.** The relationship between the  $P_N$  (A),  $E$  (B) and  $g_t$  (C) values measured by SV-C<sub>A</sub> and DV-C<sub>B</sub> on a typical sunny day (29<sup>th</sup> June 2015). The solid lines represent the regression line. The dotted lines represent the slope of results measured in the same ventilator conditions on the following day (30<sup>th</sup> June 2015). The slopes obtained from regression analysis for  $P_N$  (A),  $E$  (B) and  $g_t$  (C) are 1.15, 0.98 and 1.45, respectively. The gray lines represent a 95% confidence interval for the fit.

measurements ( $\lambda$ ) was assumed to be 1 (Linnet, 1993; Linnet, 1998). The correlation coefficients between  $P_N$  (A),  $E$  (B) and  $g_t$  (C) measured by the two chambers were 0.74, 0.82 and 0.63 ( $p < 0.01$  in all parameters), with the slopes of 1.17, 0.99 and 1.39, given by the regression analysis respectively. Incidentally, in the measurements taken on the next day (30<sup>th</sup> June 2015), we monitored  $P_N$ ,  $E$  and  $g_t$  of the same two pairs of tomato plants using two chambers with a single ventilator (SV- $C_A$  and SV- $C_B$ ). The correlation coefficients between  $P_N$ ,  $T$ , and  $g_t$  measured by the two chambers were 0.92, 0.79 and 0.58 ( $p < 0.01$  in all parameters) with the slopes of 1.15, 0.98 and 1.45, determined by the regression analysis respectively (Fig. S1). Each dotted black line in Fig. S1 was transcribed to Fig. 8. In Fig. 8, the solid black line shows the relationship between measurements taken in the SV- $C_A$  and DV- $C_B$ . Dotted lines show the relationship between measurements conducted in the SV- $C_A$  and SV- $C_B$ . Fig. 8 shows the solid black lines for each parameter partially overlap with dotted lines obtained comparing measurements both taken with single ventilators, as shown in Fig. S1. Also, in all parameters, the solid black lines were within the range of a 95% confidence interval. These results indicate that  $F$  does not have a significant effect on  $P_N$ ,  $E$  and  $g_t$ . In Fig. S1-B, points at higher  $E$  values showed significant deviations from the regression line. These large deviations are thought to be caused by pulses of inflow air with high  $[H_2O]$  entering into the SV- $C_A$  between 10:00 and 12:00 (Fig. S2) due to local humidification or ventilation. The influence of these factors was not as large for the weighted Deming regression analysis because the error is underestimated at higher values.

We can also infer that variations in the environmental factors in the chamber ( $RH$ ,  $VPD$ ,  $[CO_2]$  and air current speed) derived from low  $F$  did not affect the physiological response. As mentioned above, the  $RH$  and  $VPD$  inside the SV- $C_A$  were apparently different from the outside of the chamber. Besides, from 9:00 to 15:00, the  $RH$  and  $VPD$  were about 10% higher and 0.5 kPa lower respectively compared with that of the DV- $C_B$ . Nevertheless, the differences were not large enough to affect the  $E$  and  $g_t$  of tomato plants inside the chamber in this short-term experiment. Lu *et al.* (2015) reported that the  $P_N$ ,  $E$  and stomatal conductance of greenhouse-grown tomatoes under long-term fogging ( $VPD = 0.8$  kPa) are significantly higher than those without fogging ( $VPD = 1.4$  kPa). Furthermore, many other reports have stated that a long-term difference in  $VPD$  affects parameters such as tomato yield (Bertin *et al.*, 2000), water uptake (Trigui *et al.*, 1999) and leaf area (Gautier *et al.*, 1999). Therefore, effects of  $F$  on  $E$  and  $g_t$  of tomato plants inside the chamber may become apparent for long-term monitoring. The effect of  $10 \mu\text{mol mol}^{-1}$   $[CO_2]$  difference between the two chambers on  $P_N$  was thought to be sufficiently small. Kitaya *et al.* (2003) reported that the leaf boundary-layer resistance of sweet potato leaves decreased significantly as the air current speed increased from  $0.01$  to  $0.2 \text{ m s}^{-1}$ . However, in this experiment, the effect of different air current speeds on  $g_t$  measured by the two chambers seemed to be small. This is likely due to the extremely low speeds of the air current inside both chambers, calculated as  $F$  per ventilator ( $0.012 \text{ m s}^{-1}$  air current speed per ventilator). Kono (1987) reported that most areas in a target horticultural facility growing full-size tomato plants

