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REVIEWS OPEN ACCESS

The Essential Factor of Ventilation Rate in Prediction of Photosynthetic Rate Using the CO_2 Balance Method

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ABSTRACT

Monitoring photosynthesis is a fundamental process to improve the yield and quality of plants in a greenhouse. The CO_2 balance method is often employed to predict the photosynthetic rate of plants. We reviewed the essential parameters for predicting photosynthetic rates of plants canopy in greenhouses using the CO_2 balance method. Even in a naturally ventilated greenhouse, ventilation rate is an essential parameter for the CO_2 balance method, but it must be measured in real time as it fluctuates with weather conditions. We studied three types of ventilation rates (the tracer gas, heat balance, and water vapor balance methods). Comparing the measuring techniques of ventilation rate provided us an understanding of the strengths and weaknesses of each method. This knowledge can guide us to choosing the best method based on accuracy, device usage, practicality, and the installation budget. Most researchers have measured and controlled CO_2 concentrations in a greenhouse using an infrared gas analyzer and predicted the ventilation rates using the tracer gas method. This method is suitable for the measurement of low and closed ventilation. The estimated ventilation rate by the heat balance method is recommended for large ventilation openings. The water vapor balance method is sufficient for measuring the ventilation rate when there is a large quantity of water vapor due to plant transpiration. The reliability of this method depends on the accuracy of short-term transpiration measurements. Improved water vapor balance techniques can benefit various greenhouse applications with different ventilator configurations, owing to the flexibility and ease of use compared to those of other methods.

Keywords

heat balance, photosynthesis, tracer gas, ventilation rate, water vapor balance

1. Introduction

Photosynthesis is crucial for increasing the yield and quality of crops; therefore, a thorough understanding of photosynthesis measurement in a greenhouse environment merits special attention. Photosynthetic process responds immediately to changes in the greenhouse climate, such as alterations in light intensity, air temperature, and CO_2 concentration. Monitoring is required to determine whether the greenhouse climate is suitable to the needs of the crop. Real-time monitoring of net photosynthesis of a plant canopy in a greenhouse is crucial to elucidate and improve plant environments (Takakura *et al.*, 2017). Leaf photosynthesis is measured by determining the CO_2 uptake of a leaf using a portable gas exchanger device (Hao *et al.*, 2008). The leaf chamber method has a high accuracy level as it controls the environment (light intensity, air temperature, humidity, CO_2 concentration, and airflow rate) of the space where leaves are enclosed. However, the inner chamber is not always identical to the greenhouse environment, and is not suitable for long-term continuous measurement. To measure photosynthesis of an entire

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plant, Shimomoto *et al.* (2020) developed an open chamber method, using a transparent film supported by thin and light-weight steel frames and two ventilation fans, that measure photosynthesis and transpiration in a semi-commercial greenhouse.

There are several methods to measure photosynthesis, such as the dry matter accumulation method (Lawlor *et al.*, 1981), manometric method (Hunt, 2003), gas exchange method (Schulze, 1972; Takahashi *et al.*, 2001), and phyto-monitoring application method (Dieleman *et al.*, 2017). All forms of measurement had been reviewed for their advantages and disadvantages by Millan-Almaraz *et al.* (2009). The gas exchange method is the most utilized measurement in individual leaves, whole plants, and plant canopy for commercial equipment and experimental set-up (Schulze, 1972; Millan-Almaraz *et al.*, 2009). The gas exchange method is a suitable and convenient method for greenhouses as CO_2 exchange can be readily measured.

The CO_2 concentrations, both in the greenhouse and chamber, are determined by the gas supply from the CO_2 generator, soil respiration, the photosynthesis/respiration reaction of plants, gas movement in the greenhouse due to ventilation, and exterior CO_2 concentration level. These relationships are represented by a CO_2 balance equation, which is used to calculate the photosynthetic rate in the chamber method. However, when the photosynthetic rate prediction by the CO_2 balance method is applied to a naturally ventilated greenhouse, the ventilation rate becomes an unknown parameter as it fluctuates repeatedly depending on natural conditions. There are several techniques to predict the ventilation rate, such as tracer gas, heat balance, and water vapor balance techniques. If we could measure the parameters of the ventilation rate calculation simultaneously with the parameters of the CO_2 balance equation, it would be possible to continuously monitor the photosynthetic rate of plants, even in a naturally ventilated greenhouse.

The air exchange rate (or ventilation rate) is an important parameter of the CO_2 balance method and subsequently affects the CO_2 concentration. Accurate gas exchange rate measurements improve the accuracy of photosynthetic rate estimation. This review focuses on the photosynthetic rate measurements using CO_2 gas exchange at the greenhouse scale (including the physical and technical aspects); it also evaluates and compares the ventilation rates across tracer gas, heat balance, and water vapor balance techniques. A sufficiently detailed summary of the literature addressing photosynthesis measurement in greenhouses based on the CO_2 gas exchange and ventilation rate method is provided.

2. Measurement of photosynthetic rate in a greenhouse

A photosynthesis/transpiration measuring system (LI-6400XT, LI-6800 from LI-COR Co., USA) is commonly used to measure leaf photosynthetic rates on a fully expanded foliage. This system can control light, temperature, humidity, and CO_2 concentration in the leaf chamber (Hao *et al.*, 2008; Thwe *et al.*, 2014; Albert *et al.*, 2017). The portable photosynthetic device, LI-6400XT, measures the gas exchange of a leaf specimen or a sample isolated in a closed chamber; thus, CO_2 exchange can be measured (Schulze, 1972; Takahashi *et al.*, 2001). There are two types of gas exchange chambers: a closed chamber (where the sample is completely enclosed), and an open chamber (where air can freely enter and leave the chamber), as reported by Hunt (2003). Measurement with a portable device is done manually and does not allow on-line monitoring of crop photosynthesis (Dieleman *et al.*, 2017). For whole plant photosynthesis monitoring, Shimomoto *et al.* (2020) developed a system with an open chamber in a greenhouse to evaluate the dynamic changes in photosynthesis of crops. This system used a transparent film to cover the crops that would possibly impact the different environments inside an open chamber with the outer chamber environmental conditions. Schulze (1972) and Millan-Almaraz *et al.* (2009) reported that the gas exchange method is suitable for estimating photosynthesis in a greenhouse environment. Thus, this paper focuses only on photosynthesis measurements using the gas exchange method.

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The net canopy photosynthesis of crops measurement had been described in detail by Hand (1973a) under a daylight controlled-environment cabinet. In addition, Hand (1973b) reviewed many different methods of gas exchange measurement in which the plant enclosures can be of open, closed, or mixed systems. Furthermore, Lake et al. (1968) had applied an open system with null point compensation for CO_2, whereas the CO_2 concentration inside the greenhouse was maintained at almost the same level as the CO_2 level outside (or $\Delta CO_2 \approx 0$). Generally, the CO_2 gas exchange method was used to measure photosynthesis based on CO_2 concentration changes both inside and outside the greenhouse. It is also known as the CO_2 balance method, as presented in Eq. 1. CO_2 greenhouse fluxes are based on ventilation, CO_2 supply, CO_2 from soil respiration, changes in the greenhouse CO_2 concentration, and net photosynthesis.

$$\frac{V}{wA}\frac{dC_{in}}{dt} = I + S + \frac{G}{w}(C_{out} - C_{in}) - P_n \qquad (1)$$

where V and A are, respectively, the volume (m^3) and the cultivable ground area (m^2) of the greenhouse; dC_{in}/dt is the time change of CO_2 concentration in the greenhouse (μmol mol^{-1} min^{-1}); w is the molar volume of standard air (≈0.0224 m^3 mol^{-1} at 20 °C, 101.325 kPa); I is the CO_2 injection flux or supply (μmol m^{-2} min^{-1}); S is the CO_2 output from soil respiration (μmol m^{-2} min^{-1}); G is the ventilation flux (m^3 m^{-2} min^{-1}), C_{in} is the CO_2 concentration inside the greenhouse (μmol mol^{-1}); C_{out} is the concentration of CO_2 outside the greenhouse (μmol mol^{-1}); and P_n represents the net photosynthetic rate per floor area (μmol m^{-2} min^{-1}).

