

Understanding photosynthate translocation dynamics and regulatory mechanisms in greenhouse-grown strawberries

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Understanding photosynthate translocation dynamics and regulatory mechanisms in greenhouse-grown strawberries

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Preface

Background

Plants take carbon dioxide (CO₂) from the air through photosynthesis and convert it into carbohydrates, which serve as sources of energy and substrate for growth and maintenance. These photosynthates are translocated via phloem from leaves (sources) to various parts of the plant (sinks) (Geiger & Fondy, 1979). The growth and dry matter accumulation of non-photosynthetic organs such as fruits depend on translocated photosynthates. Therefore, photosynthate translocation (hereinafter “translocation”) is important physiological process which directly influences yield and quality in fruit production.

To ensure highly profitable and stable crop production, it is crucial to develop cropping systems that take advantage of plant eco-physiological processes. In particular, environmental control and agronomic management, which enhance plant productivity are, the primary approaches that farmers can adopt to improve yield. Photosynthesis is one of the most studied plant processes, and various environmental control techniques has been introduced to enhance photosynthesis in fruit production (Choi et al., 2015; Hidaka et al., 2016). While translocation also has been studied as a possible target for improving plant productivity (Braun et al., 2014; Hidaka et al., 2019), controlling translocation remains a significant challenge. The primary bottlenecks in applying translocation to cropping systems are the complexity of its mechanisms and the difficulties of measuring its dynamics.

Translocation involves multiple physiological processes (Taiz et al., 2015). Photosynthates produced in source leaves are allocated to various metabolic pathways. Some fraction of that is moved from mesophyll chloroplasts into the phloem through phloem loading and subsequently exported from sources. The photosynthates are then transported within the phloem and partitioned among various sinks. Finally, they are imported from the phloem into the surrounding tissues in sinks through phloem unloading. The mechanisms of the photosynthate

loading at sources and unloading at sinks can be passive, where photosynthates move through plasmodesmata by diffusion, and/or active, where photosynthates move against their concentration gradient through energy-requiring transporters. Whether the pathway is symplastic or apoplastic depends on species and organs (Braun, 2022). For the mechanism of transport in the phloem, pressure-flow hypothesis proposed by Münch (1930) is widely accepted. According to the hypothesis, the photosynthate loading at sources lowers the osmotic potential, drawing water from surrounding tissues and increasing turgor pressure in the phloem. In contrast, photosynthate unloading at sinks raises the osmotic potential and removes water, decreasing turgor pressure in the phloem. As a result, the pressure gradient in the phloem is generated, which drives photosynthates transport from source to sink. These processes are influenced by various environmental factors, though their regulatory mechanisms are not well known (Lemoine et al., 2013; Smith et al., 2018). Moreover, plants can exhibit different physiological responses to fluctuating environments, such as those in agricultural fields, compared to those observed in highly controlled and simplified environments (Annunziata et al., 2018). To develop cropping systems that optimize translocation, it is important to understand how these processes respond to environmental factors under field conditions.

Analyzing the dynamics of translocation is crucial for elucidating its regulatory mechanisms. Nevertheless, no practical and versatile method for quantifying translocation dynamics has been established, making it challenging to study translocation (Turgeon & Wolf, 2009). Ethylenediaminetetraacetic acid-based methods to collect phloem exudates do not require additional equipment but are unsuitable for evaluating translocation dynamics as cutting petioles significantly changes the source/sink balance (King & Zeevaart, 1974). Tracer methods using carbon radioactive-isotope labeling are non-invasively and can provide continuous evaluation of translocation dynamics; however, this approach requires special measurement equipment and facilities (Moorby et al., 1974; Troughton & Moorby, 1974; Hidaka et al., 2019). Evaluation based on the carbon balance requires plant destruction, but by appropriately defining

each term in the balance equation, it becomes a useful approach for obtaining continuous translocation dynamics (Ho, 1978; Araki et al., 1997; Lacointe & Minchin, 2008).

Mathematical modeling has also been applied to analyze translocation dynamics (Minchin & Lacointe, 2005). Furthermore, models that couple translocation dynamic and plant growth can be useful for successfully optimizing translocation because they allow to test the effects of cropping system on crop yield and quality. In these models, transport in the phloem is physically described based on the pressure-flow hypothesis, whereas the dynamics of photosynthate loading and unloading are not well-formulated (Jensen et al., 2012). Because the loading and unloading provide the driving force for phloem transport in the pressure-flow hypothesis, further insights into them are crucial to improve the models to simulate translocation dynamics.

Strawberry (*Fragaria × ananassa* Duch.) is commercially important fruit crop for its taste and rich nutritional value (Giampieri et al., 2012). The fruit comprises a fleshy receptacle and numerous achenes, arranged in branched clusters known as inflorescences. In protected strawberry cultivation, various environmental controls techniques, such as CO₂ enrichment and light supplementation, have been actively implemented to enhance photosynthesis (Choi et al., 2015; Hidaka et al., 2016; Yoneda et al., 2020; Zhang et al., 2020). Recently, optimizing both photosynthesis and subsequent photosynthate translocation has gained increased interest to achieve higher yield and higher quality (Hidaka et al., 2019; Miyoshi et al., 2021, 2023).

Summary of this thesis

This thesis focused on photosynthate loading and unloading among the continuous processes that constitute translocation, aiming to elucidate their dynamics and regulatory mechanisms in strawberries under fluctuating environmental conditions. Since it is currently impossible to quantify these cellular processes under such conditions, I evaluated the dynamics of loading and unloading by assuming that photosynthates export from sources and import into

sinks directly represent these processes. Understanding the physiological processes governing plant productivity is fundamental for developing cultivation systems that further enhance fruit crop yield and quality. Summary is presented in Figure P.1.

In Chapter 1, the dynamics of photosynthate loading were investigated under field conditions across three cultivation periods with different day/night cycles. I evaluated the diurnal and seasonal changes in the loading rate based on the leaf carbon balance. Furthermore, the relationship between loading dynamics and leaf carbohydrate concentrations was investigated, focusing on the conversion of photosynthates to sucrose or starch—i.e., photosynthate allocation between sucrose and starch—as a regulatory mechanism for the photosynthate loading. The content of Chapter 1 was published as Nakai et al. (2022) in *Environmental and Experimental Botany*.

In Chapter 2, photosynthate allocation between sucrose and starch was further analyzed to enhance the understanding of the regulatory mechanism of photosynthate loading. I hypothesized that the allocation during the daytime is strongly influenced not by day length but by photosynthetic activity. To test this hypothesis and explore the inherent characteristic of photosynthate allocation in strawberries, carbohydrate dynamics were studied under controlled environmental conditions using plants grown within the same cultivation period. The content of Chapter 2 was published as Nakai et al. (2023) in *Plant Growth Regulation*.

In Chapter 3, the dynamics of photosynthate unloading during fruit growth were investigated. Unloading is often suppressed when multiple sinks are present, as the sinks compete for translocated photosynthates from the source. To evaluate potential unloading dynamics, I conducted experiments under the condition that exclude the effects of sink competition by fruit removal. Furthermore, I analyzed the sink's competitive ability, termed sink strength, as the determinant of potential photosynthate unloading. The content of Chapter 3 was published as Nakai et al. (2024) in *HortScience*.

In Chapter 4, the regulatory mechanisms of photosynthate unloading were investigated

based on the hypothesis that unloading and subsequent fruit growth are determined by the balance between source strength (the capacity to supply photosynthates) and sink strength (the capacity to attract photosynthates) within the plant. To achieve this, source and sink strength were experimentally manipulated using supplemental lighting and fruit removal, and fruit growth and photosynthates accumulation were analyzed.

The final chapter presents the general conclusions and future directions of this study, highlighting fundamental findings that will contribute to the development of cultivation systems based on photosynthate translocation.

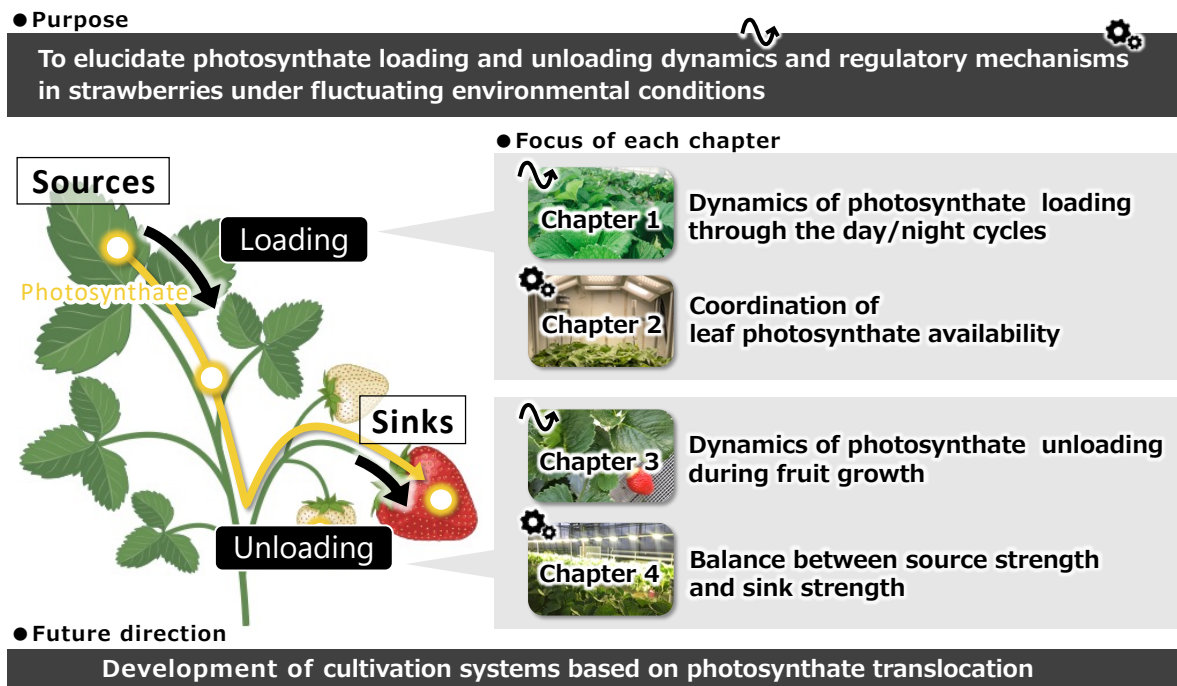


Figure P.1 Schematic diagram of summary of this study.

Chapter 1

Dynamics of photosynthate loading in leaves through the day/night cycle under field condition

1.1. Introduction

Plants coordinate the availability of photosynthates in source leaves for utilization (growth and metabolism), storage, and export via translocation through the day/night cycle (Graf et al., 2010; Taiz et al., 2015). In many species, photosynthates are converted to sucrose for immediate utilization and starch for storage. At night when no photosynthesis occurs, plants convert starch stored in leaves into sucrose for use (Geiger & Servaites, 1994; Geiger et al., 1995; Stitt et al., 2010; Stitt & Zeeman, 2012). Throughout the day, sucrose is loaded into the phloem and exported by translocation.

