Twofold Increase in Strawberry Productivity by Integration of Environmental Control and Movable Beds in a Large-scale Greenhouse

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The effect of combinational environment control, incorporating supplemental lighting and elevated CO2 concentration (maintained at 1,000 µmol mol−1) and air temperature (Tair; maintained between 15 and 27°C in the day), on growth and fruit yield of June-bearing strawberry ‘Benihoppe’ was examined under forcing culture. Supplemental lighting significantly enhanced leaf photosynthesis, and this response was further increased by elevated CO2 and Tair treatment. Flower opening on the first and second inflorescences was accelerated by supplemental lighting, and was further accelerated by elevated CO2 and Tair, and resulted in a significant increase in fruit yield. Flower number per inflorescence was significantly increased under supplemental lighting and/or elevated CO2 and Tair. Increase in flower number and shortening of the fruit maturation period caused by increasing the average air temperature under elevated CO2 and Tair resulted in a significant increase in harvested fruit number and yield. By integration of combinational environment control and 1.5-fold increase in planting density (from 8 to 12 plants m−2) with a movable bed system, the fruit yield per unit land area achieved was 10.7 kg m−2 (10.7 t / 10 a), which represented a more than two-fold increase in yield compared with that attained in a conventional stationary bed system.

Keywords: CO2 enrichment, dense planting, elevated temperature, high yield, photosynthesis, supplemental lighting

INTRODUCTION

In Japanese strawberry production, over 90% of farmers employ forcing culture in which fruits are harvested from winter to the following spring using June-bearing cultivars. However, production areas are continuously declining for Japanese strawberry production. The average yield from domestic strawberry production in 2012 was about 3 kg m−2 (t / 10a). The average yields in the main production districts of Tochigi and Fukuoka in 2012 was about 4 kg m−2 according to statistical data from the Ministry of Agriculture, Forestry and Fisheries in Japan.

To reverse the decline in Japanese strawberry production, there is an increasing trend for strawberry production in large-scale industrial facilities. Techniques to obtain consistently high yields are required in large-scale greenhouse production. In strawberry production, many factors contribute to fruit yield (Hidaka et al., 2014a). Fruit yield per unit land area is determined by the number of plants and the fruit yield per individual plant. The former is influenced by cultivation systems, and the average planting density of the conventional bench culture system with stationary beds is about 8 plants m−2 (8,000 plants / 10a) in Japan. To achieve high planting density, many types of the movable bed systems, e.g., the lateral movable type (Nagasaki et al., 2013), the circulative movable type (Hayashi et al., 2011) and the vertically movable type (Hidaka et al., 2012), have been developed. These lateral, circulative and vertically movable bed systems enable efficient use of the greenhouse space, and result in 1.5, 2.5 and 4 times planting densities as compared with the conventional bench culture system, respectively. The fruit yield per individual plant is influenced by many factors, such as unit fruit weight, fruit number per plant, flower bud, photosynthetic partitioning, leaf photosynthesis, and water and nutrient uptake by roots. These factors are affected by the environment (e.g., light intensity, photoperiod, temperature, CO2 concentration, humidity, and wind velocity) and the genetic potential of each cultivar. In our previous studies, we explored the development of a supplementary lighting technique, i.e., selection of an effective light source (Hidaka et al., 2013) and determination of the optimum photoperiod for supplemental lighting (Hidaka et al., 2014b). Furthermore, we compared the effect of supplemental lighting among cultivars and observed a remarkable increase in yield in the June-bearing cultivar ‘Benihoppe’ (Hidaka et al., 2015). To achieve an even higher increase in fruit yield, a combinational approach to environmental control, considering not only the light environment but also CO2 concentration and air temperature, for example, is required. Kawashima (1991) reported the effect of CO2 en-
richment on growth and yield of strawberries. However, almost no studies have examined the effect of combinational environment control, and the integration of combinational environment control and high planting density in strawberry production has not been reported to the best of our knowledge.

In this study, to clarify the effect of combinational environment control on strawberry growth and yield, the effects of supplemental lighting, elevation of CO₂ concentration and air temperature, and their interaction were examined. Furthermore, to achieve high productivity per unit land area, integration of combinational environment control and high planting density using a movable bed system was examined, and fruit yield per unit land area was compared with that attained under a conventional production system.

MATERIALS AND METHODS

Two experiments were conducted, one to investigate the effects of the air and light environment controls on strawberry growth and yield, and the second to demonstrate integration of these environmental controls and high planting density to increase strawberry fruit yield. The experiments were performed in 2 sections (each section was 37 m long × 9 m wide) installed in 2 separate bed systems (Fig. 1) in adjacent large-scale greenhouses (37 m long × 27 m wide × 4.5 m high) at the NARO Kyushu Okinawa Agricultural Research Center, Japan (33°18.4'N, 130°32.8'E).

Experiment I: Effects of air and light environment controls on strawberry growth and yield

Plant materials and growth conditions

The effect of air-environmental control (AEC) in conjunction with or without supplemental lighting (SL) on strawberry growth and yield was examined with a movable bed system in fiscal years of the 2012 (October 2012 to May 2013) and 2013 (October 2013 to May 2014). In early June, 45 nursery plants of the June-bearing strawberry (Fragaria × ananassa Duch.) ‘Benihoppe’ selected from mother stock plants were transplanted into plastic pots (6 cm diameter; 0.2 L volume) retaining the connection to the mother plant through the runner. The pots were filled with a mixed substrate (peat moss: coconut shells: charcoal 1:1:1).

Fig. 1  Photographs and plane figures of the conventional bed system (a, b) and the movable bed system (c, d). Stationary beds were installed in the conventional bed system. Movable hanging-beds were installed in the movable bed system, and the lateral movement of hanging beds enables 1.5 times beds per unit area as compared with those in the conventional bed system. Experiment I was conducted in the movable bed system. In experiment II, marketable fruit yield per unit area was compared between conventional bed system and movable bed system. Closed circles in the plane figures (b, d) indicate the measuring points of daily PPFD integral on the beds, and the averaged values of 9 measuring points in respective system were also shown. These PPFD measurements were conducted on the fine day in June 2014 by using the color acetate films (O-1D, Taisei E&L Co., Ltd., Tokyo, Japan).
using a portable photosynthesis and fluorescence system. The nutrient solution (OK-F-1, OAT Agrio Co., Ltd., Tokyo, Japan) with electrical conductivity of 0.6 dS m\(^{-1}\) was supplied at the rate of 300 mL d\(^{-1}\) per plant daily. From mid-August to the time of transplanting, nutrient supplementation was halted to induce anthesis, with only water supplied. On 18 September 2012 (2012 fiscal year) and 27 September 2013 (2013 fiscal year), the nursery plants, which were not confirmed previously whether the first inflorescence had differentiated in the shoot apical meristem, were transplanted into substrate-filled movable beds (30 m long \(\times\) 30 cm wide \(\times\) 80 cm high), with plants spaced 20 cm apart and with 15 cm between rows. The plants were then supplied with nutrient solution until 31 May. Substrates and nutrient solutions were the same as those used to cultivate the nursery plants, with approximately 3 L substrate per plant. Flower pollination was performed by bees. Fruit thinning was not conducted.