Several authors considered that the difference between CO_2 concentration inside and outside the greenhouse was null, and that the CO_2 respiration from soil was negligible due to plastic covering (Hand, 1973a; Hand *et al.*, 1992; Zekki *et al.*, 1999). When the null balance concept was applied (Hand, 1973a), the CO_2 concentration in the greenhouse was maintained at the same level as the ambient CO_2 concentration outside (around 350–380 μmol mol^{-1}), and the effect of the CO_2 exchanges inside and outside the greenhouse was minimized.

The net photosynthesis equation can be determined based on greenhouse CO_2 concentration and the injected CO_2 in the system. When the ventilation rate (or leakage) and the soil respiration are negligible (G, $S \approx 0$), the net photosynthesis rate is calculated by modifying Eq. 1 as follows;

$$P_n = I - \frac{V}{wA}\frac{dC_{in}}{dt} \qquad (2)$$

An infrared gas analyzer (IRGA) was used to measure the CO_2 concentrations of samples taken using a nylon tube placed within the canopy at mid-height in the greenhouse (Hand *et al.*, 1992; Zekki *et al.*, 1999). Figure 1 shows a schematic diagram of the CO_2 measurement, control, and distribution systems, as reported by Hand *et al.* (1992). Based on the equation of the CO_2 balance methods (Eq. 1) and a schematic diagram of the CO_2 measurement and control (Figure 2), there are three critical parameters: 1) changes in CO_2 concentration, 2) control of CO_2 supply, and 3) ventilation rate. The CO_2 concentration is controlled to an ambient level. The amounts of CO_2 used in the canopy zone were measured with linear mass flowmeters. The ventilation rate factor was negligible in Hand *et al.* (1992), because of the CO_2 concentration being maintained at the same level both inside and outside the greenhouse. In contrast, the ventilation rate should be added to the CO_2 balance calculation at a moderate or high ventilation rate condition, as given by Nederhoff *et al.* (1989), Ehler (1991), and Chalabi and Fernandez (1994) confirming that the ventilation rate is significant for predicting photosynthetic rate based on the CO_2 balance method. This is especially true during the late spring and summer seasons when there is high solar radiation and windows are therefore opened for temperature control.

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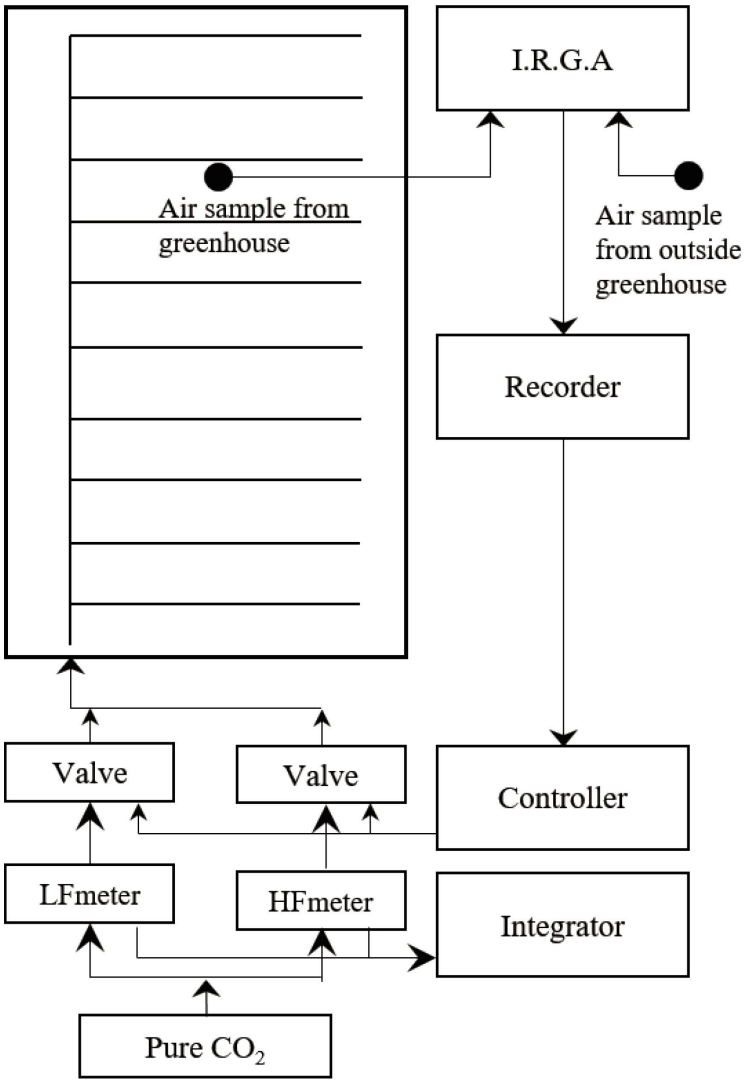

Figure 1: Schematic diagram of the CO_2 measurement, control and distribution using an infrared gas analyzer, IRGA (Hand *et al*., 1992). CO_2 supplied from the cylinders was metered through linear mass flowmeters (Teledyne Hastings-Raydist type HS) with the ranges 0–20 g CO_2 min^{-1} (low flowmeter, LF) and 0–60 g CO_2 min^{-1} (high flowmeter, HF).

Table 1 shows the photosynthetic rate in a greenhouse calculated based on a greenhouse climate system (Nederhoff *et al.*, 1989; Gijzen *et al.*, 1990; Chalabi and Fernandez, 1994; Zekki et al., 1999; Zhang and Wang, 2011; Wang *et al.*, 2013) and plant monitoring (Dieleman *et al.*, 2017). Studies have shown that ventilation rate can be calculated as an unknown parameter in the CO_2 balance equation, using the tracer gas technique with N_2O (Nederhoff *et al.*, 1989; Gijzen *et al.*, 1990; Ehler, 1991). They determined the ventilation rate using N_2O tracer gas under leakage and a small window aperture (therefore a low ventilation rate). Furthermore, Takakura *et al.* (2017) calculated the canopy photosynthesis based on the CO_2 balance and the ventilation rate using the energy balance technique under a high ventilation rate condition.

Dieleman *et al.* (2017) conducted plant monitoring based on crop measurement techniques using the Crop Photosynthesis Monitor (for measuring crop photosynthesis) and the CropObserver (for measuring

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photosynthetically active radiation and fluorescence of a large area of the crop). These monitoring systems provided reasonable estimations of crop photosynthesis when compared with those of the crop growth model (Marcelis *et al.*, 2000) at a small-scale interval measurement (15 min). However, further development of these systems is required before they will be incorporated into greenhouse climate systems.