Studies on *Arabidopsis* have demonstrated that the coordination of leaf starch availability is flexible: the leaf starch content increases during the daytime at an almost constant rate and decreases linearly at night at a rate such that starch is almost but not completely exhausted by dawn (Zeeman et al., 1998; Smith & Stitt, 2007; Gibon et al., 2009). These starch dynamics are maintained even when the day/night cycle suddenly changes (Gibon et al., 2004; Graf et al., 2010), suggesting that starch availability is optimized to prevent carbon starvation (Graf & Smith, 2011; Feugier & Satake, 2013).

In contrast, the complex coordination of leaf photosynthate availability underlying the dynamics of translocation including photosynthate loading is poorly understood. Most studies have examined the coordination in model plants (*Arabidopsis*) in controlled environments with regular step-like changes in the light environment. Therefore, the coordination in relation to photosynthate loading in horticultural crops such as strawberries grown under field conditions with diurnal light changes is not clear. Such fluctuating light environments may involve more

complex coordination processes. Indeed, Annunziata et al. (2018) emphasized an importance of plant metabolic studies to understand how plant responses to field conditions that various environmental factors vary simultaneously.

Moreover, strawberry plants develop multiple inflorescences in sequence and produce fruits around three times per year, during which they are exposed to different day/night cycles. The differences in light environments among cultivation periods likely influence the coordination of leaf photosynthate availability and relate to productivity.

The objective of this chapter was to elucidate the dynamics of photosynthate loading in relation to the coordination of photosynthate availability under field conditions with diurnal light changes. Through three different day/night cycles, leaf photosynthetic rates and carbohydrate concentrations were evaluated, and loading dynamics were estimated based on the leaf carbon balance.

1.2. Materials and methods

1.2.1. Plant materials and growth conditions

Strawberry plants (*Fragaria × ananassa* Duch. ‘Fukuoka S6’) were grown under natural light conditions in a greenhouse (20 m long × 8 m wide × 4 m high) at Ito Plant Experiment Field & Facilities, Faculty of Agriculture, Kyushu University, Japan. On October 3, 2019, the nursery plants were transplanted into cultivation beds (9.7 m long × 0.3 m wide × 0.12 m high) filled with M-1 substrate (Kaneya Co., Ltd., Aichi, Japan). A total of 96 plants were planted in two rows at intervals of 0.2 m between plants and 0.18 m between rows. Nutrient solution (OK-F-1, OAT Agrio Co., Ltd., Tokyo, Japan; an electrical conductivity = 0.6 dS m⁻¹; N:P:K = 75:40:85 mg L⁻¹) with an electrical conductivity of 0.6 dS m⁻¹ was supplied at a rate of 200 mL d⁻¹/plant. To control the greenhouse air temperature, the heater threshold temperature was set to 8°C, and the ventilation was set to 22°C. Upon the experiments, each plant used had 8 leaves

and a total of 7 with fruits and/or flowers.

1.2.2. Measurement of environmental conditions

In the greenhouse, photosynthetic photon flux density (PPFD), air temperature, air relative humidity, ambient air CO₂ concentration, and wind speed were measured using a light quantum meter (PAR-02D, Prede Co., Ltd., Tokyo, Japan), a temperature-humidity sensor (HMP60, Vaisala, Finland) installed in a forced ventilation shelter (RSVH01A1203, SCE, Inc., Hokkaido, Japan), a CO₂ sensor (GMP252, Vaisala, Finland), and an ultrasonic anemometer (81000, R. M. Young Company, USA), respectively. To investigate the environments that strawberry plants were exposed, sensors were located at the center of the cultivation beds at a height of 1.2 m (near the top of the canopy). Data were logged at 5 s intervals, and 5 min means were automatically stored using a data logger (CR1000, Campbell Scientific, USA).

1.2.3. Estimation of photosynthetic rate in a single leaf

The photosynthetic rate was estimated using the biochemical photosynthesis model (Farquhar–von Caemmerer–Berry; FvCB model) (Farquhar et al., 1980), the stomatal model (Medlyn et al., 2011), and the energy balance equation of the leaf. Model calculations were performed in R version 4.3.1 (R Core Team, 2023) in the RStudio environment (version 2023.06.0) (RStudio Team, 2023). For the photosynthetic model parameters, the maximum Rubisco carboxylation rate at 25°C ($V_{\text{cmax},25}$), the maximum rate of electron transport at 25°C ($J_{\text{max},25}$), and the residual conductance (g_0) and empirical slope (g_1) of the stomatal conductance model were determined using the R package “plantecophys” (version 1.4.4, Duursma, 2015). To estimate $V_{\text{cmax},25}$ and $J_{\text{max},25}$, the net photosynthetic rate (A)–intercellular CO₂ concentrations (C_i) (A – C_i) curves were measured using a portable open gas-exchange system (LI-6400XT, LI-COR, USA) with an LED light source chamber (6400-40, LI-COR, USA). For A – C_i curves

measurements, five or six fully expanded leaves were randomly selected from the top of the canopy a few days before the leaf sampling experiments in the three cultivation periods. The leaf temperature was set to 25°C, and the photosynthetic rate was measured sequentially at the following ambient CO₂ concentrations: 400, 300, 200, 100, 60, 400, 600, 800, 1000, 1200, and 1600 $\mu\text{mol mol}^{-1}$ under the saturated light intensity (1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The $A-C_i$ curves were fitted to the FvCB model using the “fitaci” function of “plantecophys,” and $V_{\text{cmax},25}$ and $J_{\text{max},25}$ were estimated. To estimate g_0 and g_1 , diurnal changes in photosynthetic rate, stomatal conductance, ambient air CO₂ concentration, and leaf-to-air vapor pressure deficit were measured concurrently during experiments using a portable open gas-exchange system (LI-6400XT, LI-COR, USA) with a top transparent chamber (standard leaf chamber, LI-COR, USA). Data were fitted to the stomatal model using the “fitBB” function, and g_0 and g_1 were estimated. The above parameters were averaged among each cultivation period and used to estimate the photosynthetic rate (Table 1.1). The day respiration rate (R_d) was calculated as 40% of the dark respiration rate (R_n) (Villar et al., 1995). R_n depends on temperature and can be described by an Arrhenius function. To estimate R_n , the night respiration rate was measured using a portable open gas-exchange system (LI-6400XT, LI-COR, USA) with an LED chamber in darkness (0 $\mu\text{mol m}^{-2} \text{s}^{-1}$) at various leaf temperatures (14, 16, 18, 20, 22°C) and 400 $\mu\text{mol mol}^{-1}$ ambient CO₂. Data were fitted to the Arrhenius function, and the temperature dependence of R_n ($R_{n,25}$: the night respiration rate at 25°C; $E_a(R_n)$: activation energy for R_n) was obtained. Photosynthetic rate estimation was performed as follows. First, the arbitrary stomatal conductance was set as the initial value, and the leaf temperature was obtained using the “tleaf” function of the R package “tealeaves” (version 1.0.5, Muir, 2019) using the strawberry leaf characteristic dimension of 0.064 m (Kimura et al., 2020) and the default values for the other package parameters. Second, the photosynthetic rate was estimated using the “Photosyn” function of the R package “plantecophys” using the estimated leaf temperature and photosynthetic model parameters, the temperature dependence of photosynthetic capacity

($E_a(V_{\text{cmax}})$, $E_a(J_{\text{max}})$): activation energy for V_{cmax} and J_{max} ; $\Delta S(V_{\text{cmax}})$, $\Delta S(J_{\text{max}})$): entropy factor for V_{cmax} and J_{max}) from Kimura et al. (2020) (Table 1.1), and the default values for the other package parameters. Stomatal conductance was calculated from the obtained photosynthetic rate using the stomatal model (Medlyn et al., 2011). These steps were repeated until the difference between the initial and estimated stomatal conductance was fully minimized, and finally, the leaf photosynthetic rate was estimated.

Table 1.1 Photosynthetic model parameters for estimating the photosynthetic rate of a single leaf.

Parameter	Value	Unit	Reference
$V_{\text{cmax},25}$	120.5 (Dec.) 110.4 (Apr.) 109.6 (May)	$\mu\text{mol m}^{-2} \text{s}^{-1}$	This study
$E_a(V_{\text{cmax}})$	53.2	kJ mol^{-1}	Kimura et al. (2020)
$\Delta S(V_{\text{cmax}})$	0.637	kJ K mol^{-1}	Kimura et al. (2020)
$J_{\text{max},25}$	233.6 (Dec.) 228.0 (Apr.) 204.5 (May)	$\mu\text{mol m}^{-2} \text{s}^{-1}$	This study
$E_a(J_{\text{max}})$	23.7	kJ mol^{-1}	Kimura et al. (2020)
$\Delta S(J_{\text{max}})$	0.639	kJ K mol^{-1}	Kimura et al. (2020)
$R_{\text{n},25}$	1.46	$\mu\text{mol m}^{-2} \text{s}^{-1}$	This study
$E_a(R_{\text{n}})$	53.5	kJ mol^{-1}	This study
g_0	0.0354 (Dec.) 0.0186 (Apr.) 0.0165 (May)	$\text{mol m}^{-2} \text{s}^{-1}$	This study
g_1	2.75 (Dec.) 2.83 (Apr.) 1.57 (May)	Unitless	This study

$V_{\text{cmax},25}$: the maximum Rubisco carboxylation rate at 25°C; $E_a(V_{\text{cmax}})$: activation energy for V_{cmax} ; $\Delta S(V_{\text{cmax}})$: entropy factor for V_{cmax} ; $J_{\text{max},25}$: the maximum rate of electron transport at 25°C; $E_a(J_{\text{max}})$: activation energy for J_{max} ; $\Delta S(J_{\text{max}})$: entropy factor for J_{max} ; $R_{\text{n},25}$: the night respiration rate at 25°C; $E_a(R_{\text{n}})$: activation energy for R_{n} ; g_0 : the residual conductance; g_1 : the empirical slope.

1.2.4. Leaf sample processing and analysis of carbohydrate concentrations

For the carbohydrate concentration analysis, leaf sampling experiments were conducted on two days in three cultivation periods: December 20 and 27, 2019 (Dec.); April 2 and 7, 2020 (Apr.); May 13 and 21, 2020 (May). Dawn/dusk times in the study region were around 7:20/17:15 (Dec.), 6:00/18:40 (Apr.), and 5:15/19:15 (May). Assuming that the day/night cycles were 10-h day/14-h night (Dec.), 12-h day/12-h night (Apr.), and 14-h day/10-h night cycle (May), sampling was performed at 2-h intervals in the daytime, and 2- or 4-h intervals at night. On each day, 120–165 fully expanded leaves were randomly selected from the top of the canopy. At each sampling time, a leaf disk (6-mm diameter, 28.3 mm²) was punched from every leaf, avoiding main veins (Figure 1.1), collecting sets of 15 disks, i.e., 18–19 replicates were obtained in 2 days of each cultivation period.