**Air environment and supplemental lighting conditions**

To establish appropriate values for CO\(_2\) concentration and air temperature to incorporate in the environment control treatments, preliminary measurement of leaf photosynthesis under the controlled air environment was conducted using a portable photosynthesis and fluorescence system (LI-6400XT, LI-COR, Lincoln, NE, USA). Photosynthetic rate in relation to CO\(_2\) concentrations was measured in the free expanded third leaflet of strawberry ‘Benihoppe’ under controlled environments in a leaf chamber as follows: 200, 400, 700, 1,000, 1,500 and 2,000 \(\mu\)mol mol\(^{-1}\) CO\(_2\), 25°C air temperature, 50% relative humidity, and 500 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) photosynthetic photon flux density (PPFD) in November 2011. Photosynthetic rate in strawberry ‘Sagahonoka’ under the different air temperatures and CO\(_2\) concentrations was also measured under controlled environments in a leaf chamber as follows: 10, 20 and 30°C air temperatures, 200, 400, 700, 1,000, 1,500 and 2,000 \(\mu\)mol mol\(^{-1}\) CO\(_2\), 50% relative humidity, and 400 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) PPFD on 29 December 2011. Figure 2 shows the relationship between CO\(_2\) concentration and photosynthetic rate in ‘Benihoppe’ (Fig. 2a), and in ‘Sagahonoka’ under the different air temperatures (Fig. 2b). Leaf photosynthetic rate in ‘Benihoppe’ increased with the increase in CO\(_2\) concentration, and was almost saturated at 1,000 \(\mu\)mol mol\(^{-1}\). Photosynthetic responses to CO\(_2\) concentration in ‘Sagahonoka’ were almost identical to those of ‘Benihoppe’. At a CO\(_2\) concentration less than 400 \(\mu\)mol mol\(^{-1}\), little difference in photosynthetic rate was observed between air temperatures of 10, 20 and 30°C. However, at CO\(_2\) concentrations higher than 600 \(\mu\)mol mol\(^{-1}\), photosynthetic rates at 10°C were lower than those at 20°C. Photosynthetic rates at 30°C were almost identical to those at 20°C.

On the basis of these photosynthetic responses, the CO\(_2\) concentration in the greenhouse was maintained at 1,000 \(\mu\)mol mol\(^{-1}\) with a fuel-burning CO\(_2\) generator (CG-254S1, NEPON Inc., Tokyo, Japan). To enhance the effect of elevated CO\(_2\) treatment on leaf photosynthesis, the daytime air temperature in winter in the greenhouse was maintained above 15°C with heating provided by a fuel-burning heater (HK2027TEV, NEPON Inc., Tokyo, Japan). To maintain a high CO\(_2\) concentration in a greenhouse for a prolonged period, it is necessary to restrict CO\(_2\) release through ventilation into the outside environment. No differences in leaf photosynthesis at 30 and 20°C air temperatures under elevated CO\(_2\) conditions were observed and, furthermore, it has been reported that the optimum air temperature for leaf photosynthesis of C\(_3\) plants shifted to the high temperature side under the high CO\(_2\) concentration (Long, 1991; Kawamitsu, 1996). Therefore, onset of ventilation during the day (05:00–18:00) was set at 27°C, a comparatively high temperature, to delay the start of ventilation. The night (18:00–05:00) air temperature was maintained above 8°C with heating provided by a fuel-burning heater. The elevated CO\(_2\) and \(T_d\) treatment was applied in the movable bed system (Fig. 1c, d) during the day (06:00 to 18:00) from October 2013 to May 2014 (2013 fiscal year). After April when the amount of solar radiation increased, CO\(_2\) enrichment was only conducted during 05:00 to 12:00. As a control (non-treatment) for the elevated CO\(_2\) and \(T_d\) treatment, the daytime air temperature was maintained below 26°C by ventilation, and the nighttime air temperature was maintained above 6°C with heating provided by a fuel-burning heater. The CO\(_2\):

![Fig. 2](image-url)
concentration was not controlled. The experiment without elevated CO\textsubscript{2} and \( T_\text{s} \) treatment was also conducted in the movable bed system from October 2012 to May 2013 (2012 fiscal year).

Supplemental lighting was provided employing the same light-emitting diode (LED) system described previously (Hidaka et al., 2013; 2014b; 2015). This system consisted of an LED lamp unit (LLM0312A, Stanley Electric Co., Ltd., Tokyo, Japan) coupled to an exclusive power supply (LLP0019A, Stanley Electric Co., Ltd.). This LED lighting system was placed every 30 cm at 50 cm height from the base of the plants on the cultivation bed. The 30 cm spacing between LED lamps was determined to be approximately equal the horizontal distribution of \textit{PPFD} at the plant bases. Each LED system consumed 26 W. The LED system featured peak light intensity at 450 and 550 nm as reported previously (Hidaka et al., 2013). The LED supplemental lighting was supplied for 12 h per day from 06:00 to 18:00 (lighting) with timer switches (TB22101, Panasonic Corp., Osaka, Japan), and controls received no supplemental lighting (non-lighting). The lighting and non-lighting treatments were conducted under different air-environment treatments of elevated CO\textsubscript{2} and \( T_\text{s} \) (2013 fiscal year) and non-treatment (2012 fiscal year), respectively.

\textbf{Measurement of environmental conditions and leaf photosynthesis}

During the experiment, we monitored \textit{PPFD}, air temperature and CO\textsubscript{2} concentration inside and outside the greenhouse. Quantum sensors (PAR-02, Prede Co., Ltd., Tokyo, Japan) were placed 20 cm above the plant base under the lighting and non-lighting conditions, and additionally placed outside the greenhouse at 90 cm above the ground surface to measure \textit{PPFD}. Temperature and CO\textsubscript{2} recorders (TR-76Ui, T&D Corporation, Nagano, Japan) were placed in the center of the greenhouse and outside the greenhouse. Data for \textit{PPFD}, air temperature and CO\textsubscript{2} concentration were automatically recorded every 10 min.

In May, 2013 and 2014, we measured leaf photosynthesis in the lighting and non-lighting treatments under the elevated CO\textsubscript{2} and \( T_\text{s} \) treatment and non-treatment using the portable photosynthesis and fluorescence system. Photosynthetic rates of leaves were measured using the chamber head with a natural light window. Leaves were placed in the chamber head positioned horizontally at 10 cm above the plant base. Measurements were recorded under the following controlled environmental conditions: 25°C air temperature, 50% relative humidity, and 400 \( \mu \text{mol} \text{ mol}^{-1} \) CO\textsubscript{2} in the non-treatment and 1,000 \( \mu \text{mol} \text{ mol}^{-1} \) CO\textsubscript{2} in the elevated CO\textsubscript{2} treatment. Measurements were conducted on cloudy days from 10:00 to 16:00 using the fully expanded third leaflet of strawberry ‘Benihoppe’ grown under the elevated CO\textsubscript{2} and \( T_\text{s} \) treatment and non-treatment. We analyzed 4 plants per treatment. The \textit{PPFD} and photosynthetic rate in the leaf chamber were recorded simultaneously.