Table 1: Summary of reviewed literature suggesting photosynthesis rate measurement and simulation in the greenhouse system from 1989 to 2017

Year	Photosynthesis (measurement/model)	Measurement methods of ventilation rate	References
1989	Dynamic simulation model using the CO_2 balance method	Tracer gas using N_2O	Nederhoff *et al.* (1989)
1990	Measurement of photosynthesis using a CO_2 balance method every 30 min	Tracer gas using N_2O	Gijzen *et al.* (1990)
1991	An hourly net photosynthesis measurement using CO_2 balance method at a low ventilation rate	Tracer gas	Ehler (1991)
1994	Estimation of canopy photosynthesis based on CO_2 balance method	The using model developed by Fernandez and Bailey (1992); function of wind speed and the opening angle	Chalabi and Fernandez (1994)
1999	Measurement of net photosynthetic rate using the CO_2 balance method	Negligible (null CO_2 balance)	Zekki *et al.* (1999)
2017	Monitoring of a plant canopy using CO_2 balance technique	Energy balance method	Takakura *et al.* (2017)
1991	The black box model of the photosynthesis simulation model	-	Ehler (1991)
1994	Simulation of canopy photosynthesis based on mechanistic models (Acock model, and Challa and Schapendonk model)	-	Chalabi and Fernandez (1994)
1999	Simulation of photosynthesis using the TOMGRO model which integrates Acock's model	-	Zekki *et al.*, (1999)
2011	Simulation of photosynthesis based on simple leaf photosynthesis after the careful consideration of leaf area index and light density distribution in the greenhouse and considered the influence of the environment (temperature, CO_2 concentration, and moisture).	-	Zhang and Wang (2011)
2013	Model of monitoring photosynthesis based on CO_2 concentration and photosynthesis data measurements analyzed using the back-propagation neural network model.	-	Wang *et al.* (2013)

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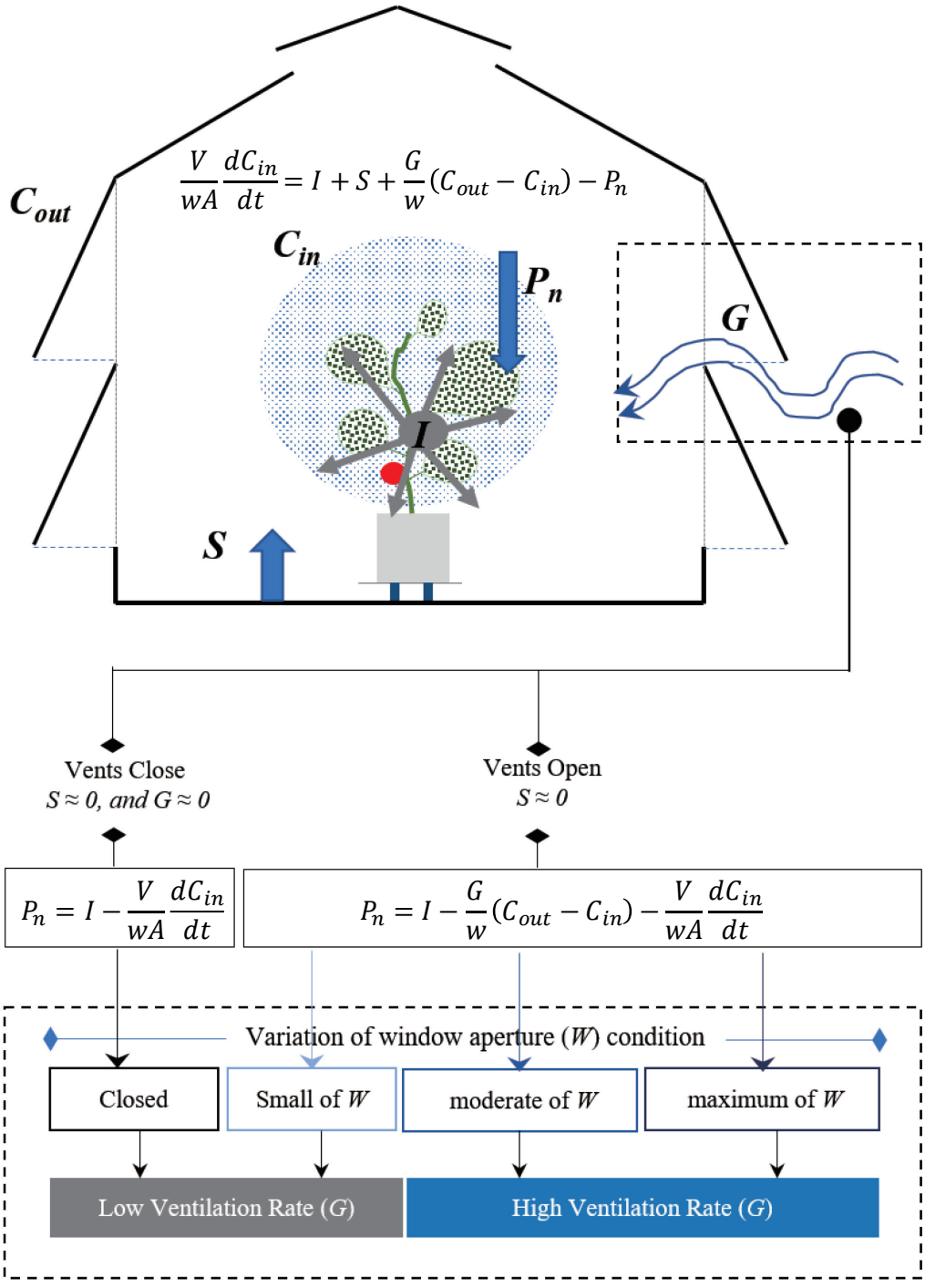

Figure 2: Schematic diagram of the CO_2 balance method with CO_2 supply (I) in a greenhouse for estimating the photosynthetic rate (P_n). The changes of concentration between outside and inside ($C_{out} - C_{in}$) and the changes of inside concentration during the time of measurement (dC_{in}) in total volume (V) per floor area of greenhouse (A), are affected by window aperture (W) condition, as presented by ventilation rate value (G). Assuming the CO_2 release from the soil was zero ($S \approx 0$) due to being covered by plastic and using the hydroponic cultivation method (with soilless culture).

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3. CO_2 measurements in greenhouse

CO_2 fertilization effectively promotes photosynthesis in cases where the CO_2 concentration in the community is higher than that in the outside air. CO_2 depletion of 5–10% below ambient is typical for an adequately ventilated greenhouse on warm and sunny days. CO_2 concentration can further decrease in winter and spring due to closed windows in greenhouses (Hand, 1985); hence, it is crucial to measure the CO_2 concentration accurately. Accurate CO_2 concentration data in a greenhouse is critical for controller design and actuator control. Many growers operate the actuators based only on their experience (Rodríguez *et al.*, 2015). The development of sensor technology has allowed the objective monitoring of greenhouse parameters, but accurate measurements of CO_2 concentration are difficult because CO_2 is not homogenous in the greenhouse.

The most common equipment used for CO_2 measurement is the infrared gas analyzer (IRGA) (Hand, 1973a; Nederhoff *et al.*, 1989; Ehler, 1991; Chalabi and Fernandez, 1994; Zekki *et al.*, 1999). This sensor uses an infrared emitter-photodetector pair whose light beam measures the concentration of gas molecules in the air. In addition to using the IRGA, the CO_2 concentration can be determined using a simple CO_2 engine K30 sensor (Senseair AB Co., Sweden), as shown by Takakura *et al.* (2017).

In a CO_2 monitoring system, the spatial distribution of CO_2 concentration, which is relevant to crop growth, needs to be considered (Li *et al.*, 2018). Analyzing this spatial distribution is complex and requires a specific tool. Computational fluid dynamics (CFD) involves simulating flow fields based on the conservation equations of mass and energy. It is suitable for analyzing non-uniform data and has been used successfully in prior studies. CFD can also explore the CO_2 distribution within a greenhouse for optimal control (Boulard *et al.*, 2017).

The differences in vertical CO_2 distribution are not significant in a greenhouse (Li *et al.*, 2018). Therefore, CO_2 sensors are placed in locations where they are easy to operate. However, in some sophisticated greenhouses, horizontal or vertical changes in CO_2 concentration were complex. In general, the vertical distribution of CO_2 concentration is low in the canopy, while it is high air above the canopy and the soil surface (Chalabi and Fernandez, 1994; Kim *et al.*, 2015). The horizontal CO_2 concentration at the height of the canopy was relatively uniform. Therefore, the sensor is commonly placed approximately at the height of the crop canopy (Li *et al.*, 2018).