Samples were stored at –80°C until the carbohydrate analysis. Soluble sugars (sucrose, glucose, and fructose) were extracted using the method described by Nishizawa (1994), with some modifications. Freeze-dried leaf disk samples were ground into powder using a ball mill at 30 Hz for 15 min, and three extraction steps were performed using 0.8 mL, 0.6 mL, and 0.6 mL of 80% aqueous ethanol (v/v) at 82°C for 5 min, respectively. Ethanol solutions were transferred to covered test tubes before the third extraction. The homogenates were centrifuged at 12,000 min^{–1} and 24°C for 5 min after the first and second extractions and at 4,000 min^{–1} at room temperature for 15 min after the third extraction. The pellet (ethanol insoluble fractions) was used for the starch concentration analysis, and the pooled ethanolic extract was evaporated to dryness and resolubilized in 1.5 mL of distilled water for the soluble sugar concentration analysis. The solution was centrifuged at 15,000 min^{–1} and 4°C for 5 min, and the upper layer (the aqueous phase) was filtered through a 0.45-μm membrane filter and used for the high-performance liquid chromatography (HPLC) analysis. Soluble sugar concentrations were measured using the HPLC system (Shimadzu Corp., Japan) following Fujiwara et al. (1999)

with some modifications. The HPLC system consisted of an online degassing unit (DGU-20A), pump unit (LC-20AD), auto-sampler (SIL-20A), refractive index detector (RID-10A), and column oven (CTO-20A). The LC-Solution software (Shimadzu Corp., Japan) was used for controls and data acquisition. Experiments were performed on the column (Shim-pack SCR-101N, Shimadzu Corp., Japan) (300 mm $\times$ 7.9 mm i.d., 10 μ m). The mobile phase was ultrapure water at a flow rate of 0.7 mL min⁻¹ at 40°C, and the injection volume was 20 μ L. Starch concentration was analyzed using a Total Starch Assay kit (K-TSTA, Megazyme, Ireland) according to the manufacturer's protocol with slight modifications. The pellet was suspended in 3 mL of sodium acetate (100 mM, pH 5.0), and 30 μ L of α -amylase (2500 U mL⁻¹) was added. Samples were vortexed and then placed in a dry bath incubator at 100°C where they were removed and vortexed for 5 s after 2, 5, and 10 min. After 15 min, samples were vortexed for 5 s and placed in a 50°C water bath for 5 min to equilibrate. Next, 30 μ L of amyloglucosidase was added to the samples, which were vortexed for 3 s before incubation at 50°C for 30 min. Sample aliquots (2 mL) were transferred to microfuge tubes and centrifuged at 13,000 min⁻¹ and 24°C for 5 min. The colorimetric analysis reagent for the K-TSTA (GOPOD) was added to a 50 μ L aliquot of the supernatant and placed in a 50°C water bath for 20 min. Starch was quantified as glucose equivalents based on the glucose-GOPOD standard curve using a spectrophotometer (UV-1800, Shimadzu Corp., Japan) at 510 nm. For comparative purposes, carbohydrate concentrations were expressed as moles of carbon per unit leaf area.



Figure 1.1 Leaf sampling process.

1.2.5. Calculation of photosynthate loading rate

Net photosynthesis was defined as the net carbon inflow to leaves with consideration of carbon loss via respiration. Therefore, the loading rate of photosynthates was calculated using the leaf carbon balance equation (Eq. 1.1; De Swaef et al., 2013):

$$Load = Photo - \frac{dCarbon}{dt} \quad (1.1)$$

Load is the loading rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), *Photo* is the net photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), and *Carbon* is the carbohydrate carbon content in leaves ($\mu\text{mol m}^{-2}$). Assuming the main carbohydrates contained in strawberry leaves are sucrose, glucose, fructose, and starch, except for cell wall components (Nishizawa, 1994), Eq. 1.1 converts to the following equation (Eq. 1.2):

$$Load = Photo - \frac{dSuc}{dt} - \frac{dGlu}{dt} - \frac{dFru}{dt} - \frac{dSta}{dt} \quad (1.2)$$

Suc, *Glu*, *Fru*, and *Sta* are sucrose, glucose, fructose, and starch concentration ($\mu\text{mol m}^{-2}$), respectively. The average photosynthetic rate for each leaf sampling interval was used as *Photo* in Eq. 1.2.

1.2.6. Calculation of relative contribution of carbon sources to loading

Dividing both sides of Eq. 1.2 by *Load*, it converts to Eq. 1.3:

$$1 = RC_{\text{Photo}} - RC_{\text{Suc}} - RC_{\text{Glu}} - RC_{\text{Fru}} - RC_{\text{Sta}} \quad (1.3)$$

RC_{Photo} , RC_{Suc} , RC_{Glu} , RC_{Fru} , and RC_{Sta} are the relative indices that compare the effects of photosynthesis, sucrose, glucose, fructose, and starch on photosynthate loading, respectively. In this study, they were defined as the contribution ratio to loading and calculated per time interval.

1.2.7. Statistical analyses

Statistical analyses were performed in R version 4.3.1 (R Core Team, 2023) in the RStudio environment (version 2023.06.0) (RStudio Team, 2023). The significance of the correlations between sucrose concentration and loading rate was checked with Pearson's correlation test using the *cor* function of the R stats package (R Core Team, 2023).

1.3. Results and discussion

1.3.1. Growth conditions and leaf photosynthesis

Figure 1.2 shows the diurnal changes in PPFD, air temperature, and photosynthetic rate in three cultivation periods. PPFD increased after dawn and peaked 6–8 h later, reaching daily maxima of 630 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Dec.), 1410 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Apr.), and 1690 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (May). PPFD temporarily dropped around 8 h after dawn in common in all cultivation period, which would be due to interception of solar radiation by the frame material of the greenhouse. Air temperature began to increase according to solar radiation (represented as PPFD) in the early morning, remaining in the range of 15–25°C (regulated by temperature control equipment), except for one night in Dec. with a minimum below 5°C. The photosynthetic rate (estimated

using models) changed depending on PPFD in Dec., in addition to ambient air CO₂ concentration in Apr. and May. Decrease of PPFD by interception mentioned above was also reflected to the estimated photosynthetic rate. The daily maximum photosynthetic rate in every cultivation period was approximately 15 $\mu\text{mol C m}^{-2} \text{ s}^{-1}$, although photosynthetic model parameters ($V_{\text{cmax},25}$, $J_{\text{max},25}$; Table 1.1) decreased in order from Dec. to May. Possible reasons for this are as follows: leaf temperature related to both solar radiation (represented as PPFD) and air temperature tended to generally increase from Dec. to May, which would induce an increase in photosynthetic capacities (V_{cmax} , J_{max}). Additionally, when the photosynthetic rate was daily maximum, PPFD would reach the light saturation point, as suggested by Hidaka et al. (2013). Consequently, the photosynthetic rate would be strongly affected by the CO₂ concentration, which remained at approximately 450 $\mu\text{mol mol}^{-1}$ during the daytime across all cultivation periods, leading the relatively constant rates of photosynthesis. The maximum was similar to that reported previously for strawberry plants (Hidaka et al., 2013; Choi et al., 2016).

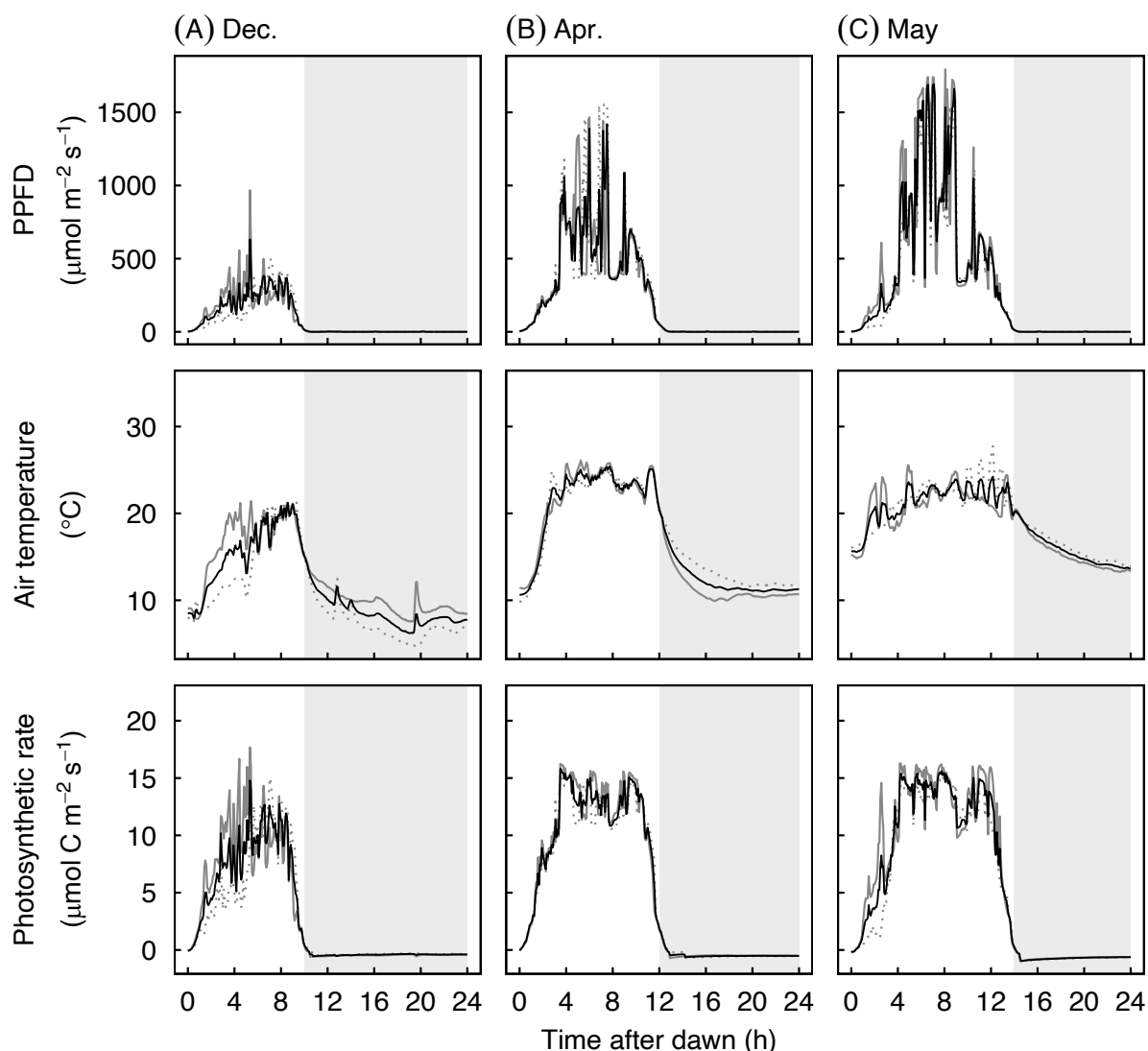


Figure 1.2 Diurnal changes in photosynthetic photon flux density (PPFD), air temperature, and estimated leaf photosynthetic rate from dawn in Dec. (A), Apr. (B), and May (C). The day/night cycles were roughly 10-h day/14-h night (Dec., A), 12-h day/12-h night (Apr., B), and 14-h day/10-h night (May, C). Day and night are indicated by white and gray background colors, respectively. Data from two observation dates in each cultivation period are shown using a gray solid line (the first day) and a gray dotted line (the second day); means are shown using a black solid line.