\textbf{Analyses of flowering characteristics, yield, and fruit quality}

To analyze the effect of supplemental lighting and air-environment control on flowering, we recorded the flowering date, which is the date on which the first flower opened, of the first and second inflorescences, and the number of flowers in each inflorescence. Furthermore, for the first inflorescence, the dates of fruit harvest were also recorded. These data for flowering and harvesting dates were used to evaluate the fruit maturation period, which is the number of days after anthesis to harvest. We analyzed 5 plants per treatment.

To determine the effects of supplemental lighting and air-environment control on yield, marketable fruit (fruit fresh weight \( \geq 6 \text{ g} \)) were harvested from 5 plants in each treatment. For the fruit harvested from the first inflorescence, the following fruit quality parameters were analyzed: soluble solids content (SSC), titratable acidity (TA), and fruit firmness (FF). The SSC and TA of fruit juice and the FF of fruit were measured using a digital refractometer (PAL-1, Atago Co., Ltd., Tokyo, Japan), a coulometric acidity meter (CAM-500, Kyoto Electronics Manufacturing Co., Ltd., Kyoto, Japan) and a penetrometer (RX-2, Aikoh Engineering Co., Ltd., Osaka, Japan), respectively. For fruit quality analysis, 8 fruits per treatment were used.

\textbf{Growth analysis}

To analyze the long-term responses of leaf photosynthesis and the partitioning of photosynthates to plant organs, growth analysis was conducted. Plant growth was divided into 2 stages, 1) before fruit set and 2) after fruit set, and then the growth characteristics in these 2 stages were analyzed. Five plants of each treatment were harvested at 3 time points for growth analysis as follows: just after the transplanting (26 September 2012 and 4 October 2013); during fruit set (27 November 2012 and 2 December 2013); and after fruit set (26 February 2013 and 2014). The harvested plants were separated into leaf, crown, peduncle, fruit and root. Each part was dried for 48 h at 80°C in a circulation drier, cooled to room temperature, and then weighed. We calculated the leaf area and the growth characteristic parameters specific leaf weight (SLW; an index of leaf thickness), crop growth rate (\( \text{CGR} \); the dry matter production rate per unit ground area), leaf area index (\( \text{LAI} \); the leaf area per ground area), net assimilation rate (\( \text{NAR} \); the dry matter production rate per unit leaf area), shoot growth rate (\( \text{GR}_{s} \); the dry matter partition rate to shoots per unit ground area), root growth rate (\( \text{GR}_{r} \); the dry matter partition rate to roots per unit ground area) and fruit growth rate (\( \text{GR}_{f} \); the dry matter partition rate to fruit per unit ground area) following the method used in our previous study (Hidaka et al., 2015).

\textbf{Experiment II: Integration of environmental controls and movable bed system to increase yield}

\textbf{Cultivation system}

The movable hanging-bed system, which was introduced by Nagasaki et al. (2013), was used to achieve a high yield of strawberry fruit (Fig. 1c, d). In this system, 9 movable hanging beds (30 m long \( \times \) 30 cm wide \( \times \) 80 cm high) were installed in the greenhouse. Each bed is hung from beams (2.8 m high) and attached to a DC24V gear-motor. By rotation of the gear-motor, lateral movement of the hanging beds is achieved through the rack and pinion operation, and this can widen the aisle between adjacent
increased strawberry yield

Analyses of yield, growth characteristics, and leaf photosynthesis

To demonstrate the high yield productivity of strawberries, the yields per unit area under the integrated treatment of combinational environment control and higher planting density (12 plants m\(^{-2}\)) with the movable hanging-bed system (integration) were compared with those attained without environmental control and at the standard planting density (8 plants m\(^{-2}\)) with a conventional bed system (convention). The yield per unit area (g m\(^{-2}\)) was calculated as yield per plant (g/plant) \times planting density (plant number m\(^{-2}\)) for each treatment. The data for yield per plant with environmental control were obtained from plants grown in the movable bed system, as shown in Fig. 1c and d. On the movable bed system, plants were cultivated under the combined treatment with supplemental lighting, elevated CO\(_2\) and \(T_s\), and crown temperature control. The supplemental lighting and elevated CO\(_2\) and \(T_s\), treatments were applied as described for Experiment I. We previously observed that maintaining the temperature of the crown, which is the organ containing the shoot apical meristem, at about 20°C remarkably promoted the onset of anthesis, resulting in an increase in fruit yield of strawberry grown in a forcing culture (Dan et al., 2015). In the present study, the crown temperature treatment was conducted by controlling the temperature of water flowing in a polyvinyl chloride tube set to the crown. The water temperature in the tube was maintained at about 20°C by switching the cooling and/or heating with chiller (RKE3750A-V-SP, Orion Machinery Co., Ltd., Nagano, Japan) seasonally, and the crown temperature treatment was conducted from just after transplanting to the last day of cultivation. Data for yield per plant in the treatment lacking air-environment control were obtained from plants grown in the conventional bed system, as shown in Fig. 1a and b. In this system, plants were cultivated in the greenhouse where the air temperature was only controlled by setting the threshold temperatures for ventilation and heating at 26 and 6°C, respectively. The plants in the demonstration treatment and control were cultivated identically, i.e., in terms of cultivar, transplanting date, and water and fertilizer management, to those in Experiment I in the 2013 fiscal year. Fruit yield and growth characteristics of plants were also measured by identical methods to those employed in Experiment I.

To examine the effect of long-term treatment with elevated CO\(_2\) and \(T_s\) on leaf photosynthetic activity, the relationship between photosynthetic rate and intercellular CO\(_2\) concentration (A-C curve) was measured using the portable photosynthesis and fluorescence system. Measurements were conducted on the free expanded third leaflet of plants grown under the integration and convention treatments on 5–6 December 2013 (approximately 2 months after application of the elevated CO\(_2\) and \(T_s\) treatment). The environment in the leaf chamber was controlled as follows: 0, 50, 100, 150, 300, 400, 600, 800 or 1,000 \(\mu\)mol mol\(^{-1}\) CO\(_2\), 25°C air temperature, 50% relative humidity, and 1,000 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) PPFD. The initial slope of the A-C curve indicates the carboxylation capacity of Rubisco (Farquhar et al., 1980). We calculated the initial slope of the A-C curve for Ci less than 200 \(\mu\)mol mol\(^{-1}\) with reference to the method of Araya et al. (2006).