The control of CO_2 was actuated by a valve for injecting CO_2 fertilizer in a short time to maintain the desired CO_2 concentration level. Then, the canopy photosynthesis rate using the CO_2 balance can be predicted with a short-time interval calculation (Figure 3). Studies have calculated the photosynthetic rate between and 5–15 min (Ehler, 1991; Chalabi and Fernandez, 1994; Zekki *et al.*, 1999) based on the CO_2 balance method and tracer gas analysis (Figure 3). Takakura *et al.* (2017) calculated the canopy photosynthesis every minute using the CO_2 balance and the energy balance method. However, there was a problem with scattered data due to frequent changes in the ventilation amount. Advanced technology is needed to control the CO_2 concentration supply based on real-time CO_2 concentration conditions, ventilation rate prediction, and photosynthesis calculations.

Li *et al.* (2018) categorized various control methods for CO_2 concentration: conventional control (ON-OFF control), proportional-integral-differential (PID) control; modern control (non-linear, robust, and optimal control); intelligent control (fuzzy, neural network control); hybrid control (fuzzy PID, neural network PID, fuzzy neural network) and others such as CFD and machine vision. Lee *et al.* (2000) conducted a PID algorithm in a tunnel-type chamber to maintain the temperature gradient from the inlet to the outlet by automatic ventilation rate control.

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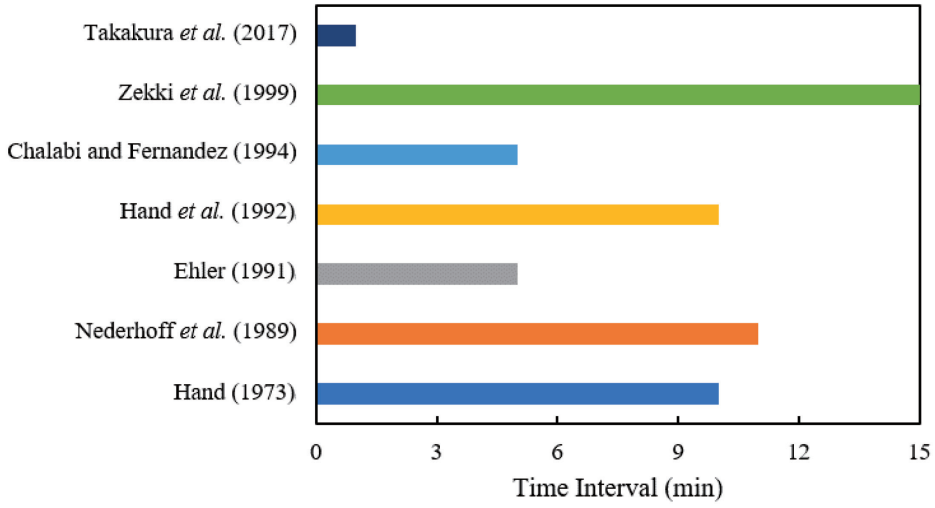

Figure 3: The interval time of photosynthetic rate measurement in different sources based on the CO_2 balance method

Based on the mass CO_2 balance model literature, as shown in Table 2 and Figure 3, it is clear that the CO_2 balance using the null balance method is intended to work at low ventilation rates (Zekki *et al.*, 1999; Hand *et al.*, 1992) and at long-interval calculations of photosynthesis (10–15 min). In contrast, the ventilation rate should be added to the CO_2 balance calculation at moderate or high ventilation rate conditions with interval calculations of photosynthesis between 5–11 min, as shown in Nederhoff *et al.* (1989), Ehler (1991), and Chalabi and Fernandez (1994).

CO_2 loss still exists even when the ventilation rate is assumed to be zero (as in the null balance concept), but the amount of CO_2 loss by this process is small compared with the amount at higher ventilation rate conditions. Hence, infiltration should not be neglected, even with closed windows. Therefore, it is vital to add and evaluate the ventilation rate performance used in the CO_2 balance equations.

The calculated photosynthetic rate using the CO_2 balance has not yet been validated in a naturally ventilated greenhouse. It is essential to compare and verify the measurement of photosynthesis rate based on 1) the CO_2 balance method with ventilation rate prediction and 2) the leaf chamber method. Moreover, there is a lack of information on accurate and continuous measurement techniques for ventilation as a parameter of the CO_2 balance method. Therefore, further research is needed under different window apertures, from low to high ventilation rates.

4. Ventilation rate prediction in greenhouse

An essential tool for controlling greenhouse microclimate is ventilation. Air exchange between the interior and exterior of a greenhouse influences environmental conditions such as temperature, humidity, and CO_2 concentration, thereby influencing the development and production of the crop.

The measurement of the ventilation rate in a greenhouse involves various environmental parameters. It is a complex mechanism, including heat transfer processes of conduction, convection, and/or radiation. There is no single method to measure the ventilation rate because it is not only influenced by the microclimate and the presence of crop conditions, but it is also affected by the structure and design of the greenhouse.

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Various techniques have been used to measure and predict the ventilation rate, such as the tracer gas method (Boulard and Draoui, 1995; Papadakis *et al*., 1996; Baptista *et al*., 1999), heat balance method (Fernandez and Bailey, 1992; Demrati *et al*., 2001; Harmanto *et al*., 2006a), and water vapor balance method (Boulard and Draoui, 1995; Harmanto *et al*., 2006a).

Table 2: Summary of reviewed literature suggesting photosynthesis rate measurement using the CO_2 balance method

CO_2 concentration		Measurement method	Ventilator	Model Comparison	Authors
µmol mol^{-1}	device	of ventilation rate			
350	IRGA	- (null balance method)	Closed all-day	TOMGRO model (a mechanistic model)	Zekki *et al.* (1999)
350	IRGA	- (null balance method)	Closed and small ventilator	Empirically based on a quadratic regression mechanistic model as developed by Thornley *et al.* (1992)	Hand *et al.* (1992)
350–1000	IRGA	Model as developed by Fernandez and Bailey, 1992)	Small ventilator	Mechanistic model: Acock *et al*., (1978) and Challa and Schapendonk (1984)	Chalabi and Fernandez (1994)
350–1500	IRGA	Tracer gas (N_2O)	Small ventilation	The SUCROS model (Gijzen and Ten Cate, 1988)	Nederhoff *et al.* (1989)
350–1000	DGT infrared CO_2 scanner (IRGA)	Tracer gas (N_2O)	Low and high ventilation	The black box model of the photosynthesis simulation model	Ehler (1991)
400–1200	IRGA	Tracer gas (N_2O)	Low ventilation	-	Hand (1973a)
No information	CO_2 engine K30	Energy balance method	Low ventilation	-	Takakura *et al.* (2017)

4.1 Tracer gas technique

The tracer gas technique is the most common technique for measuring the ventilation rate and leakage rates [Nederhoff *et al*. (1985), Fernandez and Bailey (1992), Boulard and Draoui (1995), Kittas *et al*. (1996), Baptista *et al*. (1999), Kittas *et al*. (2002), and Katsoulas *et al*. (2006)]. It works based on a mass balance method or the principle of mass conservation and is mostly used to estimate ventilation rates directly.

Various gases have been used as tracer gases, such as sulfur hexafluoride (SF_6), methane (CH_4), argon 41, Krypton 85, nitrous oxide (N_2O), and carbon dioxide (CO_2). For ventilation rate analysis in a greenhouse, these gases should have the following characteristics: workable at low concentrations, nontoxic, non-flammable, inert, not naturally occurring in air, and weighing close to the average molecular weight of the air component (Goedhart *et al*., 1984; Sherman, 1990). The most frequently used are CO_2 (Nederhoff et al., 1985; Boulard and Baile, 1995) and N_2O (Fernandez and Bailey, 1992; Baptista *et al*., 1999; Kittas *et al*., 2002; Katsoulas *et al*., 2006), and there are some advantages and disadvantages relating to both of these.