1.3.2. Leaf carbohydrate concentration

Figure 1.3 shows the diurnal changes in sucrose, glucose, fructose, and starch concentrations in leaves in three cultivation periods. Sucrose concentration in Dec. began to increase at dawn from approximately 75 mmol C m^{-2} , peaked 2–4 h before dusk at 160 mmol

C m⁻², and then decreased linearly to 100 mmol C m⁻² by the next dawn. The dynamics were similar in Apr., whereas, in May, the sucrose concentration was generally higher than those in other cultivation periods, with a daily maximum of approximately 180 mmol C m⁻². The glucose concentration was almost constant throughout the day at approximately 10 mmol C m⁻² in Dec. and 25 mmol C m⁻² in Apr. and May. Similarly, fructose concentration was almost constant throughout the day at approximately 50 mmol C m⁻² in Dec. and 70 mmol C m⁻² in Apr. and May. In all three cultivation periods, starch began to accumulate 2–4 h after the sucrose concentration increased. In Dec., the starch concentration increased from approximately 20 mmol C m⁻² to 60 mmol C m⁻², peaking at dusk, and then decreasing linearly until the next dawn. Although the starch level was the same at dawn in Apr., it peaked at 140 mmol C m⁻², more than double that in Dec. In May, the starch concentration was generally higher than those in other cultivation periods, reaching a daily maximum of 220 mmol C m⁻².

The observed sucrose concentrations were much larger than those previously reported in *Arabidopsis* leaves (Gibon et al., 2004). Additionally, the sucrose concentration in strawberry fluctuated significantly during the day, differing from *Arabidopsis*, in which sucrose dynamics were mostly stable and low (~0.5–1.5 mmol C m⁻²; Smith et al., 2004). Since sucrose is less reactive than glucose and fructose, it is the most common carbohydrate form for translocation in plants (Arnold, 1968) and storage in leaves of some plant species (Lunn & Hatch, 1995). In strawberry leaves, photosynthetic assimilates are translocated in the form of sucrose (Forney & Breen, 1985c; Nishizawa, 1994). The results of the present study suggest strawberry leaves have a greater sucrose storage capacity than *Arabidopsis*, which could contribute to differences in the coordination of leaf photosynthate availability. In contrast, reducing sugars, such as glucose and fructose, are more reactive; thus, they typically act as osmolytes (Hartmann & Trumbore, 2016) and are hardly accumulated in leaves. This is reflected in the relatively steady dynamics of glucose and fructose observed in the present study. The linear decrease of starch concentrations recorded from dusk in each cultivation period was consistent with the results

previously reported for *Arabidopsis* leaves (Graf et al., 2010). They suggested that the rate of starch degradation at night was adjusted to the night length by circadian control. The results of the present study suggest that the coordination of leaf starch availability in the nighttime in strawberry is under circadian control as well. Additionally, this perspective provides the attractive possibility that strawberry plants can adapt to differences in day/night cycles among cultivation periods.

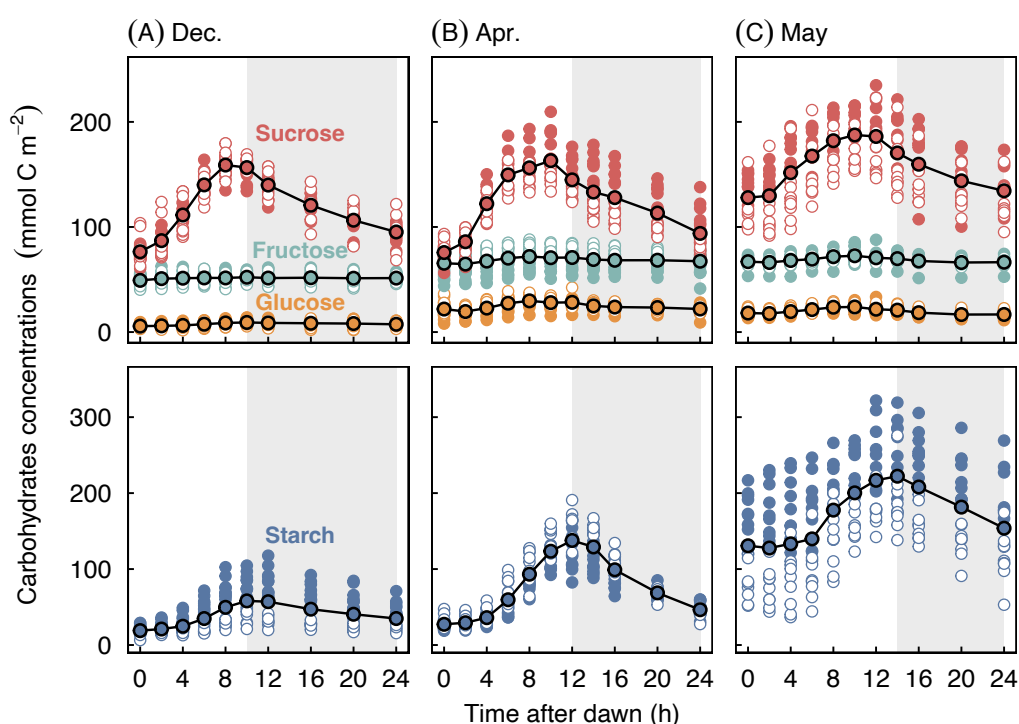


Figure 1.3 Diurnal changes in concentrations of leaf sucrose, glucose, fructose, and starch from dawn in Dec. (A), Apr. (B), and May (C). The day/night cycles were roughly 10-h day/14-h night (Dec., A), 12-h day/12-h night (Apr., B), and 14-h day/10-h night (May, C). Day and night are indicated by white and gray background colors, respectively. Data from two observation dates ($n=18-19$) in each cultivation period are shown using closed symbols (the first day) and open symbols (the second day); means are shown using black-edged symbols and a solid line.

1.3.3. Photosynthate loading rate

Figure 1.4 shows the diurnal changes in loading rates in three cultivation periods. The loading rate was calculated using Eq. 1.2, which assumed that the photosynthetic rate was

common in all replicates, and the carbohydrate concentrations were represented as individual values for each replicate. Therefore, some scatter was detected among the calculated values due to individual differences in carbohydrate concentrations (Figure 1.3). The loading rate was near zero ($\sim 0\text{--}2 \mu\text{mol C m}^{-2} \text{ s}^{-1}$) at dawn in every cultivation period. Similar to the sucrose concentration, the loading rate in Dec. and Apr. tended to increase in the daytime, peaking 2–4 h before dusk and then dropping to reach the same level as previous dawn. In May, the loading rate increased from the early morning to 2–4 h before dusk with some fluctuations and then dropped at dusk and remained low until dawn, similar to that in other cultivation periods. The daily maxima were approximately $6 \mu\text{mol C m}^{-2} \text{ s}^{-1}$ (Dec.), $10 \mu\text{mol C m}^{-2} \text{ s}^{-1}$ (Apr.), and $12 \mu\text{mol C m}^{-2} \text{ s}^{-1}$ (May). The reason why the loading rate in the daytime generally increased from Dec. to May may be an increase in cumulated photosynthesis in leaves (approximately 260, 450, and 500 mmol C m^{-2} in Dec., Apr., and May, respectively), as suggested in Miyoshi et al. (2021). In addition, high temperature is able to activate photosynthate turnover in leaves, which may trigger an increase in the loading rate.

The loading rate tended to increase even as the photosynthetic rate stagnated or decreased in the afternoon in the three cultivation periods with different day/night cycles. These results suggest that loading rate in the daytime does not depend only on photosynthesis but might be affected by the coordination of leaf photosynthate availability. In contrast, only stored photosynthate are available at night, representing a significant decline in leaf photosynthate availability for translocation compared to that in the daytime. Therefore, the loading rate could be greatly limited by leaf photosynthate storage, consequently reducing the rate to the low levels observed at night in the present study.

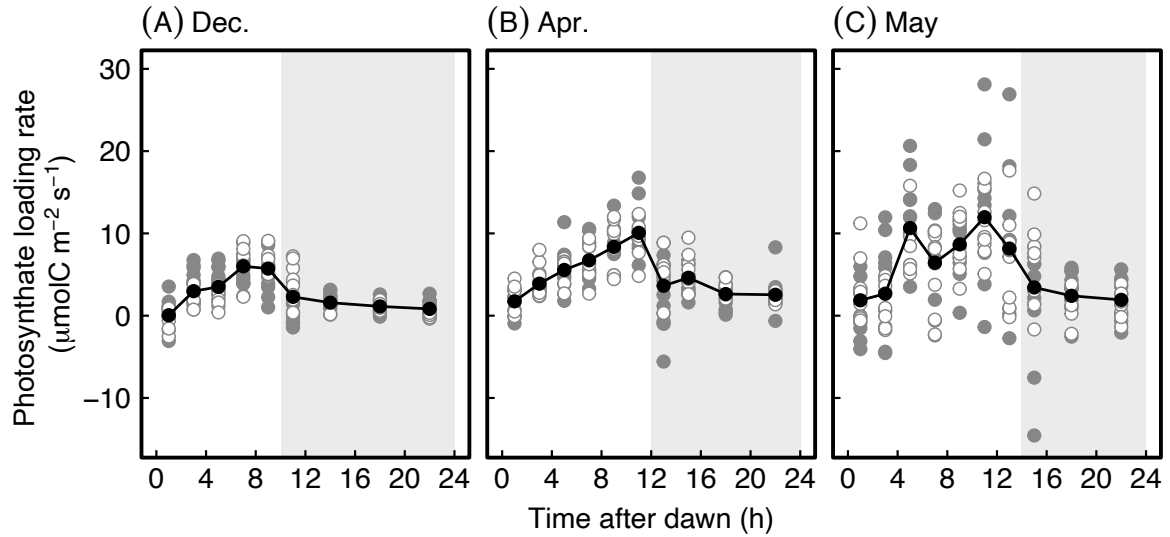


Figure 1.4 Diurnal changes in photosynthate loading rates from dawn in Dec. (A), Apr. (B), and May (C). The day/night cycles were roughly 10-h day/14-h night (Dec., A), 12-h day/12-h night (Apr., B), and 14-h day/10-h night (May, C). Day and night are indicated by white and gray background colors, respectively. Data from two observation dates ($n=18-19$) in each cultivation period are shown using gray symbols (the first day) and white symbols (the second day); means are shown using black symbols and a solid line.

1.3.4. Relative contribution of carbon sources to loading

Figure 1.5 shows the diurnal changes in the relative contributions of photosynthesis, sucrose, glucose, fructose, and starch to loading, providing evidence for the relative importance of carbon sources in translocation. In the daytime, the relative contribution of photosynthesis exceeded one, whereas the contributions of carbohydrates were negative. This indicates that translocation was usually supported by photosynthesis, and photosynthesis also supported carbohydrate synthesis during the daytime. Moreover, a positive contribution of photosynthesis was observed just after dawn, consistent with Hidaka et al. (2019), who reported that photosynthates are quickly (~ 40 min) translocated from the leaf to the petiole in strawberry plants. At night, the relative contribution of carbohydrates exceeded one, and that of photosynthesis was negative. These results indicate that carbohydrates accumulated in leaves supported translocation and leaf respiration at night.