Statistical analysis

In Experiment I, we analyzed the data obtained under the AEC and SL treatments by two-way analysis of variance (ANOVA). The significance of differences between means among the respective treatments was tested using the Tukey-Kramer test. In Experiment II, the significance of differences between means among the control and treatments were tested with Student’s t-test. All statistical analyses were performed using the SAS Ver 9.2 statistical software (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Experiment I: Effects of air and light environment controls on strawberry growth and yield

Figure 3 shows diurnal changes in PPFD (a), monthly changes in PPFD integral (b) and yearly changes in PPFD integral (c) under the supplemental lighting (lighting) and non-lighting treatments, and outside the greenhouse. With regard to diurnal changes, PPFD in the lighting treatment drastically increased with the onset of supplemental lighting, and fluctuated between 500 and 600 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) during 06:00 to 10:00. From 10:00 to 15:00, PPFD in the lighting treatment fluctuated consistent with the outside PPFD, and thereafter fluctuated between 500 and 600 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) during 15:00 to 18:00. Especially in the periods from 06:00 to 10:00 and 15:00 to 18:00, PPFD in the lighting treatment was remarkably high compared with those recorded outside the greenhouse and in the non-lighting, PPFD in the non-lighting fluctuated at about half the values recorded outside. In terms of monthly changes, the PPFD integral in the lighting treatment was higher than those recorded outside the greenhouse and in the non-lighting treatment during December to May and, especially during December to February, the value was remarkably higher than that outside. The values of the PPFD integral in the non-lighting treatment fluctuated at about half the values recorded outside. A low-light environment in winter is a typical meteorological phenomenon in the northern part of Kyushu Island. Therefore, the positive effect of supplemental lighting on the PPFD integral was pronounced in winter. Yearly PPFD integral values in the non-lighting treatment were half those recorded outside the greenhouse. Higashide et al. (2014) reported that light transmission of a large-scale greenhouse, which was built to meet building and fire standards, was only 50–60% because of light blockage by film, ribs and structural frames. Our experimental large-scale greenhouse was also built to meet
building and fire standards, and furthermore the movable bed system (Fig. 1c, d) has many more structural frames for hanging the movable beds compared with those in the conventional bed system (Fig. 1a, b). These might have been responsible for the PPFD integrals in the non-lighting treatment being half those recorded in the outside environment. The large difference in PPFD integral values between 2012 and 2013 was not observed outside the greenhouse. From these results, it was considered that the effect of annual variation in the outside PPFD integral on the environmental controls of Experiment 1 in the 2012 and 2013 fiscal years was negligible.

Figure 4 shows diurnal changes in air temperature (a) and CO$_2$ concentration (b), and monthly changes in the daytime average values of air temperature (c) and CO$_2$ concentration (d) in the elevated CO$_2$ and $T_a$ treatment, non-treatment, and outside the greenhouse in winter. In diurnal variation, the air temperature of non-treatment gradually increased from 00:00 to 08:00, and thereafter fluctuated around 10°C until 08:00. After 08:00, the air temperature increased drastically and attained 26°C at 11:00, and thereafter fluctuated around 26°C from 11:00 to 16:00 and subsequently decreased with the decline in outside air temperature. In elevated CO$_2$ and $T_a$ treatment, the air temperature quickly increased with initiation of early morning heating at 05:00, and fluctuated around 15°C from 05:00 to 08:00. After 08:00, the air temperature drastically increased and attained 28°C at 11:00, and thereafter fluctuated around 27°C between 11:00 and 16:00. Therefore, the air temperatures in the elevated CO$_2$ and $T_a$ treatment in the morning were remarkably high compared with those of the non-treatment. The CO$_2$ concentration in the non-treatment gradually increased from about 550 to 600 μmol mol$^{-1}$ from 00:00 to 08:00, and thereafter decreased to 450 μmol mol$^{-1}$ at 12:00. Subsequently, it gradually increased from 450 to 540 μmol mol$^{-1}$ from 12:00 to 24:00. In the elevated CO$_2$ and $T_a$ treatment, the CO$_2$ concentration quickly increased with the start of CO$_2$ enrichment at 05:00, and fluctuated around approximately 1,000 μmol mol$^{-1}$ between 05:00 and 18:00. Thereafter the CO$_2$ concentration gradually decreased to 700 μmol mol$^{-1}$ from 18:00 to 24:00. Daytime average air temperature in the non-treatment was about 18°C from December to February. After February, the air temperature linearly increased with increasing PPFD integral outside (Fig. 3b), and attained about 25°C in May. The average air temperatures in the elevated CO$_2$ and $T_a$ treatment were about 1.5, 3 and 4°C higher than values of the non-treatment in December, January to February, and March to May, respectively. Daytime average CO$_2$ concentration in the non-treatment fluctuated from 450 to 490 μmol mol$^{-1}$ from December to February, and then gradually decreased to 425 μmol mol$^{-1}$ in May. The average CO$_2$ concentrations in the elevated CO$_2$ and $T_a$ treatment fluctuated between about 800 and 850 μmol mol$^{-1}$ from December to February, which were about 300–400 μmol mol$^{-1}$ higher compared with those of the non-treatment. After February, average CO$_2$ concentrations in the elevated CO$_2$ and $T_a$ treatment decreased to 480 μmol mol$^{-1}$ in May, which reduced the extent of the difference in average values between the elevated CO$_2$ and $T_a$ and non-treatment. It is considered that this decrease in average CO$_2$ concentration in the elevated CO$_2$ and $T_a$ treatment was caused by increase in the ventilation frequency to reduce the daytime air temperature in the greenhouse after March. Especially in April and May, average CO$_2$ concentrations in the elevated CO$_2$ and $T_a$ treatment remarkably decreased because CO$_2$ enrichment was conducted only in the morning.

From these results of daily and monthly changes in air temperature and CO$_2$ concentration, and leaf photosynthesis measurements (Fig. 2b), it is considered that the daytime CO$_2$ concentration and air temperature of the elevated CO$_2$ and $T_a$ treatment in winter were more effective environments to accelerate leaf photosynthesis than those in the non-treatment.

Figure 5 shows PPFD (a) and photosynthetic rate (b) of the fully expanded third leaflet under the supplemental lighting treatment among the different air-environment control treatments. With supplemental lighting on cloudy daytime, third leaflets were exposed to increased PPFD of 400
INCREASED STRAWBERRY YIELD

Supplemental lighting resulted in an increased photosynthetic rate more than 2-fold higher than that of non-lighting plants under elevated CO2 and TA treatment and non-treatment. The CO2 elevation treatment accelerated leaf photosynthesis about 2 times higher than that of non-treatment under lighting, however, in the non-lighting treatment, the photosynthetic rate of elevated CO2 treatment was lower than that of non-treatment under lighting (<100 mol m\(^{-2}\) s\(^{-1}\)). Therefore, it is considered that an adequate light level is essential to induce the positive effect of CO2 enrichment on leaf photosynthesis.