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Baptista *et al*. (1999) suggested N_2O as a tracer gas because it meets all the above requirements and is not influenced by the photosynthesis and respiration of the plants. A tracer gas with N_2O is inert for plants and mixes well with air; however, water vapor can influence the infrared absorption used for tracing N_2O gas (Goedhart *et al*., 1984). In addition, it requires additional operational costs for the grower.

CO_2 can be used as a tracer gas, but it is necessary to measure the CO_2 concentration within the greenhouse and also the CO_2 release rate from the soil. CO_2 is also influenced by the CO_2 exchange of the plants; however, this problem can be solved, as proposed by Nederhoff *et al*. (1985), by using a practical method for measuring leakage and low-level ventilation greenhouses based on CO_2 as a tracer gas. In addition, they suggested that the proposed method could be used in practice after comparing with the infrared gas analyzer. There are particular advantages of using CO_2 as a tracer gas. It not only stimulates plant growth but also determines the ventilation. Thus, both types of gas have advantages and disadvantages, but the CO_2 gas is more practical and cost-efficient.

There are two kinds of approaches when using tracer gas: the static method and the dynamic method. Bot (1983) used the static method or continuous injection; Baptista *et al*., 1999; Fernandez and Bailey (1993), and Boulard and Draoui (1995), injected gas at a constant rate into a greenhouse until an equilibrium concentration was reached. The gas supply and sampling system must be distributed around the greenhouse to obtain good dispersion of the gas and uniform sampling of the air. Unfortunately, this method requires a high consumption of tracer gas.

In contrast, the dynamic method, or pulse injection, uses less tracer gas to estimate the ventilation rate. This method is known as the decay rate method because it measures the decay rate of a tracer gas concentration from an initial level. This method has been used by Nederhoff and Vegter (1994), Fernandez and Bailey (1992), Baptista *et al.* (1999), Muñoz *et al*. (1999), and Kuroyanagi *et al.* (2014). The tracer gas was injected and uniformly distributed in the greenhouse until a specific pre-determined concentration was reached. The decay rate in the gas concentration was then measured. When CO_2 concentration level had decreased to 10–20% of the initial value, another pulse of gas was injected, and the decay was measured. The ventilation rate (G) can be calculated from the following equation (Nederhoff *et al*., 1985):

$$N = \frac{3600}{t_1} \ln \left[\frac{C_{in}(t_0) - C_{out}}{C_{in}(t_1) - C_{out}}\right] \quad (3)$$

Assuming that G and C_{out} are constant over the observed time interval, in which C_{in} (t_0) is the initial CO_2 concentration at $t = 0$, C_{in} (t_1) is the CO_2 concentration measured at $t = t_1$, and the factor 3600 is introduced because the ventilation rate is defined in h^{-1} and t in seconds. N is the greenhouse air exchange rate expressed as times per hour of exchange of the greenhouse volume (h^{-1}). The greenhouse air exchange rate per hour (N) can be converted to the ventilation rate value, G ($m^3\ m^{-2}\ min^{-1}$) as follows:

$$G = \frac{1}{60} \frac{N V_g}{A_f} \quad (4)$$

where, V_g is the greenhouse volume (m^3), and A_f is the greenhouse floor area (m^2).

For accurate ventilation rate values based on the decay rate method with CO_2, Nederhoff *et al*. (1985) suggested supplying CO_2 when the concentration fell to 1000 µmol mol^{-1}. CO_2 was injected up to 2000 µmol mol^{-1}, because at concentrations between 1000 and 2000 µmol mol^{-1}, CO_2 concentration has little effect on photosynthesis; and, for accurate measurements, the concentration of the tracer gas inside the greenhouse must be significantly higher than that in the outside environment ($\pm$ 330 µmol mol^{-1}).

Results from the tracer gas technique in closed or low-level ventilation was in agreement with other methods for estimating the ventilation rate, as mentioned by Katsoulas *et al*. (2006) and Boulard and Draoui (1995) (Table 3). At the leakage rate condition, the ventilation rate via the tracer gas method was comparable with that using IRGA

and the theoretical model (pressure difference and wind pressure model), as shown in Table 3. However, there is little research about applying this technique to a maximum ventilation open area, such as that during summer. Therefore, it is better to reduce the CO_2 concentration enrichment strategy in a maximum ventilation opening area, but it is still sufficient to predict the ventilation rate based on the decay rate approach.

There are two ways to predict the ventilation rate based on the heat balance method, that used by Katsoulas *et al.* (2006) and Yasutake *et al*. (2017), and the water vapor balance technique by Boulard and Draoui (1995). Both methods have good results, although the accuracy is slightly less than that with tracer gas, at a window aperture range of 0–20%. A correlation between tracer gas and heat balance under opening vents between 0–20% was weak because the ventilation rate value fairly deviated from the target line which the heat balance value was higher than the tracer gas value (Yasutake *et al*., 2017). In contrast, the water vapor balance method had a slightly higher gradient value than that of the continuous and decay rate methods. This may be due to the estimation of plant transpiration from a limited number of plants (Boulard and Draoui, 1995).

Overall, tracer gas is the most widely used ventilation measurement method in conjunction with either the constant flow or the decay rate. Many authors have shown that the tracer gas at leakage and low ventilation conditions showed excellent performance; however, there is a lack of information on applications under maximum ventilation conditions, such as in summer.

In summer, ventilation removes energy and prevents excessively high temperatures. The tracer gas presented numerous disadvantages in large-scale greenhouses (Demrati *et al*., 2001), and the mixing problem was a primary potential source of error when the tracer gas method was used. This problem is particularly significant when a large area and low natural ventilation rate is used, because when there is large opening, it can be challenging to get the right mixing gas. This will affect an increase in operational costs for gas consumption. Simultaneously, the tracer gas consumption method results in a considerably inefficient use of gases when the window aperture is too large. Therefore, it needs to be determined how large the opening of the ventilation can be, to still predict the air exchange rate accurately using the tracer gas approach. It is necessary to conduct and evaluate the application of the tracer at maximum level ventilation conditions under both roof and side vent configurations. Moreover, it should be compared with other methods comprehensively, such as the heat balance and water vapor balance method.

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Table 3: Application of tracer gas in different window apertures