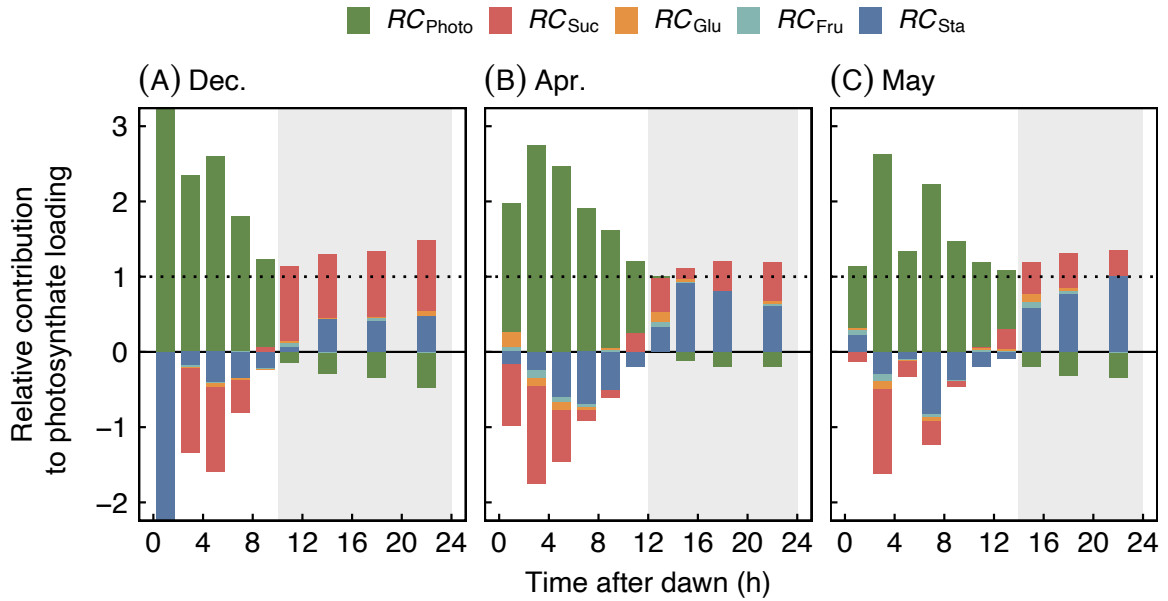


Figure 1.5 Diurnal changes in the relative contributions of photosynthesis (RC_{Photo}), sucrose (RC_{Suc}), glucose (RC_{Glu}), fructose (RC_{Fru}), and starch (RC_{Sta}) to loading. The day/night cycles were roughly 10-h day/14-h night (Dec., A), 12-h day/12-h night (Apr., B), and 14-h day/10-h night (May, C). Day and night are indicated by white and gray background colors, respectively. The total height of each column equals 1. A portion of the first column in A is omitted because the value was extremely large due to the low loading rate.

1.3.5. Effects of leaf sucrose and starch concentration on photosynthate availability coordination

Figure 1.6 shows the relationships between leaf sucrose concentration and starch concentration in the daytime and nighttime. Considering all cultivation periods together, starch concentration increased gradually in the daytime when sucrose concentration was less than 150 mmol C m⁻² but increased rapidly when the sucrose concentration exceeded 150 mmol C m⁻². The plateau-like pattern observed at dusk reflected the time difference in the onset of declines in sucrose and starch concentrations. In contrast, the starch concentration decreased linearly with sucrose concentration at night. Figure 1.7 shows the relationships between leaf sucrose concentration and loading rate in the daytime and nighttime. The loading rate was significantly

positively correlated with sucrose concentration in the daytime ($r = 0.84$, $p < 0.001$ for the average data from two observation dates; $r = 0.81$, $p < 0.001$ for the full data), but the relationship was not significant at night ($r = 0.45$, $p = 0.162$ for the average data from two observation dates; $r = 0.11$, $p = 0.633$ for the full data). These results suggest that leaf sucrose concentration affects the coordination of leaf photosynthate availability for storage and loading in the daytime, but the effect is not outstanding at night.

Moorby & Jarman (1975) proposed that plants coordinate leaf photosynthate availability by allocating photosynthates to conceptual sucrose and starch “storage pools” for storage and a sucrose “labile pool” for loading. Based on this concept, the following can be inferred from the results of the present study: In the daytime when sufficient photosynthates are produced, the allocation of photosynthates to starch storage pools increases when leaf sucrose concentration exceeds the capacity of sucrose storage and/or labile pools, as shown by the biphasic increase in starch concentration in Figure 1.6. Additionally, photosynthates allocated in the sucrose labile pool should increase with leaf sucrose concentration, increasing the loading rate. These effects of leaf sucrose concentration on the coordination of photosynthate availability in the daytime are consistent with the overflow hypothesis that starch is synthesized when the photosynthetic rate exceeds the loading rate and sucrose storage capacity of the leaf (Cseke et al., 1984; Stitt et al., 2010). In contrast, available photosynthates in the nighttime are supplied from both sucrose and starch storage pools. This is reflected in the linear decrease in sucrose concentration with starch observed at night (Figure 1.6), even though starch should be converted into sucrose. At night, available photosynthates depend on their storage at dusk, resulting in lower photosynthate availability for loading than that in the daytime. As mentioned above, some investigations on *Arabidopsis* showed that plants adjusted the photosynthate supply rate from the starch storage pool to the night length via circadian control (Graf et al., 2010; Graf & Smith, 2011). Similarly, circadian control may affect the rate of photosynthate supply from the storage pool in strawberry. Therefore, the coordination of leaf photosynthate availability at night would

be affected by leaf photosynthate storage and circadian control rather than sucrose concentration, which is consistent with the low impact of loading rate on sucrose concentration observed in this study (Figure 1.7).

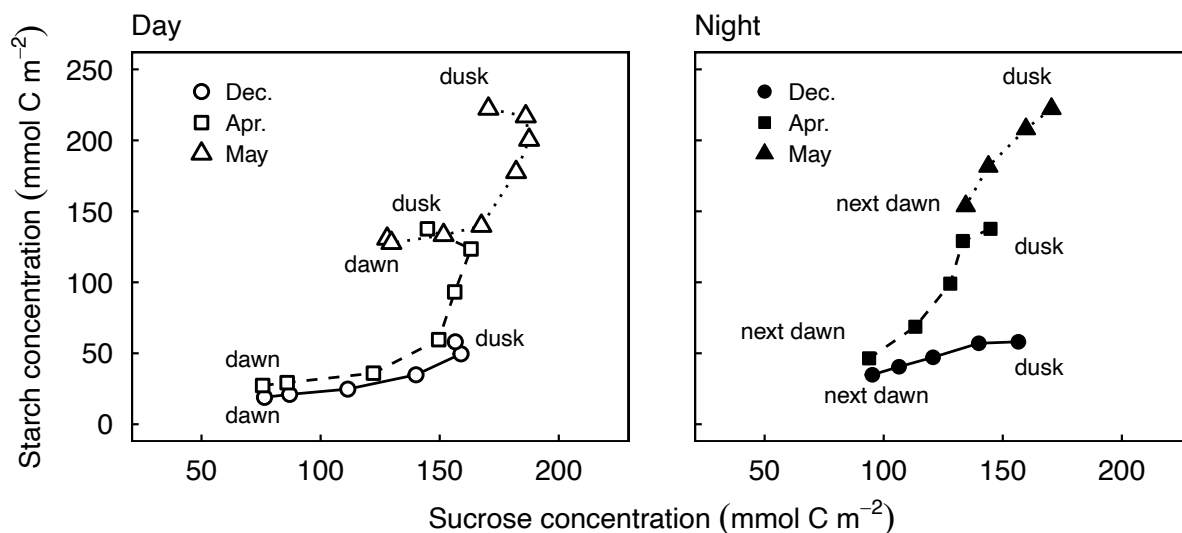


Figure 1.6 Relationships between sucrose and starch concentrations (Dec.: circle; Apr.: square; May: triangle) in the daytime (white symbols) and nighttime (black symbols). Plots are connected in order of time per each cultivation period.

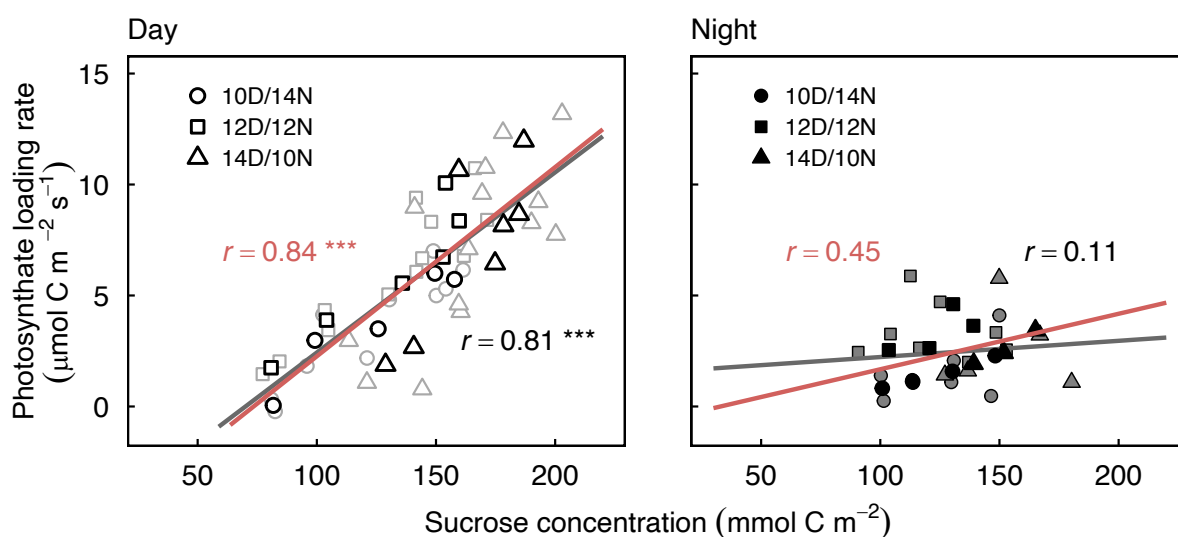


Figure 1.7 Relationships between sucrose concentrations and loading rates (Dec.: circle; Apr.: square; May: triangle) in the daytime (white symbols for the average data from two observation dates; gray symbols for the full data) and nighttime (black symbols for the average data from two observation dates; gray symbols for the full data). Red solid lines denote regression lines

for the average data from two observation dates, and black lines denote that for the full data. Pearson's correlation coefficients (r) are shown, and three asterisks (***) indicate p -values less than 0.001.

1.4. Conclusion

In this chapter, the dynamics of photosynthate loading were evaluated based on the leaf carbon balance, and the effects of photosynthate availability coordination on these dynamics were analyzed in strawberries grown under field conditions. During the daytime, when photosynthesis is the primary source of available photosynthates, it contributes to both photosynthate loading and storage of sucrose and starch. Increasing sucrose concentration enhance loading, and when it exceeded 150 mmol m^{-2} , starch synthesis became significant. In the nighttime, when available photosynthates are supplied by sucrose and starch stored during the daytime, starch concentrations decreased linearly, and the loading rate was lower than the daytime level.

These results suggest that the coordination of photosynthate availability through allocation between sucrose and starch regulates loading dynamics during the daytime; however, this would not apply at night. Although this study provided considerable insight into the regulatory mechanism of photosynthate loading, it may be insufficient to elucidate an inherent characteristic of strawberry leaves. A potential issue is that the experiments were conducted across three different day/night cycles. According to studies on *Arabidopsis*, circadian control can affect the coordination of photosynthate availability not only during the nighttime but throughout the day. Therefore, the next chapter addresses this issue to further understanding of the regulatory mechanism of photosynthate loading.