Table 1 shows the effect of air-environment control and supplemental lighting on flowering characteristics. In the first and second inflorescences, the anthesis and flower number were significantly affected by supplemental lighting and air-environment controls. In the first inflorescence, supplemental lighting accelerated the onset of flowering compared with that of the non-lighting. Furthermore, the earliness of anthesis with supplemental lighting was additionally promoted by elevated CO2 and TA treatment. Consequently, flowering date of plants receiving supplemental lighting and elevated CO2 and TA treatments was about 10 d earlier than that of non-lighting and non-treated plants. Elevated CO2 and TA treatment also promoted anthesis under the non-lighting condition. Flower number was increased by supplemental lighting, and was further boosted by elevated CO2 and TA treatment. Flower number in plants treated with supplemental lighting and elevated CO2 and TA was about 6 flowers as many as that of non-lighting with non-treatment. Elevated CO2 and TA treatment also increased flower number under the non-lighting condition. The onset of anthesis and flower number in the

Fig. 4 Diurnal changes in air temperature (a) and CO2 concentration (b), and monthly changes in daytime average values of air temperature (c) and CO2 concentration (d) under the elevated CO2 and air temperature treatment (elevated CO2 & TA), and the non-treatment of air environment. The data of diurnal changes in the elevated CO2 & TA and outside were obtained by measurements in December 29 in 2013, and in the non-treatment were obtained in December 27 in 2012, respectively. The data of monthly changes in the elevated CO2 & TA were obtained by measurements in fiscal year 2013 (December in 2013 to May in 2014), and in the non-treatment were obtained in fiscal year 2012 (December in 2012 to May in 2013), respectively. The daytime data of air temperature and CO2 concentration from 06:00 to 18:00 were used to calculate the average values.

Fig. 5 PPFD (a) and photosynthetic rate (b) of fully expanded third leaflet under the different treatments of air-environment control (elevated CO2 or non-treatment) among the supplemental lighting treatments (lighting or non-lighting). Data are mean ± S.E. (n=4). Results of two-way ANOVA with supplemental lighting (SL) and air-environment control (AEC) as independent variables and their interaction (SL × AEC) for each dependent variable are shown in each panel. Different letters indicate significant differences by Tukey-Kramer test among 4 treatments (P<0.05). Photosynthetic rate was measured at the controlled environment of air temperature 25°C, relative humidity 50% and CO2 concentration of 1,000 (elevated CO2) or 400 (non-treatment) μmol mol\(^{-1}\) with a portable photosynthesis and fluorescence system (LI-6400XT, LI-COR) on cloudy daytime.

μmol m\(^{-2}\) s\(^{-1}\) compared with that in the non-lighting at leaf heights of 10 cm above the plant base. Supplemental lighting resulted in an increased photosynthetic rate more than 2-fold higher than that of non-lighting plants under the elevated CO2 and TA treatment and non-treatment. The CO2 elevation treatment accelerated leaf photosynthesis about 2 times higher than that of non-treatment under lighting, however, in the non-lighting treatment, the photosynthetic rate of elevated CO2 treatment was lower than that of non-treatment by the low light environment below 100 μmol m\(^{-2}\) s\(^{-1}\). Therefore, it is considered that an adequate light level is essential to induce the positive effect of CO2 enrichment on leaf photosynthesis.

Table 1 shows the effect of air-environment control and supplemental lighting on flowering characteristics. In the first and second inflorescences, the anthesis and flower number were significantly affected by supplemental lighting and air-environment controls. In the first inflorescence, supplemental lighting accelerated the onset of flowering compared with that of the non-lighting. Furthermore, the earliness of anthesis with supplemental lighting was additionally promoted by elevated CO2 and TA treatment. Consequently, flowering date of plants receiving supplemental lighting and elevated CO2 and TA treatments was about 10 d earlier than that of non-lighting and non-treated plants. Elevated CO2 and TA treatment also promoted anthesis under the non-lighting condition. Flower number was increased by supplemental lighting, and was further boosted by elevated CO2 and TA treatment. Flower number in plants treated with supplemental lighting and elevated CO2 and TA was about 6 flowers as many as that of non-lighting with non-treatment. Elevated CO2 and TA treatment also increased flower number under the non-lighting condition. The onset of anthesis and flower number in the
second inflorescence in response to supplemental lighting and/or air-environment control showed the same pattern as that observed in the first inflorescence.

In our previous studies, it was observed that the higher photosynthetic activity of plants stimulated by 12 h illumination with high-irradiance LED resulted in a shortened period from flower bud differentiation to anthesis through acceleration of plant growth (Hidaka et al., 2014b; 2015). The acceleration of flower opening in response to supplemental lighting and elevated CO\(_2\) and \(T_a\) treatment is hypothesized to be caused by acceleration of plant growth through increase in leaf photosynthesis, and further acceleration of growth rate by increase in the average air temperature might also contribute to the acceleration of flower opening in the elevated CO\(_2\) and \(T_a\) treatment.

Sung and Chen (1991) reported that acceleration of leaf photosynthesis increased fruit set per plant. Therefore, an increase in flower number in the first and second inflorescences under both supplemental lighting and elevated CO\(_2\) and \(T_a\) treatment should be induced by a higher allocation of carbohydrates, which are produced abundantly with increased leaf photosynthesis, to bud primordia.

Table 2 shows the effect of air-environment control and supplemental lighting on SSC, TA, FF, and the maturation period of fruits. Supplemental lighting resulted in higher SSC values compared with the non-lighting in the presence or absence of elevated CO\(_2\) and \(T_a\) treatment. Treatment with elevated CO\(_2\) and \(T_a\) decreased SSC values both in the presence or absence of supplemental lighting. Supplemental lighting increased TA both with and without elevated CO\(_2\) and \(T_a\) treatment. The observed increases in SSC may also be caused by higher allocation of photosynthesize to fruits through acceleration of leaf photosynthesis under LED lighting; this finding confirmed results from our previous studies (Hidaka et al., 2013; 2014b; 2015). Although leaf photosynthesis under elevated CO\(_2\) was higher than that in non-treated plants under supplemental lighting (Fig. 5b), a decrease in SSC was observed in the elevated CO\(_2\) and \(T_a\) treatment compared with that of non-treated plants. According to Kumakura and Shishido (1994), increasing average air temperature shortens the maturation period of strawberry fruits, and this brings the low SSC of fruits through shortening the period of photosynthesize accumulation in the fruits. In the present study, the fruit maturation period under elevated CO\(_2\) and \(T_a\) was significantly shorter than that of non-treated plants. Therefore, it is considered that the lower SSC of fruits under elevated CO\(_2\) and \(T_a\) was caused by shortening of the fruit maturation period by the increase in average air temperature as shown in Fig. 4c. The increased in fruit TA under supplemental lighting would be caused by the high sugar accumulation to fruits as observed in our previous study (Hidaka et al., 2015).