Tracer gas	Comparative method	*r**	Greenhouse type	Opening window (*W*, %)	*N* (h^{-1})	*G* ($m^3\ m^{-2}\ min^{-1}$)	Source
N_2O	Decay Rate Pressure difference	0.82	Four-spans Glasshouse (A_f, 12.8×16.0 m^2) Roof (leeside) vent (max. 42°)	0%[b] 10%[b] 20%[b]	0.33–0.53 7.78–19.43 16.14–37.37 (1–5 m s^{-1})	0.025 – 0.116 0.590 –1.474 1.224 – 2.835	Baptista *et al.* (1999)
N_2O	Decay Rate Energy balance	0.86	Single-span of arch-shaped roof (A_f, 8×20 m^2) Side and roof vents with the screen (50% porosity)	0%[d] no screen 0% With screen 11.2%[d] roof 17.0%[d] side 28.2%[d] Roof and side	0.85 (0.94) 1.83 (0.64) 2.97–7.73 6.39–19.64 15.73–56.52	0.051 (0.056) 0.109 (0.038) 0.177–0.461 0.381–1.170 0.938–3.368	Katsoulas *et al.* (2006)
N_2O	Decay rate Theoretical model (wind pressure)	0.93	Three-span tunnel-type greenhouse (A_f, 19.2×12 m^2) Roof vents with screen 45%)	9.4%[d] (0.6 m)	5.43–36.18	0.226–1.508	Muñoz *et al.* (1999)
N_2O CO_2	Decay rate (N_2O) Continuous injection (CO_2) Water vapor balance	0.68 0.68 0.60	Two-span type greenhouse (Filclair) (A_f, 2×6.5 m × 32 m)	0% 8%	0.004–0.04 0.12–0.15	0.0003–0.002 0.007–0.588	Boulard and Draoui (1995)
CO_2	Decay rate IRGA device	0.9 (±1%)	Venlo type (A_f, 6×9.6 m^2) Roof (leeside) vent (max. 0.6m):	0%[a] 14%[a]	0.25–0.50 2.70–4.80 (2–4 m s^{-1})	0.012–0.023 0.127–0.227	Nederhoff *et al. (1985)*
CO_2	CO_2 balance Energy balance	0.39	Single-span (A_f, 7.5×20 m^2) side and roof vents	0%[c] 20%[c]	<= 0.50 6.00–7.50 (2–4 m s^{-1})	<= 0.026 0.312–0.390	Yasutake *et al.* (2017)
SF_6	None	-	Two spans of arch-shaped roof (A_f, 9.7×18.4 m^2)	0%	0.27–0.46 (2–4 m s^{-1})	0.021–0.035	Kuroyanagi *et al.* (2014)
Average value at close ventilation at wind speed 1 – 2 m s^{-1}				0%	0.37 ± 0.12	0.023 ± 0.007	
Average value at close ventilation at wind speed 4 – 5 m s^{-1}				0%	0.81 ± 0.29	0.051 ± 0.020	

Note: *The Pearson correlation coefficient for tracer gas and comparative method; a) percentage opening of maximum opening (0.6 m); b) the ventilator aperture is expressed as a percentage of the maximum aperture (42°); c) the opening area is presented as the ratio of the coverage area to the window opening; and d) total opening area per greenhouse floor area.

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4.2 Heat balance method

The heat balance method was developed using microclimate data. Some models concentrated on a naturally ventilated greenhouse with a small opening on the roof or on both roof and side configurations. The predicted model was validated with the experimental data, and the result was fitted between them (Table 4) (Fernandez and Bailey, 1992; Katsoulas *et al*., 2006; Yasutake *et al*., 2017).

The heat balance method uses either static or dynamic models (Roy *et al*., 2002). The dynamic model is more accurate than that is the static model, because the latter looks at only a few parameters. Several dynamic models have been developed (Teitel and Tanny, 1999; Wang and Boulard, 2000; Roy *et al*., 2002). The greenhouse heat balance is the sum of the gains and losses during a specified period. This method assumes a steady state and uses energy conservation technique, i.e., heat gains are equal to heat losses. The energy content is affected by heat gains and losses, determined by the change in temperature. The heat exchange between the inside and outside of a greenhouse is a complex mechanism involving the processes of radiation, conduction, convection, and latent heat.

Fernandez and Bailey (1992) developed a model based on solar energy, considering the stored energy inside the greenhouse that involved the cover, inside air, crop, and soil. Demrati *et al*. (2001) proposed a model based on a global energy balance of the greenhouse. They considered all parameters involved in the heat transfer processes occurring in the greenhouse. For instance, with net solar radiation as the input heat flux, and heat fluxes through the soil, material cover, and ventilation system as heat loss, the following equation is used to estimate the ventilation rate:

$$G = \frac{A_f\,(R_n - F_s) - A_c\,[K\,\Delta T + C_h\,\Delta T_c]}{\rho_a\,[c_p\,\Delta T + L\,\Delta H_A\,]} \tag{5}$$

$$K = a + bv \tag{6}$$

$$C_h = 1.75\,(T_c - T_{in})^{0.333} \tag{7}$$

where G is the volume flow rate ($m^3\ s^{-1}$); A_f and A_c are the greenhouse floor and cover area (m^2); R_n is the net radiation ($W\ m^{-2}$); F_s is the thermal flux at soil surface ($W\ m^{-2}$); K is the global sensible heat loss coefficient through the greenhouse material cover ($W\ m^{-2}\ K^{-1}$); ΔT is the air temperature difference between inside and outside (°C); A_c is the surface area of the roof (m^2); C_h is the convective heat exchange coefficient between inside air and the material cover ($W\ m^{-2}\ K^{-1}$); ΔT_c is the temperature difference between inside air temperature (T_{in}) and the material cover (T_c) (°C); ρ_a is the air density ($kg\ m^{-3}$); c_p is the air specific heat at constant pressure ($J\ kg^{-1}\ K^{-1}$); L is the latent heat of water vaporization ($J\ kg^{-1}$); ΔH_A is the absolute humidity difference between inside and outside air ($kg\ kg^{-1}$); v is the wind speed ($m\ s^{-1}$), and a and b are constants. Demrati *et al*. (2001) used constant values for a of 1.44 $W\ m^{-2}\ K^{-1}$ and b of 0.12 $J\ m^{-2}\ K^{-1}$, when considering the greenhouse floor area surface as the surface unit.

The ventilation rate value prediction is affected by the dimensions and type of greenhouse. A multi-spans-type greenhouse has a higher value than a single-type greenhouse (Figure 4 and Table 4). Large-scale greenhouses (Figure 4b) have more natural ventilation than the small-scale single-span greenhouse type (Figure 4a), even if the outdoor wind speed is the same. The ratio of the side and roof vents to the floor area (W %) of the larger-scale greenhouse became smaller even when the vents were widened. However, the small greenhouse had a larger W, but the air exchange rate was low because of the effect of temperature difference ventilation from the side vents to the skylight. Therefore, it was necessary to enlarge the window aperture and force ventilation.

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Table 4: The heat balance method performance under a different type of greenhouse and window apertures

Greenhouse Type	Comparative method	Vents configuration	Window aperture	W [c] (%)	N (h^{-1})	R^2[d]	Sources
Multispan (4 spans) (A_f = 12.8×33 m^2; V_g = 1,523 m^3)[a]	Tracer Gas (N_2O)	Flap Roof	0% (0°)[b]	0	0.8	0.06	Fernandez and Bailey (1992)
			10% (3.9°)	2	3.2	0.47	
			20% (7.8°)	5	4.8	0.32	
			30% (11.6°)	7	6.4	0.41	
			40% (15.4°)	10	7.4	0.81	
Large multi-span Canarian type greenhouse (A_f=10,000 m^2; 6–7 m height)	Theoretical model (wind and stack effect)	Both roof and side vents	9%	9	9.8	0.86	Demrati *et al.* (2001)
Single-span plastic greenhouse (A_f=150 m^2; V_g = 468.2 m^3)	Tracer gas (CO_2 balance)	Both side and roof	0%	0	1.9	0.39	Yasutake *et al.*, (2017)
			20%	40	3.8	0.39	
Single-span plastic greenhouse (A_f=160 m^2; V_g = 572 m^3)	Tracer gas (N_2O)	Closed	0%	0	0.5	0.76	Katsoulas *et al.* (2006)
		Side only	17%	17	1.7		
		Roof	11%	11	2.5		
		Roof and Side	28.2%	28	2.6		

Note: a) A_f is the greenhouse floor area, V_g is the greenhouse volume, b) the percentage of window aperture based on ratio angle of opening and maximum angle of the opening ventilator; c) W is window aperture in %. It was calculated based on the ratio between the total opening area and the greenhouse floor area, and d) R^2 is the coefficient of determination (R-squared).

Figure 5 shows that the correlation value of the air exchange rate between the heat balance method and the tracer gas method (using N_2O) increased linearly with the enlargement of the roof vents in the large-scale greenhouse. Fernandez and Bailey (1992) noted that measurement precision increased with the length of the measurement time scale and vent opening. For higher ventilation conditions, it was found that the energy balance gives better results for larger ventilator apertures (Fernandez and Bailey, 1992; Baptista *et al*., 2001).