(LAI = 3.5) are close to windless (less than  $0.05 \text{ m s}^{-1}$  under the condition of natural ventilation and  $1.0 \text{ m s}^{-1}$  external wind speed). There has also been a report that the air current speed in the greenhouse depends on LAI and the external wind speed (Wang *et al.*, 1999). Based on these reports, it may be better to consider the chamber air current speed as a condition that can significantly affect the physiological response of tomato plants.

In this experiment, we investigated the effect of  $F$  on the environmental factors in the chamber and plants inside an on-site monitoring chamber. This experiment was performed by concurrently monitoring two pairs of tomatoes using two chambers with different ventilator airflow rates. To analyze the dependence of  $P_N$ ,  $E$  and  $g_t$  on the airflow, we employed weighted Deming regression. This simultaneous monitoring method, undertaken in multiple chambers, and weighted Deming regression analysis can be used to ascertain whether measurement conditions are appropriate for on-site monitoring. Therefore, this method could be useful for the application of our chamber system in commercial cultivation systems.

In this paper, we discussed the practical setting of the on-site measurement, as the first step for the practical use of this system in the agricultural production site. As the second step, we need to examine the possibility of 1) dynamic control of  $F$  according to air temperature or  $RH$  inside the chamber, 2) the difference in the location and structure (glass or vinyl in covering material, high or low in height of the ridge, etc.) of greenhouse, 3) the difference in the growing position within a greenhouse, 4) the difference in growing season and so on. Moreover, to spread this system widely, we also need to discuss the utilizing methods, such as the plant diagnosis by referencing past data obtained by this system and the control of fogging system by detecting the stomatal closure in the various situations. In late years, the smart agriculture demonstration project has been being promoted in our country and we have already started verifying the system under the various conditions throughout the project. Therefore, it is expected that the knowledge about the utilization of the monitoring system is the accumulated by the verification of the system under the various environmental conditions.

## Conclusion

In this study, we conducted simultaneous measurements of the  $P_N$ ,  $E$  and  $g_t$  of tomato plants with two open-bottom chambers and weight Deming regression analysis. We evaluated the on-site effect of  $F$  on environmental conditions and physiological responses of tomato plants inside the chamber. As a result, the  $RH$  and  $VPD$  inside SV- $C_A$  ( $0.36 \text{ m}^3 \text{ min}^{-1}$ ) was about 10% higher and 0.5 kPa lower than those inside the DV- $C_B$  ( $0.72 \text{ m}^3 \text{ min}^{-1}$ ). However, the effect of climatic differences on  $P_N$ ,  $E$  and  $g_t$ , arising from variations in  $F$ , was found to be negligible for short-term experiments. Additionally, in DV- $C_B$ , the  $T$ ,  $RH$  and  $VPD$  differences between the inside and outside of the chamber were sufficiently small. This result indicates that the chamber measurements were conducted under conditions equivalent to the conditions outside of the chamber. We have demonstrated that, through simultaneous measurements in multiple chambers and the application of weighted Deming regression, appropriate measurement conditions can be determined for plants monitored



on agricultural production sites. The results of this analysis provide insight into the applicability of our chamber system in commercial greenhouses. As a next step, throughout the verification of the system under the various environmental conditions, the accumulation of the knowledge about the utilization of the monitoring system is expected.

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