Figure 6 shows average fruit weight, number of fruits, and marketable yield under the air-environment control and supplemental lighting treatments. Supplemental lighting resulted in significantly increased average fruit weight, which was about 1.2–1.4 times higher than that in the non-lighting. Under supplemental lighting, the average fruit weight was significantly decreased by elevated CO\(_2\) and
T area. Supplemental lighting resulted in an increase in the number of fruits, and the fruit number in the early season (December to February) was about 1.7 and 1.5 times higher than non-lighting in the presence or absence of elevated CO\textsubscript{2} and T\textsubscript{a}. Through the entire fruiting season (December to May), fruit number under supplemental lighting was about 1.7 and 1.4 times higher than non-lighting in the presence or absence of elevated CO\textsubscript{2} and T\textsubscript{a} treatment. The elevated CO\textsubscript{2} and T\textsubscript{a} also resulted in an increase in the number of fruits, and the fruits number in the early season was about 1.8 and 2 times higher than non-treated plants under non-lighting and lighting. Through the entire fruiting season, fruit number under elevated CO\textsubscript{2} and T\textsubscript{a} treatment was about 1.2 and 1.5 times higher than non-treatment under non-lighting and lighting. By the combinational treatment of supplemental lighting with elevated CO\textsubscript{2} & T\textsubscript{a}, the number of fruits in the respective early and entire fruiting season increased to 3.1 and 2.1 times of those under non-lighting with non-treatment. Marketable yield per plant was also increased by supplemental lighting, and the values in the early and the entire fruiting seasons were about 1.9 and 2.2 times higher than non-lighting under non-treatment or elevated CO\textsubscript{2} and T\textsubscript{a} treatment. The elevated CO\textsubscript{2} and T\textsubscript{a} treatment also increased in marketable yield, and the values in the early season was about 1.8 and 2.1 times higher than non-treatment under non-lighting or lighting. The fruits yields of elevated CO\textsubscript{2} and T\textsubscript{a} through entire season were about 1.2 times higher than non-treatment under non-lighting or lighting. By the combinational treatment of supplemental lighting with elevated CO\textsubscript{2} & T\textsubscript{a}, the marketable yield in the respective early and entire fruiting season was increased to 3.9 and 2.4 times of those under non-lighting with non-treatment.

Although supplemental lighting and elevated CO\textsubscript{2} and T\textsubscript{a} enhanced leaf photosynthesis, the average fruit weight was increased under supplemental lighting but was decreased under elevated CO\textsubscript{2} and T\textsubscript{a} treatment. Roussos et al. (2009) observed that fruit size can be enhanced by increasing the supply of photosynthesate to fruit. Therefore, we attribute the observed increase in average fruit weight under supplemental lighting to higher allocation of photosynthesate to fruit. Furthermore, Kumakura and Shishido (1994) reported that increasing average air temperature decreased fruit weight due to shortening of the fruit maturation period. Therefore, the decrease in average fruit weight under elevated CO\textsubscript{2} and T\textsubscript{a} may have been caused by reduction in the fruit maturation period with increasing average air temperature, similar to the decrease in fruit SSC.

The number of fruits per plant was increased by supplemental lighting and elevated CO\textsubscript{2} and T\textsubscript{a}, which reflects the increased fruit number per inflorescence (Table 1) and acceleration of the harvest time of fruit. In the early season from December to February, elevated CO\textsubscript{2} and T\textsubscript{a} treatment increased the number of harvested fruits, and these numbers of harvested fruits (14 and 23 fruits in non-lighting and lighting) under elevated CO\textsubscript{2} and T\textsubscript{a} were more than all flower numbers of first inflorescence (Table 1), while in spite of the number of harvested fruits under non-treatment were less than all flower numbers of first inflorescence. Therefore, the remarkable increase in fruit number under elevated CO\textsubscript{2} and T\textsubscript{a} in the early season reflected the advance in harvest time due to the shortened fruit maturation period with increasing average air temperature. This positive effect of elevated CO\textsubscript{2} and T\textsubscript{a} on fruit number in the early season was decreased in the whole fruiting season. The average air temperature in the elevated CO\textsubscript{2} and T\textsubscript{a} treatment and the non-treatment after March drastically increased (Fig. 4c), and this led to the decrease in average CO\textsubscript{2} concentration under the elevated CO\textsubscript{2} and T\textsubscript{a} treatment due to the increase in ventilation frequency after March (Fig. 4d). Therefore, the depression in the positive effect of elevated CO\textsubscript{2} and T\textsubscript{a} on fruit number over the whole fruiting season may be attributed to the decrease in leaf photosynthesis with decreasing average CO\textsubscript{2} concentration after March. On the other hand, the positive effect of supplemental lighting on fruit number in the early season was maintained at similar levels over the entire fruiting season. This might reflect the comparatively high PPFD integral throughout the fruiting season in the supplemental lighting.
treatment (Fig. 3b).

The marketable yield fundamentally reflected fruit number more than the average fruit weight, and showed an almost identical pattern as fruit number. In the early fruiting season, when the market price of strawberry fruit is high, the elevated CO$_2$ and $T_a$ treatment led to an approximately twofold increase in fruit yield, but this positive effect decreased after March. The positive effect of supplemental lighting on fruit yield was maintained at an almost twofold increase over the whole fruiting season. Combined application of supplemental lighting and elevated CO$_2$ and $T_a$ would generate an approximately fourfold increase (2 times in lighting $\times$ 2 times in elevated CO$_2$ and $T_a$) in fruit yield compared with the fruit yield in untreated plants in the early season. Over the whole season, this combined treatment resulted in a 2.4-times increase (2 times in lighting $\times$ 1.2 times in elevated CO$_2$ and $T_a$) in fruit yield compared with the untreated plants.

Figure 7 shows NAR, LAI, SLW, CGR, GR$_S$, and GR$_R$ before fruit set under the supplemental lighting and air-environment control treatments. Supplemental lighting resulted in significantly higher NAR in the presence or absence of elevated CO$_2$ and $T_a$. Elevated CO$_2$ and $T_a$ had no significant effect on NAR either in the presence or absence of supplemental lighting. Supplemental lighting had no effect on LAI, although elevated CO$_2$ and $T_a$ treatment resulted in significantly higher LAI. Conversely, SLW was significantly increased by supplemental lighting, but it was not affected by elevated CO$_2$ and $T_a$ treatment. CGR and GR$_S$ showed similar patterns to that of LAI. Supplemental lighting resulted in a significant increase in GR$_S$, but in contrast GR$_R$ was significantly decreased by elevated CO$_2$ and $T_a$ treatment.

The significant increase in NAR under supplemental lighting may be caused by the enhanced leaf photosynthetic rate (Fig. 5). The higher NAR of illuminated leaves would have affected SLW rather than LAI. Chabot and Chabot (1977) detected increases in leaf thickness and SLW under high light conditions, and suggested that the observed leaf thickening was related to increases in mesophyll cell size and amounts of mesophyll tissue produced. Thus, enhanced leaf photosynthesis under supplemental lighting may have contributed to leaf thickening rather than an enlargement in leaf area before fruit set. A significant increase in LAI was only observed with elevated CO$_2$ and $T_a$ treatment, which suggests that the increase in LAI was not caused by enhanced leaf photosynthesis but some other physiological factors. Erwin et al. (1991) reported that alternation of the difference between day and night temperatures (DIF) affects plant morphogenesis, and observed an increase in leaf area as DIF increased (day temperature $>$ night temperature) in Fuchsia $\times$ hybrida plants. This morphogenetic phenomenon may be induced by the phytohormone gibberellin (Stavang et al., 2010). Paroussi
et al. (2002) reported that leaf area of June-bearing strawberries was greater in response to treatment with a high concentration of gibberellins rather than a low concentration. In the present study, the average daytime air temperature was higher in elevated CO$_2$ and $T_s$ treatment than non-treatment due to early morning heating and the high temperature threshold for initiation of ventilation. This led to the higher DIF in the elevated CO$_2$ and $T_s$ treatment compared with the non-treatment (data not shown). Therefore, the significant increase in LAI with elevated CO$_2$ and $T_s$ may be caused by the increased DIF through gibberellin signaling. It can be considered that the difference of effect of elevated CO$_2$ and $T_s$ treatment between NAR (calculated by CGR / LAI) and leaf photosynthetic rate (Fig 5b) was caused by the remarkable increase in LAI through plant morphogenetic phenomenon under the increased DIF.