However, the R-squared values presented the strength of the relationship between the estimated air exchange rate via the heat balance method and the measured tracer gas using N_2O, indicating a weak correlation at below 10 % of the window aperture. This was also found in a small-scale greenhouse, as mentioned by Yasutake *et al*. (2017). Fernandez and Bailey (1992) elucidated that a significant error occurred when the window condition was closed or there was a leakage rate condition, and this was caused by an error in other components of the energy balance method compared to the energy lost by ventilation. Therefore, it is challenging to correlate measurement and prediction with heat balance in the closed window condition or the smallest opening area (angles).

It is evident that the heat balance method can be used to predict the ventilation rate under a small window aperture. It should be determined what the minimum solar radiation and window opening levels would be that could still allow for a reasonable prediction of the air exchange rate. Further study is needed on the heat balance method, especially in the lower ventilation rate conditions.

Several authors calculated the air exchange rate using the heat balance method with daily measurement data (Demrati *et al*., 2001; Harmanto *et al*., 2006a) and found good prediction values. One of the goals of monitoring the ventilation rate is to monitor the photosynthetic rate. As explained before, continuous monitoring of photosynthesis should be employed in a short-term interval.

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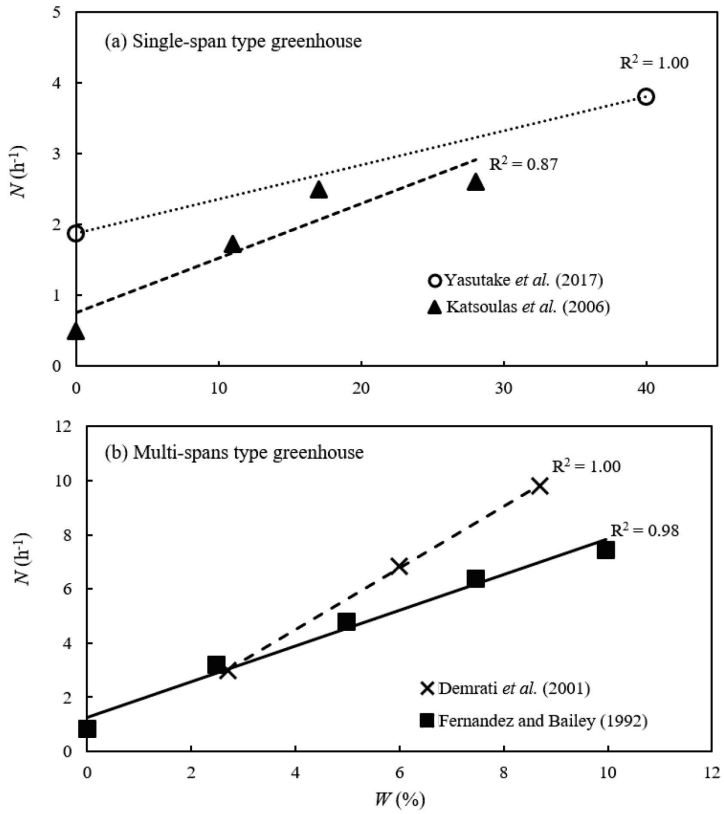

Figure 4: Single-span and multi-spans greenhouse air exchange rate (N) value under different window apertures (W) (the ratio between total opening areas with greenhouse floor area)

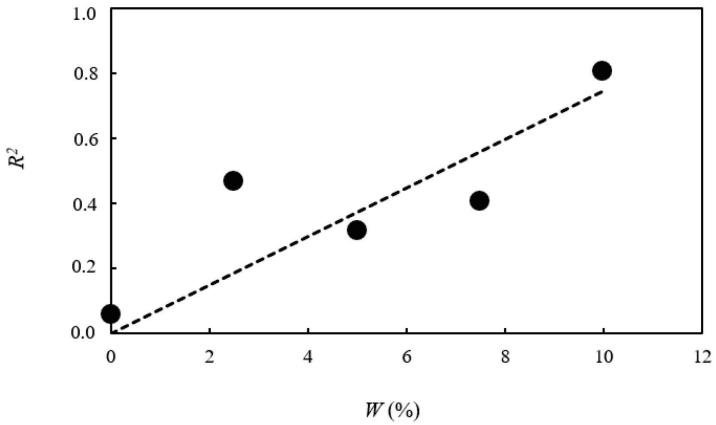

Figure 5: R-squared explains the strength of the relationship between the predicted air exchange rate via the heat balance method and the measured value by tracer gas method using N_2O at multi-span greenhouse (4 spans) under different of window apertures (W). The greenhouse floor area was 422 m^2 and roof vents were applicable (Fernandez and Bailey, 1992).

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Yasutake *et al*. (2017) found that a single-span type greenhouse with a ventilation opening area up to 20 % of the total opening area resulted in a ventilation rate of below four air exchanges per hour. However, the coefficient of determination value was low (R^2 = 0.39) compared with the Katsoulas *et al*. (2006) predicted value (R^2=0.76). Additionally, at leakage or the smallest opening area, the predicted value of Katsoulas *et al*. (2006) was more accurate than that by Yasutake *et al*. (2017).

The accuracy of different results between the two studies, Katsoulas *et al*. (2006) and Yasutake *et al*. (2017), might be affected by the ventilation rate methods used. Yasutake *et al.* (2017) conducted tracer gas using CO_2 with the K30 engine sensor and Katsoulas *et al.* (2006) used N_2O with an IRGA. Moreover, the predicted value conducted by Katsoulas *et al*. (2006) used the heat balance method proposed by Demrati et al. (2001). In contrast, Demrati *et al.* (2001) and Katsoulas *et al*. (2006) considered all parameters involved in the heat transfer processes occurring in the greenhouse. Hence, it is better to consider all parameters of heat transfer when using the heat balance approach to compute the ventilation rate. Further experiments are needed on the heat balance method under the different opening areas, from leakage rate condition to a maximum opening area of the ventilation system, and in different light intensities (or seasons).

4.3 Water vapor balance

The water vapor balance method is a quick and straightforward method to estimate the ventilation rate in a greenhouse. This method uses water vapor from the evapotranspiration process and air-specific humidity in the greenhouse. Many authors have neglected soil evaporation due to the presence of continuous plastic mulch on the soil surface or soilless culture (hydroponic systems) as well as condensation within the greenhouse. The following equation is used to estimate the ventilation rate (Boulard and Draoui, 1995):

$$\rho V_g \frac{dp_{in}}{dt} = \rho G(t) [p_{out}(t) - p_{in}(t)] + ET(t) \quad (8)$$

Here, ρ is air density (kg m^{-3}), p_{in} and p_{out} are absolute humidity (kg kg^{-1}), and ET is evapotranspiration rate (kg s^{-1}). All the measurements were automatically recorded each minute, and averaged over 1 h, then the above equation was used to calculate G and N as a simple function of p_{in}, p_{out}, and ET.

This method was previously adopted by Boulard and Draoui (1995) in a two-span plastic naturally ventilated greenhouse with roof vents only. The water vapor balance method was in good agreement with other methods using tracer gas, that is, CO_2 or N_2O. Harmanto *et al*. (2006b) pointed out that the water vapor balance method had a better estimation accuracy when measured in a greenhouse cultivated with mature plants and with a smaller ventilation opening area. Table 5 shows that the method has been applied in various types of compartments with good results, such as multi-span greenhouses with continuous roof vents only (Boulard and Draoui, 1995; Kittas *et al*., 2002), those with continuous roof and side vent configurations (Mashonjowa *et al*., 2010), units with a screen house (Harmanto *et al*., 2006b; Rigakis *et al*., 2015), and compartments with growth chambers (Li *et al*., 2012).