Chapter 2

Coordination of leaf photosynthate availability in response to photosynthetic activity

2.1. Introduction

Photosynthetically fixed carbon is allocated to multiple metabolic pathways and converted into various forms of carbohydrates in leaves (Taiz et al., 2015). Sucrose and starch are the most common photosynthetic end products (Lunn & Hatch, 1995; McClain & Sharkey, 2019). In many species, including the model plant *Arabidopsis*, carbon from atmospheric CO₂ is fixed via the Calvin–Benson cycle in chloroplasts, whose intermediate products (triose phosphate) remain in the chloroplasts for starch synthesis or are exported to the cytosol for sucrose synthesis. Sucrose synthesized in the cytosol of leaves is loaded into the phloem throughout the day and is used for growth, storage, and metabolism in the sinks (Geiger & Servaites, 1994; Smith & Stitt, 2007; Stitt & Zeeman, 2012). Meanwhile, starch is stored in source leaves during the day and subsequently degraded to sucrose and remobilized at nighttime for translocation, growth, and maintenance. Because starch accumulation competes with sucrose synthesis for translocation, the photosynthate allocation between sucrose and starch in leaves can regulate the dynamics of photosynthate loading and determines plant productivity (MacNeill et al., 2017).

Regarding the coordination of photosynthate allocation between sucrose and starch in leaves, two hypotheses have been proposed (Stitt et al., 2010; Mengin et al., 2017). The first hypothesis (overflow hypothesis) (Cseke et al., 1984; Stitt, 1990) proposes that starch is synthesized as an overflow product when the photosynthetic rate exceeds the loading rate and sucrose storage capacity in source leaves. The second hypothesis proposes that starch accumulates in leaves to maintain a consistent supply of sucrose and avoid carbon starvation at

night. The second hypothesis has been emphasized in numerous studies on *Arabidopsis* (Smith & Stitt, 2007; Gibon et al., 2009; Graf & Smith, 2011; Sulpice et al., 2014). These studies demonstrated that even under conditions of low photosynthetic activity, *Arabidopsis* accumulated a portion of photosynthates as starch, and the starch reserve was almost, but not entirely, depleted by circadian regulation at the next dawn. Although various biochemical reactions related to the regulation of sucrose and starch synthesis are known (Stitt et al., 2010; Taiz et al., 2015), the mechanisms by which photosynthate allocation between sucrose and starch is regulated in both hypotheses are not entirely clear.

Furthermore, the role of sucrose and starch in photosynthate allocation differs substantially across species (Streb & Zeeman, 2012; Smith & Zeeman, 2020). As previously mentioned, *Arabidopsis* predominantly accumulates starch in source leaves but rarely any sucrose (Annunziata et al., 2018). In contrast, cereal crops such as rice, wheat, and barley predominantly accumulate and use sucrose as a nighttime resource instead of starch (Gordon et al., 1980; Rösti et al., 2007). The ratio of starch to sucrose in leaves reflects the differences in their roles and is closely related to the coordination of photosynthate allocation (Dong & Beckles, 2019).

I found that strawberry accumulated sucrose and starch throughout the day and used both at night and that the synthesis of starch was remarkable when the sucrose concentration per unit leaf area was greater than $150 \text{ mmol C m}^{-2}$ in Chapter 1, and these results were consistent with the hypothesis that starch is an overflow product (Cseke et al., 1984; Stitt, 1990). However, these results were obtained from three cultivation periods with different day lengths in the field. It has been proposed that the photosynthate allocation to starch in some species, like *Arabidopsis*, varies with day length (Stitt & Zeeman, 2012; Mengin et al., 2017). Therefore, it is possible that the relationship between photosynthetic activity and photosynthates allocation of sampled plants varies according to season; consequently, the study in Chapter 1 was insufficient to confirm how the photosynthate allocation between sucrose and starch are regulated in strawberry leaves.

This chapter aimed to determine whether the relationships between sucrose and starch dynamics in leaves suggested in Chapter 1 were an inherent characteristic in strawberry plants and whether starch served as an overflow product in the coordination of photosynthate allocation. To avoid seasonal effects, experiments were conducted under controlled environmental conditions using strawberry plants grown within the same cultivation period. The carbohydrates concentrations in strawberry leaves were measured in conditions of high or low photosynthetic activity, and the relationship between sucrose and starch dynamics was investigated.

2.2. Materials and methods

2.2.1. Plant materials and growth conditions

Similar to Chapter 1, strawberry plants (*Fragaria* × *ananassa* Duch. ‘Fukuoka S6’) grown under natural light conditions were used as plant material. The nursery plants were transplanted into plastic pots (28.0 cm long × 14.5 cm wide × 11.8 cm high) filled with M-1 substrate (Kaneya Co., Ltd., Aichi, Japan) on September 24, 2021; 16 plants were planted (2 in each pot) and then placed on cultivation beds (1.2 m high). The other growth conditions are similar to those in Chapter 1.

2.2.2. Experimental condition and treatments

The experiments were conducted on December 15 and 23, 2021, in a plant growth chamber (HNM-S11, Koito Electric Industries, Ltd., Shizuoka, Japan; growth area: 60.0 cm long × 90.0 cm wide × 90.0 cm high) that can control air temperature and relative humidity. Nine dimmable LED floodlights (HMFD45EW1SV12H-RM (40Y), Kyoritsu Densho Co., Ltd., Miyazaki, Japan) were used as the light source (Figure 2.1).

The light condition was controlled to provide two treatments: one with high photosynthetic

activity treatment (High) that replicated the greenhouse light condition on a clear day in December and another with low photosynthetic activity treatment (Low) that replicated a rainy day in December. The maximum photosynthetic photon flux density (PPFD) was set to approximately 970 and 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for the High and Low treatments, respectively, by manually operating the dimmers in a stepwise manner from dawn to dusk. Both treatments had the same dawn/dusk times of 7:15/17:15, air temperature of 20°C during the day (7:15–17:15) and 12°C at night (17:15–7:15 the following day), and relative humidity of 70% during the entire day.



Figure 2.1 Photograph of the plant growth chamber where nine LED floodlights were installed above the strawberry plants. A temperature–humidity sensor with a forced ventilation shelter and a CO₂ sensor were also installed in the chamber.

2.2.3. Measurement of environmental conditions and leaf temperature

PPFD was measured using a light quantum meter (PAR-02D, Prede Co., Ltd., Tokyo, Japan) placed at a canopy height of 35.0 cm from the growth area floor of the chamber. Air relative humidity and ambient air CO₂ concentration were measured using a temperature-humidity sensor (HMP60, Vaisala, Finland) installed in a forced ventilation shelter

(RSVH01A1203, SCE, Inc., Hokkaido, Japan) and a CO₂ sensor (GMP252, Vaisala, Finland), respectively, which were placed at a height of 50 cm (above the canopy). Leaf temperature was measured using a T-type thermocouple (0.1-mm diameter) fixed to an arbitrary leaf surface. Data were logged at 5-s intervals using a data-logger (CR1000X, Campbell Scientific, USA) and 5-min means were automatically stored in it.

2.2.4. Estimation of photosynthetic activity

To ensure that the treatments were appropriately set, the photosynthetic rate in a single leaf was used as an indicator of photosynthetic activity. The estimation model of single-leaf photosynthesis under sunlight, described in Chapter 1, was used with some modifications. The model was based on the biochemical photosynthesis model (Farquhar et al., 1980) and the stomatal model (Medlyn et al., 2011). Regarding the modification from Chapter 1, the actual measured leaf temperature was used instead of the estimated value using the energy balance equation of the leaf, as LED produces less heat than sunlight. The estimation was performed using the R package “plantecophys” (version 1.4.4, Duursma, 2015) in R version 4.3.1 (R Core Team, 2023) in the RStudio environment (version 2023.06.0) (RStudio Team, 2023). The photosynthetic model parameters used for estimation were obtained using a portable open gas-exchange system (LI-6400XT, LI-COR, USA) as described by Kimura et al. (2020) and Chapter 1 and are shown in Table 2.1.

Table 2.1 List of parameters for estimation of single-leaf photosynthetic rate.

Parameters	Definitions	Values	Unit	Reference
$V_{\text{cmax},25}$	The maximum Rubisco carboxylation rate at 25°C	77.8	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Estimated in this study
$E_a(V_{\text{cmax}})$	Activation energy for V_{cmax}	53.2	kJ mol^{-1}	Kimura et al. (2020)

$\Delta S(V_{\text{cmax}})$	Entropy factor for V_{cmax}	0.637	kJ K mol^{-1}	Kimura et al. (2020)
$J_{\text{max},25}$	The maximum rate of electron transport at 25°C	210.546	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Estimated in this study
$E_a(J_{\text{max}})$	Activation energy for J_{max}	23.7	kJ mol^{-1}	Kimura et al. (2020)
$\Delta S(J_{\text{max}})$	Entropy factor for J_{max}	0.639	kJ K mol^{-1}	Kimura et al. (2020)
$R_{\text{n},25}$	The night respiration rate at 25°C	1.36	$\mu\text{mol m}^{-2} \text{s}^{-1}$	Estimated in this study
$E_a(R_{\text{n}})$	Activation energy for R_{n}	35.6	kJ mol^{-1}	Estimated in this study
g_0	Residual stomatal conductance	0.0449	$\text{mol m}^{-2} \text{s}^{-1}$	Estimated in this study
g_1	Empirical constant of stomatal conductance model	2.90	Unitless	Estimated in this study

2.2.5. Leaf sample processing and analysis of carbohydrate concentrations

Each treatment experiment was conducted on eight plants that had been transferred to the plant growth chamber the night before the experiment. A total of 48 fully expanded leaves were selected from the 8 plants, and 1 leaf disk (6-mm diameter, 28.3 mm²) of every selected leaf was sampled from the intercostal regions (i.e., the lamina between major veins) throughout the day. Sampling was performed 10 times within 24 h at 2-h intervals during the day (7:15, dawn; 9:15; 11:15; 13:15; 15:15) and at 2- or 4-h intervals during the night (17:15, dusk; 19:15; 23:15; 3:15; 7:15). To ensure that sufficient amounts of carbohydrates were available for analysis, the 48 leaf disks collected at each sampling time were grouped into sets of 12, i.e., 4 biological replicates were obtained in each treatment. All samples were stored at –80°C until further

analysis.

The concentrations of the most abundant carbohydrates in strawberry leaves—sucrose, glucose, fructose, and starch—were quantified following the methods described in Chapter 1 with minor modifications according to the sample size: the amount of sodium acetate was changed from 3 mL to 2 mL, and the amount of α -amylase and amyloglucosidase were from 30 μ L to 25 μ L.

2.2.6. Statistical analyses

Statistical analyses were performed R version 4.3.1 (R Core Team, 2023) in the RStudio environment (version 2023.06.0) (RStudio Team, 2023). The carbohydrate concentration data were statistically expressed as means $\pm$ standard error ($n = 4$) and subjected to Student's t -test to identify significant differences between two treatments at each sampling time point.