Dry matter partitioning to shoots ($GR_s$) and roots ($GR_r$) before fruit set was changed by treatment with supplemental lighting and elevated CO$_2$ and $T_s$. With elevated CO$_2$ and $T_s$ treatment, 59% shoot vs 41% root dry matter partitioning rates were remarkably changed to 94% shoot vs 6% root partitioning without supplemental lighting. With supplemental lighting, 50% shoot vs 50% root partitioning rates were changed to 86% shoot vs 14% root by elevated CO$_2$ & $T_s$ treatment. This remarkable increase in dry matter partitioning to shoots under elevated CO$_2$ and $T_s$ may be caused by the increase in LAI. Comparison of the lighting and non-lighting treatments showed that supplemental lighting increased dry matter partitioning to roots. Therefore, before fruit set, leaf thickening and high photosynthetic partitioning to roots under supplemental lighting, and leaf expansion and high photosynthetic partitioning to shoots under elevated CO$_2$ and $T_s$ were observed.

Figure 8 shows NAR, LAI, SLW, CGR, $GR_s$, $GR_r$ and $GR_F$ after fruit set under treatment with elevated CO$_2$ and $T_s$ and supplemental lighting. Supplemental lighting resulted in significantly higher NAR in the presence and absence of elevated CO$_2$ and $T_s$. In contrast, elevated CO$_2$ and $T_s$ treatment, tended to decrease NAR with and without supplemental lighting. Results of two-way ANOVA with supplemental lighting (SL) and air-environment control (AEC) as independent variables and their interaction (SL × AEC) for each dependent variable are shown in each panel. NS, not significant ($P \geq 0.05$). Different letters indicate significant differences by Tukey-Kramer test among 4 treatments ($P < 0.05$).
supplemental lighting, but the differences were not significant. Supplemental lighting had little effect on \( \text{LAI} \), whereas elevated CO\(_2\) and \( T_s \) treatment resulted in significantly higher \( \text{LAI} \). \( \text{SLW} \) was significantly increased by supplemental lighting, but was significantly decreased by elevated CO\(_2\) and \( T_s \) treatment. Both \( \text{CGR} \) and \( \text{GR} \) were significantly increased by supplemental lighting, and this positive effect was further increased by elevated CO\(_2\) and \( T_s \) treatment. Supplemental lighting increased \( \text{GR} \), but only in combination with elevated CO\(_2\) and \( T_s \) treatment.

The significant increases in \( \text{NAR} \) and \( \text{SLW} \) under supplemental lighting may be caused by enhanced leaf photosynthesis similar to the results observed before fruit set (Fig. 7a, c). The significant increase in \( \text{LAI} \), which reflects leaf expansion, under elevated CO\(_2\) and \( T_s \) is also likely to be caused by the same mechanism as observed before fruit set (Fig. 7b). The significant decrease in \( \text{SLW} \), which reflects leaf thickness, under elevated CO\(_2\) and \( T_s \) may be caused by a trade-off relationship between leaf area expansion and leaf thickening as the leaf growth under the high sink demand of fruits. The positive effect of supplemental lighting on \( \text{GR} \), which is an index of the rate of photosynthate partitioning to fruit, was highest under the elevated CO\(_2\) & \( T_s \) treatment. This can be attributed to an increase in fruit yield during the early fruiting season through increase in flower number per inflorescence and early harvesting caused by enhanced leaf photosynthesis and increased average air temperature as discussed above. The percentages of dry matter partitioning to fruit in all treatments exceeded 60%. Although the percentages of dry matter partitioning to roots before fruit set were about 41%, 50% and 40% without supplemental lighting and elevated CO\(_2\) and \( T_s \), supplemental lighting alone, and under elevated CO\(_2\) and \( T_s \) with supplemental lighting treatments, after fruit set partitioning to roots decreased to less than 10% in all treatments. Therefore, after fruit set, most photosynthates were translocated to the fruit despite the increasing amount of photosynthates that resulted from supplemental lighting and elevated CO\(_2\) and \( T_s \). This difference was probably caused by the markedly higher sink strength of fruit than other organs (shoots and roots) as was seen in our previous study (Hidaka et al., 2014b; 2015).

**Experiment II: Integration of environmental controls and movable bed system to increase yield**

Figure 9 shows average fruit weight, number of fruits per unit area and marketable yield per unit area under the conventional cultivation method (convention) or the newly demonstrated method (integration). In convention, plants were grown on the conventional bed system (see Fig. 1a, b), which enables 8 plants \( \text{m}^2 \). In integration, plants were grown on the movable bed system (see Fig. 1c, d), which enables 12 plants \( \text{m}^2 \), with the combinational environmental controls of supplemental lighting, elevated CO\(_2\) & \( T_s \), and crown temperature treatments. Data are mean ± S.E. (\( n=6 \)). *, ***, indicate significant differences at \( P<0.05, P<0.001 \) by \( t \)-test among treatments.

![Fig. 9](image-url) Average fruit weight, number of fruits per unit area and marketable yield per unit area under the conventional cultivation method (convention) or the newly demonstrated method (integration). In convention, plants were grown on the conventional bed system (see Fig. 1a, b), which enables 8 plants \( \text{m}^2 \). In integration, plants were grown on the movable bed system (see Fig. 1c, d), which enables 12 plants \( \text{m}^2 \), with the combinational environmental controls of supplemental lighting, elevated CO\(_2\) & \( T_s \), and crown temperature treatments. Data are mean ± S.E. (\( n=6 \)). *, ***, indicate significant differences at \( P<0.05, P<0.001 \) by \( t \)-test among treatments.

was 333 fruits \( \text{m}^2 \) in the convention and 668 fruits \( \text{m}^2 \) in the integration. The number of fruits per plant was 41.7 fruits / plant in the convention and 55.7 fruits / plant in the integration. Therefore, the twofold increase in fruit number per unit area in the integration treatment may result from the 1.3-times increase in fruit number per plant \( \times 1.5 \)-times higher planting density. Marketable fruit yield per unit area over the entire season was 4.6 kg \( \text{m}^{-2} \) (4.6 t / 10 a) in the convention and 10.7 kg \( \text{m}^{-2} \) (10.7 t / 10 a) in the integration (a more than twofold increase). Marketable yield per plant was 577 g / plant in the convention and 888 g / plant in the integration. This 1.5 times increase in fruit yield per plant may result from the 1.15-times increase in average fruit weight \( \times 1.3 \)-times increase in fruit number per plant under the combination treatment of supplemental lighting, elevated CO\(_2\) and \( T_s \), and crown temperature treatment. Therefore, the 2.3-times increase in marketable fruit yield per unit area in the integration treatment may be caused by the 1.5-times increase in marketable yield per plant \( \times 1.5 \)-times higher planting density.