Figure 6 illustrates the performance of the water vapor balance method for predicting the air exchange rate (N) in a greenhouse cultivated with tomato plants. Mainly large-scale greenhouses with multiple spans are shown, except for one source from Harmanto *et al*. (2006b) which uses screen houses. In general, it is clear that the ventilation rate increases with the width of the window opening (W). Most of the results in this graph were measured under small opening conditions (<10 % of W) or ventilation rates below 20 air exchange rates per hour in multi-span greenhouses equipped with vents, both on the sides and/or above only.

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Information on the performance of the water vapor balance method is quite limited under maximum opening ventilation conditions. Further studies are needed to evaluate the ventilation performance of single-span type greenhouses with varying window apertures.

There are some disadvantages of the water vapor balance method, for instance the overestimation of ventilation rate at night, and errors in scaling-up crop transpiration from a single plant (or a few plants) to whole canopy transpiration (Boulard and Draoui, 1995; Mashonjowa *et al*., 2010). An increase in the transpiration error might be present due to the sensor used in the research. The transpiration rate was measured using a stem heat balance sensor (sap flow gauges) installed on the main stems of the crops. This sensor is handy, although sap-flow is not precisely the same as transpiration, and it requires non-ideal calibration (Akutsu *et al*., 2015). In addition, Kittas *et al*. (2002) noted error values because of either direct solar radiation penetration to the lysimeter by the window opening or from temporary shading of the lysimeter by the greenhouse frame.

Overall, the ventilation rate using the water vapor balance method on the mature plant has a good prediction value; however, it is challenging to predict the rate for young plants, possibly due to the error of crop transpiration rate caused by small plants, or less uniformity of humidity in the greenhouse (Harmanto *et al*., 2006b).

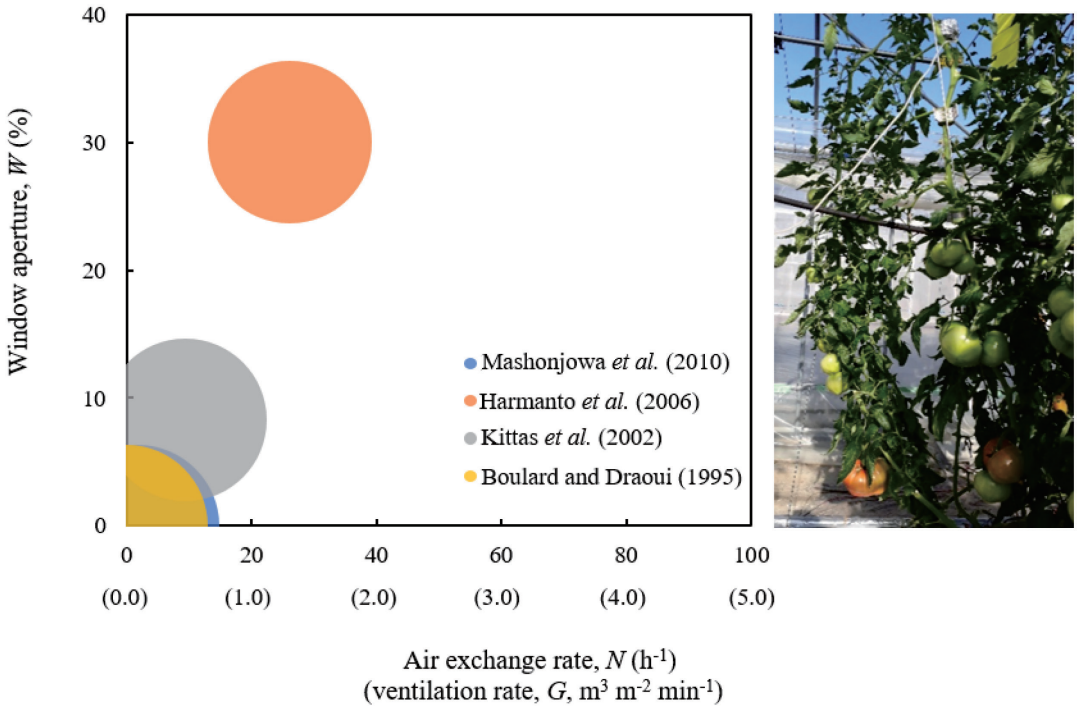

Figure 6: Bubble scattered chart of the air exchange rate using water vapor balance method in a single-span and multi-span greenhouse type cultivated with tomato crops

The water vapor balance was mainly affected by transpiration, which is determined by environmental factors, mainly radiation, vapor deficit (Jolliet and Bailey, 1992; Katsoulas *et al*., 2001), and air velocity (Jolliet and Bailey, 1992; Thongbai *et al*., 2010). Transpiration from leaves is regulated by stomatal conductance (G_s). The G_s are determined by the water potential in the plant, which depends on the balance between root water absorption and

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transpiration. Since stomata also mediate CO_2 diffusion into the leaves, maintaining a low VPD (0.39–0.40 kPa) could manage the G_s to the optimum value and improve the effect of CO_2 fertilization between 350–1000 µmol mol^{-1}, as reported by Zabri and Burrage (1998). Thongbai *et al*. (2010) considered that an increase in CO_2 concentration up to the outside concentration (350–450 µmol mol^{-1}) and/or air circulation in a ventilated greenhouse (1 m s^{-1}) could increase the net photosynthetic rate significantly and maintain the transpiration processes. Therefore, it is essential to keep the inside greenhouse climate at optimum conditions for the crops, especially keeping the vapor pressure deficit parameter at optimum conditions because it can improve the photosynthesis and transpiration processes under CO_2 application.

Continuous crop transpiration measurement information is essential not only for the need to know the condition of the plants and the whole greenhouse condition, but also for estimating the ventilation rate using the water vapor balance approach. Various devices have been applied for measuring transpiration, such as an electronic scale, a sap flow sensor, and a flow meter, as presented in Table 5. A weigh scale minimizes the errors of scaling up from a few plants to the whole canopy transpiration, and permits the use of water flow measurement in the greenhouse. Akutsu *et al*. (2015) conducted a direct transpiration measurement on a tomato crop stand using artificial leaves made of towel and paper filters. The report showed agreement with data measured by a weighing device.

In the context of ventilation rate monitoring using the water vapor balance technique, an electronic weighing device was most commonly used by researchers, followed by sap flow measurements. The weighing method has reasonably good accuracy (Leperen and Madery, 1994) but is expensive for the growers, is measured only on some plants, and does not have the ability to describe the entire transpiration rate in the greenhouse. It needs to be determined how to measure and monitor transpiration in a greenhouse continuously. Further study is also required to validate the weight and sap flow measurements that are applied to one or several plants for the calculation of the overall transpiration rate of plants in the greenhouse.

5. Conclusions and future work

The CO_2 balance method for a greenhouse is applied to the photosynthetic rate of the plant canopy by accurately measuring the ventilation rate and the difference between internal and external CO_2 concentrations. The reliability of CO_2 concentration control depends on the measurement error of the CO_2 sensor and the operating characteristics of the CO_2 supply device, which consists of a porous tube, flow meter, valve, and cylinder. The ventilation rate changes depend on the climatic conditions and window apertures, and it is necessary to continuously measure the, rate by the tracer gas method, the heat balance method, or the water vapor balance method for application to the CO_2 balance method.

The tracer gas method is highly reliable as a ventilation rate measurement method, but a large amount of tracer gas is required for use in a greenhouse with a large ventilation volume for long-term measurement. In particular, CO_2 gas is not suitable as a tracer gas for ventilation in a greenhouse. Therefore, the evaluation of greenhouse ventilation using alternative methods (heat balance and water vapor balance methods) should be conducted to estimate the photosynthetic rate using the CO_2 balance method under various window apertures, and compare then with the tracer gas method.

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