2.3. Results and discussion

2.3.1. Environmental conditions and leaf temperature

To assess how photosynthetic activity affects leaf carbohydrate dynamics, sampling experiments were conducted in two treatments with different light conditions. Figure 2.2 shows the diurnal changes in PPFD (A) and leaf temperature (B) in the two treatments. The daily maximum PPFD for the High and Low treatments was approximately 970 and 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively; however, the PPFD was generally higher in the High treatment than the Low treatment. The leaf temperature mainly varied depending on the air temperature set (day: 20°C; night: 12°C). In the High treatment, PPFD had a minimal effect on leaf temperature when its value exceeded 750 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The local fluctuations in leaf temperature observed throughout the day, such as 4 and 12 h after dawn, were probably caused by the opening and closing of the chamber's door for sampling.

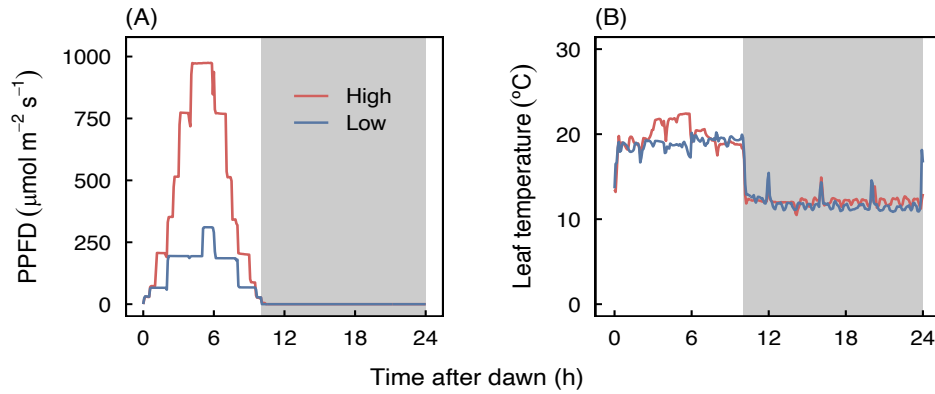


Figure 2.2 Diurnal changes in photosynthetic photon flux density (PPFD) (A) and leaf temperature (B) from dawn in two treatments: high photosynthetic activity treatment (High; red line) and low photosynthetic activity treatment (Low; blue line). Shaded regions represent the nighttime.

2.3.2. Single-leaf photosynthetic rate

The estimated photosynthetic rate changed mainly depending on PPFD (Figure 2.3). The photosynthetic rate remained generally higher in the High treatment than in the Low treatment throughout the day, indicating that the treatments were set properly.

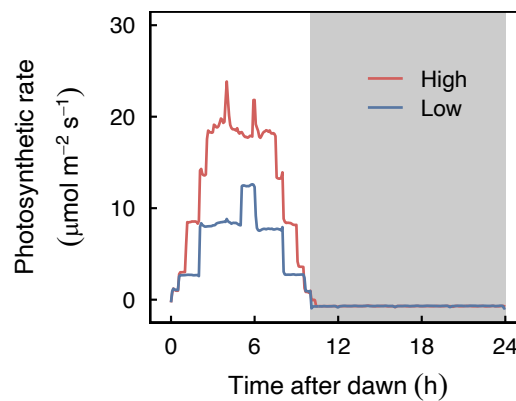


Figure 2.3 Diurnal change in estimated single-leaf photosynthetic rate from dawn in two treatments: high photosynthetic activity treatment (High; red line) and low photosynthetic activity treatment (Low; blue line). Shaded regions represent the nighttime.

2.3.3. Leaf carbohydrate concentration

The diurnal changes in leaf carbohydrate concentrations in the two treatments reflected a difference in photosynthetic activity (Figure 2.4). The sucrose concentration in the High treatment was approximately 75 mmol C m^{-2} at dawn. It immediately increased at dawn, peaked at $170 \text{ mmol C m}^{-2}$ 4 h before dusk, and then decreased in a linear manner to 95 mmol C m^{-2} until the following dawn. The sucrose concentration in the Low treatment was slightly higher immediately after dawn than that in the High treatment, but it was significantly lower from 4 h after dawn to the following dawn. It peaked at $130 \text{ mmol C m}^{-2}$ and then decreased to 70 mmol C m^{-2} . The glucose concentration in the High treatment was significantly higher than that in the Low treatment at some time points; however, it remained almost constant throughout the day at approximately 25 mmol C m^{-2} in both treatments. The fructose concentration remained nearly constant throughout the day (at approximately 80 mmol C m^{-2}) in both treatments and did not differ significantly between the treatments. The starch concentration in the High treatment started to increase a little behind the increase in sucrose concentration. It increased from approximately 25 mmol C m^{-2} to $135 \text{ mmol C m}^{-2}$, peaked at dusk, and then decreased in a linear manner to approximately 50 mmol C m^{-2} until the following dawn. Although the starch concentration in the Low treatment was slightly higher immediately after dawn than that in the High treatment, similar to sucrose, it was significantly lower than that in the High treatment from 6 h after dawn to the following dawn, with the daily maximum being only about 40 mmol C m^{-2} , less than a third of that in the High treatment.

I previously found similarities in the dynamics of carbohydrates during three different cultivation periods under field conditions as described in Chapter 1. Specifically, sucrose concentration began to increase at dawn and peaked 2–4 h before dusk, glucose and fructose concentrations both remained constant throughout the day, and starch concentration increased significantly after sucrose concentration did and peaked at dusk. These current findings are

consistent with the previously reported findings except for the trends in starch concentrations in the Low treatment. The concentration of total carbohydrates (sucrose, glucose, fructose, and starch) in the High treatment was markedly higher than that in the Low treatment, which proves that photosynthesis occurred more actively in the High treatment than in the Low treatment. Thus, the present study results suggest that photosynthates were allocated to both sucrose and starch under high photosynthetic activity conditions, whereas they were preferentially allocated to sucrose than starch under low photosynthetic activity conditions. Conversely, glucose and fructose showed stable dynamics throughout the day, suggesting that their roles as osmolytes may be maintained even if photosynthetic activity fluctuates.

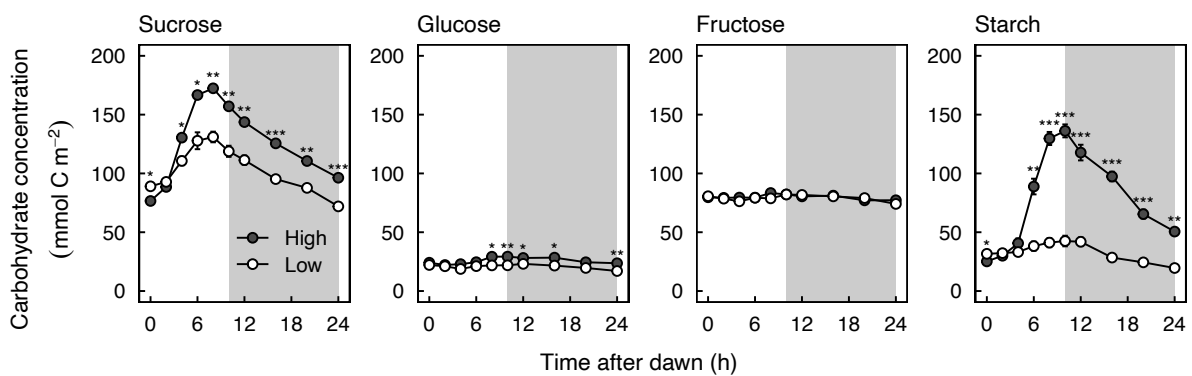


Figure 2.4 Diurnal changes in leaf carbohydrate concentrations from dawn in two treatments: high photosynthetic activity treatment (High; closed circles) and low photosynthetic activity treatment (Low; opened circles). Shaded regions represent the nighttime. Error bars represent the standard error of the mean values ($n = 4$). Significant differences between treatments are indicated by asterisks (Student's t -test): * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

2.3.4. Relationships between sucrose and starch concentrations

The relationships between sucrose and starch concentrations in the daytime and nighttime (Figure 2.5) were investigated based on the diurnal changes in leaf carbohydrate concentrations (Figure 2.4). The phased increase in starch concentration in the High treatment depended on the sucrose concentration during the day; it increased gradually from approximately 25 to 50 mmol

C m⁻² until the sucrose concentration reached about 150 mmol C m⁻², then increased rapidly to approximately 135 mmol C m⁻² until 2 h before dusk. In the Low treatment, sucrose concentration did not reach 150 mmol C m⁻², and the starch concentration increased gradually from approximately 25 to 50 mmol C m⁻², following a similar pattern to that in the High treatment. The starch concentration in the nighttime decreased linearly with sucrose concentration in both treatments. These results are consistent with those in Chapter 1, which suggest a biphasic increase in starch concentration during the daytime and the effects of sucrose concentration on the carbon allocation in strawberry leaves. However, as mentioned above, the experiments in Chapter 1 were conducted under field conditions over three different cultivation periods; therefore, the previous findings could not exclude the possibility that other seasonal factors, in addition to photosynthetic activity, may have affected the relationship between sucrose and starch concentrations when considering the coordination of photosynthate allocation. In this chapter, the difference in photosynthetic activity during the same cultivation period confirmed the dynamics of sucrose and starch. The current findings provide additional evidence that photosynthates are allocated preferentially to sucrose than starch when leaf sucrose concentration is less than the storage capacity of 150 mmol C m⁻², whereas they are allocated to starch as an overflow product when photosynthesis is active and sucrose concentration exceeds the storage capacity.

As previously mentioned, the mechanisms underlying the coordination of photosynthate allocation are not fully clarified. photosynthate allocation between sucrose and starch is directly linked to the distribution of triose phosphate between the chloroplasts and cytosol. Triose phosphates and their precursor, 3-phosphoglycerate (3-PGA), are transported from the chloroplast into the cytosol in exchange of inorganic phosphates (Pi) in the cytosol via the triose phosphate/phosphate translocator (TPT). Regarding the overflow hypothesis, starch synthesis is activated through the following mechanisms: when photosynthetic activity is high and sucrose and/or its intermediates accumulate in the cytosol, sucrose phosphate synthase (SPS)

or fructose-1,6-bisphosphatase (key enzymes in the sucrose synthesis pathway in the cytosol) is inactivated (Stitt, 1990; Huber & Huber, 1996). This results in the inhibition of the entire pathway of sucrose synthesis and a decrease in the release of Pi in the cytosol. As the transport activity of TPT depends on the availability of Pi in the cytosol, the exchange of triose phosphates/3-PGA and Pi is inhibited, leading to the starvation of Pi and accumulation of triose phosphates and 3-PGA in the chloroplast. Because ADP-glucose pyrophosphorylase, the first enzyme in the starch synthesis pathway, is activated by 3-PGA and inhibited by Pi (Ballicora et al., 2004), starch synthesis is consequently activated. These mechanisms can explain the results of the present study that starch concentration rapidly increased when sucrose accumulated under conditions of high photosynthetic activity. However, the feedback regulation of sucrose described above does not fully explain the mechanism of carbon allocation (Smith & Stitt, 2007), and other mechanisms such as circadian control can contribute to the coordination of photosynthate allocation (Graf et al., 2010; Graf & Smith, 2011; Sulpice et al., 2014). This study aimed to investigate photosynthate allocation based on carbohydrate dynamics. However, the analysis of enzyme activity and the concentrations of intermediates involved in these mechanisms would be useful to further elucidate the coordination of photosynthate allocation in strawberry leaves.