Figure 10 shows leaf area, \( \text{SLW} \) and total dry matter of respective organs (leaves, crown and roots) of plants grown

![Figure 10](image-url) Leaf area, \( \text{SLW} \) and total dry matter of respective organs (leaves, crown and roots) of plants grown.
INCREASED STRAWBERRY YIELD

under the conventional cultivation method (convention) or the newly demonstrated method (integration). Leaf area and SLW in the integration were 1.5- and 1.3-times higher than those in the convention, respectively. These increases of leaf area and SLW in the integration would be caused by the same reason as observed in the results under the elevated CO₂ and T₀, and supplemental lighting in Experiment I (Fig. 8). Total dry matter in the integration was 2-times higher than that in convention. Furthermore, marked increases in dry matter were observed for leaves, crowns, and roots (1.8, 3, and 2.1 times higher, respectively), in the integration compared with the convention. Therefore, supplemental lighting, elevated CO₂ & T₀, and crown temperature treatment in the integration treatment were indicated to enhance leaf photosynthesis, resulting in the promotion of plant growth, and this would account for the more than twofold increase in fruit yield observed.

In our previous study (Hidaka et al., 2014b), long-term exposure of strawberry plants to the optimal environment for leaf photosynthesis induced downregulation of leaf photosynthesis under the condition of plants limited the sink demand. In this study, we also investigated the effect of long-term environment control on leaf photosynthetic capacity. Figure 11 shows the relationships between ambient air CO₂ concentration and photosynthetic rate (A) of leaves (a), intercellular CO₂ concentration (C) and A of leaves (b), and the initial slope of A-C curve (c) of strawberry plants grown in treatments of convention or integration. Data are mean ± S.E. (n=3), * indicate significant differences at P<0.05, ** at P<0.01 by t-test among treatments. Photosynthetic rates were measured at the controlled environment of CO₂ concentration (0, 50, 100, 150, 300, 400, 600, 800, 1,000 µmol mol⁻¹), air temperature 25°C, relative humidity 50% and PPFD 1,000 µmol m⁻² s⁻¹ with a portable photosynthesis and fluorescence system (LI-6400XT, LI-COR) on cloudy daytime. Values of initial slope of A-C, curve (c) were calculated by the slope of regression line of A-C (b) for the range of C below 200 µmol mol⁻¹.
CO₂ enrichment was initiated in October, before anthesis of Chabot, B. F., Chabot, J. F. 1977. Effects of light and temperature. Araya, T., Noguchi, K., Terashima, I. 2006. Effects of carbohydrate accumulation on photosynthesis differ between sink and source leaves of Phaseolus vulgaris L. Plant Cell Physiol. 47: 644–652. Chabot, B. F., Chabot, J. F. 1977. Effects of light and temperature on leaf anatomy and photosynthesis in Fragaria vesca. Oecologia 26: 363–377. Dan, K., Sageno, W., Nakahara, S., Goto, N., Iwasaki, K., Takano, I., Okimura, M., Hidaka, K., Takayama, T., Imamura, H. 2015. Experiment on the crown-temperature control technique in forcing culture of strawberries in Miyagi. In “Strawberries: Cultivation, Antioxidant Properties and Health Benefits” (ed. by Malone, N.). Nova Science Publishers, New York. p 309–342. Hidaka, K., Miyoshi, Y., Ito, E., Kitano, M. 2014a. Vertically-moving cultivation and local environment control for high strawberry yield. In “Strawberries: Cultivation, Antioxidant Properties and Health Benefits” (ed. by Malone, N.). Nova Science Publishers, New York. p 309–342. Hidaka, K., Araki, T., Miyoshi, Y., Dan, K., Imamura, H., Kitano, M., Sameshima, K., Okimura, M. 2014b. Effect of photoperiod of supplemental lighting with light-emitting diodes on growth and yield of strawberry. Environ. Control Biol. 52: 63–71. Higashide, T., Oshio, T., Nukaya, T., Yasuba, K., Nakano, A., Suzuki, K., Ohmori, H., Kaneko, S. 2014. Light transmissi- on of a greenhouse (NARO Tsukuba factory farm) built to meet building and fire standards. Bull. Natl. Inst. Veg. Tea Sci. 13: 27–33. Kawanishi, Y. 1996. Elevated CO₂ and C₃, C₄ photosynthesis. (in Japanese text) Environ. Control Biol. 34: 3–9. Kawashima, N. 1991. Studies on the CO₂ enrichment in a green- house (3) Effect on the growth of strawberry. (in Japanese text with English summary) Bull. Nara Agric. Exp. Sta. 22: 65–72. Kumakura, H., Shishido, Y. 1994. The effect of daytime, night-time, and mean diurnal temperature on the growth of ‘Morioka-16’ strawberry fruit and plants. (in Japanese text with English summary) J. Jpn. Soc. Hortic. Sci. 62: 827–832. Long, S. P. 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO₂ concentration: Has its importance been underestimated? Plant Cell Environ. 14: 729–739. Nagasaki, Y., Hayashi, S., Nakamoto, Y., Kawashima, H., Kohno, Y. 2013. Development of a table-top cultivation system for robot strawberry harvesting. JARQ 47: 165–169. Paroussi, G., Voyiatzis, D. G., Paroussis, E., Drogoudi, P. D. 2002. Growth, flowering and yield responses to GA₃ of strawberry grown under different environmental conditions. Sci. Hortic. 96: 103–113. Qian, T., Dieleman, J. A., Elings, A., Marcelis, L. F. M. 2012. Leaf photosynthetic and morphological responses to elevated CO₂ concentration and altered fruit number in the semi-closed greenhouse. Sci. Hortic. 145: 1–9. Roussos, P. A., Denaxa, N-K., Damakaris, T. 2009. Strawberry fruit quality attributes after application of plant growth stimulat- ing compounds. Sci. Hortic. 119: 138–146. Stavang, J. A., Pettersen, R. I., Wendell, M., Solhaug, K. A., Junttila, O., Moe, R., Olsen, J. E. 2010. Thermoperiodic growth control by gibberellin does not involve changes in photosynthetic or respiratory capacities in pea. J. Exp. Bot. 61: 1015–1029. Sung, F. J. M., Chen, J. I. 1991. Gas exchange rate and yield re- sponse of strawberry to carbon dioxide enrichment. Sci. Hortic. 48: 241–251.

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This lower sink strength of plants under CO₂ enrichment is suggested to cause the downregulation of leaf photosynthesis. Thus, it is expected that further increase in fruit yield could be achieved by adjusting the timing of initiation of CO₂ enrichment to correspond with the sink strength of plants.

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