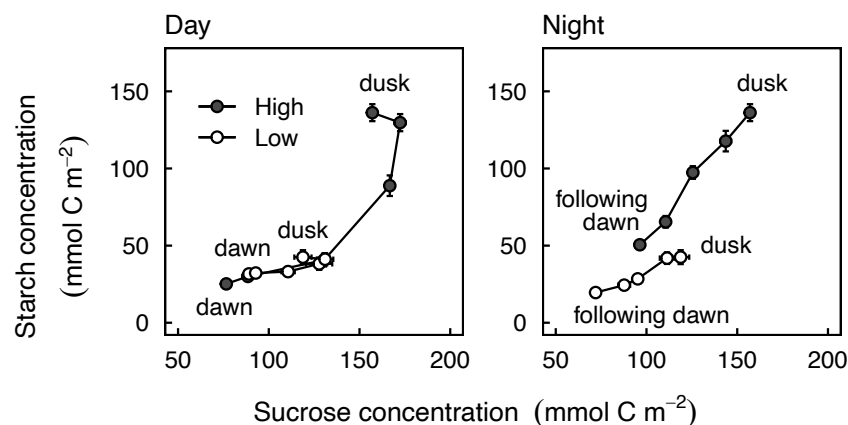


Figure 2.5 Relationships between sucrose and starch concentrations in two treatments: high photosynthetic activity treatment (High; closed circles) and low photosynthetic activity

treatment (Low; opened circles). Plots are connected in order of time per treatment. Error bars represent the standard error of the mean values ($n = 4$).

2.4. Conclusion

In this chapter, the relationships between sucrose and starch dynamics in strawberry leaves were investigated under a controlled environment with high and low photosynthetic activity. The findings strongly suggest that in the coordination of carbon allocation in source leaves, starch serves as an overflow product at a sucrose concentration of $150 \text{ mmol C m}^{-2}$ established as a threshold. Although the relationship between sucrose and starch likely varies depending on the strawberry variety and/or cultivation conditions, the fact that both experiments—one in the field environment with different day lengths according to the season and the other in a controlled environment with same day lengths but with two different photosynthetic activities—showed consistent results strongly suggests that the relationship is an inherent characteristic of strawberry plants. Further research is required to elucidate the molecular mechanism underlying photosynthate allocation; however, the present study will advance the understanding of the regulatory mechanism of photosynthate loading.

Chapter 3

Photosynthate unloading dynamics based on potential growth and carbohydrate accumulation in fruit

3.1. Introduction

Fruit size and sugar contents are major determinants of fruit quality. These characteristics change during fruit growth and ripening, and they are primarily determined by the genotype but can also be influenced by the environment and agronomic management (Gündüz & Özdemir, 2014).

Fruit growth largely depends on the photosynthate unloading and water inflow through vascular tissues (phloem and xylem). Photosynthates translocated from sources are unloaded from the phloem into the fruits, where a portion is utilized as energy and substrate for growth and maintenance, while the remainder accumulates. Accumulated photosynthates create the water potential gradient between the fruits and the parent plants, which serves as the driving force for water import into the fruit via the xylem, thereby facilitating expansive growth.

Fruits achieve their genetically determined potential growth only under sufficient supply of photosynthates and water and optimal environmental conditions. Photosynthate supply strongly relies on photosynthesis in source leaves and subsequent photosynthate loading. Photosynthesis is influenced by environmental conditions such as light intensity, air temperature, and ambient CO₂ concentration (Sun et al., 2012; Hidaka et al., 2013), which in turn affects the loading as discussed in Chapters 1 and 2. Water uptake is influenced by soil water content, and water loss through plant transpiration, which is affected by air vapor pressure deficit. Several studies have reported that fruits grown under resource-limited conditions, such as insufficient light, water stress, and high salinity, showed decreased growth rate and quality

(e.g., water content and fresh weight) compared to those grown under favorable conditions (Mitchell et al., 1991; Krauss et al., 2006; Morandi et al., 2011).

Furthermore, photosynthate unloading to individual fruit is influenced not only by supply from sources but also by competition among sinks (Ho, 1984; Blanke, 2009). When the sink demand for photosynthates exceeds the supply from sources, photosynthates are unevenly distributed among sinks. As a result, there is a reduction in photosynthate unloading, which cannot achieve their potential growth. In many fruit crops, such as apple, tomato, and peach, flower and fruit thinning has been reported to improve the quality (e.g., size and weight) of remaining fruits (Link, 2000).

The competitive ability of a sink to unload photosynthates is defined as “sink strength” (Ho, 1988). Fruit sink strength can be quantified as the potential growth rate corresponding to the photosynthate unloading rate under non-limiting conditions, which is generally achieved by increasing photosynthesis and/or reducing fruit numbers to minimize competition (Dejong & Grossman, 1995; Marcelis, 1996; T. Li et al., 2015). Sink strength has been defined as the product of sink size and sink activity, which are generally stated as fruit fresh/dry weight and relative growth rate. Ho (1988) proposed an alternative and more physiological approach, in which sink size reflects physical constraints such as cell number, while sink activity reflects physiological processes such as phloem unloading, metabolism, and imported sugar compartmentation. However, several studies suggested that cell number was not dominant of fruit size. Although there is a debate about a suitable measure of sink size and activity because the mechanisms to determine sink strength are unclear (Marcelis, 1996; Rosati et al., 2020), the former is practical due to its ease of measurement.

Sink strength varies during fruit growth; therefore, it has been used as a parameter in fruit growth models (Heuvelink, 1999; Vos et al., 2010; Zhu et al., 2019). These models used potential growth rate as sink strength to simulate photosynthate partitioning among fruits and subsequent unloading process. They are useful to understand the way in which environment

factors and agronomic practices can influence each fruit's growth, as well as to support decision-making in environmental control and agronomic practices.

In strawberry, similarly to other fruit crops, resource-limited conditions, such as light (Watson et al., 2002; Hidaka et al., 2012) and water (Liu et al., 2007) deficits, lead to a decrease in fruit growth and quality. Furthermore, carbon isotope studies have suggested that fruits within a given inflorescence compete for photosynthates and fruit sink strength changes during growth and varies depending on inflorescence position (Nishizawa & Hori, 1988; Hidaka et al., 2019; Miyoshi et al., 2021). However, the majority of previous studies was performed in the presence of multiple fruits, thus they did not observe the potential growth. Since fruit growth in these studies results from photosynthate competition, they are insufficient to discuss the dynamics of photosynthate unloading and its determinant, sink strength. To my knowledge, Miura et al. (1990) is the only study that investigated fruit growth with one fruit per inflorescence; however, they discussed the growth rate based on fruit length rather than weight, which does not directly assess photosynthate accumulation.

The objectives of this chapter were to elucidate the dynamics of photosynthate unloading and sink strength that are not influenced by competition among fruits. In this study, potential growth and carbohydrate accumulation under non-limiting conditions were evaluated following removing to achieve one fruit per inflorescence. Sink strength was analyzed based on fruit weight.

3.2. Materials and methods

3.2.1. Plant materials and growth conditions

Similar to Chapters 1 and 2, strawberry plants (*Fragaria × ananassa* Duch. 'Fukuoka S6') grown under natural light in a greenhouse were used as plant material. On September 27 and 28, 2022, nursery plants were transplanted into cultivation beds (9.7 m long × 0.3 m wide ×

0.12 m high) filled with commercial soil mixture ICHIGO BAIDO (Green Sangyo Co. Ltd., Saga, Japan). A total of 141 plants were planted in two rows, with a separation of 0.2 m between plants and 0.18 m between rows. The other growth conditions are similar to those in Chapters 1 and 2.

To establish non-limiting conditions for evaluating potential growth, the first flower on the primary inflorescence of each plant was hand-pollinated at anthesis, and subsequent flowers were removed before anthesis. The number of fully expanded leaves per individual was adjusted to eight during the experiments.

3.2.2. Measurement of environmental conditions

Inside the greenhouse, air temperature and relative humidity and photosynthetic photon flux density (PPFD) were measured and recorded at the same time intervals using the same sensors and data logger as described in Chapter 1. Daily averaged air temperature and relative humidity and daily cumulative PPFD were calculated in R version 4.3.1 (R Core Team, 2023).

3.2.3. Fruit harvest

A total of 141 flowers that bloomed between November 19 and 30, 2022 were tagged at anthesis, and the fruit growth stage was identified as the number of days after anthesis (DAA). Fruits were harvested at eleven different growth stages: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, and 55 DAA. For each stage, 12–14 fruits were collected, and growth characteristics were measured. Each fruit served as a biological replicate.

3.2.4. Measurement of parameters related to fruit size and weight

Immediately following harvest, the dimensions of the major axis (length: L , mm), medium axis (width: W , mm), and minor axis (thickness: T , mm) of fruits were measured using a digital

caliper (E05-150 130-720, As-One Co., Osaka, Japan) after removing the calyx. Fruit volume was estimated using the following equation (Eq. 3.1):

$$V = 4.0 * (L * W * T * 10^{-4}) + 2.0724 \quad (3.1)$$

where V is fruit volume (cm^3). The equation was empirically elaborated based on the same cultivar used in this study (data not shown). The fresh weight (FW, g) of each fruit was measured, followed by cutting into small pieces and immediate freezing in liquid nitrogen. The frozen samples were ground to fine powder with liquid nitrogen using a mortar and pestle or a cool mill (TK-CM20S, Tokken, Chiba, Japan), followed by storage at -80°C for further analysis. Fruit dry weight (DW, g) was calculated based on dry matter percentages obtained by the lyophilization of pre-weighted frozen powder and measured FW values. Fruit water content (FWC, %) was calculated based on the difference between FW and DW as follows (Eq. 3.2):

$$\text{FWC} = (\text{FW} - \text{DW}) / \text{FW} * 100 \quad (3.2)$$

3.2.5. Analysis of fruit carbohydrate concentrations and carbon content

In this study, to investigate carbohydrate accumulation during fruit growth, the concentrations based on FW and contents in whole fruits of the major soluble carbohydrates (sucrose, glucose, and fructose) and starch in strawberry fruits were analyzed.

Soluble carbohydrates were extracted following the procedures described in Souleyre et al. (2004) with some modifications, and concentrations were determined using the high-performance liquid chromatography (HPLC) analysis described in Chapter 1. Approximately 20 mg of fruit frozen powder were weighted and mixed with 0.8 mL 80% (v/v) ethanol in a thermostatic bath at 82°C for 5 min. After centrifugation at $20,630 g_n$ for 10 min at room temperature, and the supernatant containing soluble carbohydrates was pooled. The extraction procedure was then repeated four times using 0.6 mL 80% (v/v) ethanol instead of 0.8 mL. The pooled supernatants were evaporated to dryness, and the residue was dissolved in 3.2 mL of

distilled water. The solution was centrifuged at 20,630 g_n and 4°C for 5 min, and the supernatant was filtered through a 0.45- μ m membrane filter and used for HPLC analysis. Similar to Chapter 1 and 2, starch concentration was determined using a Total Starch Assay kit (K-TSTA, Megazyme, Bray, Ireland) enzymatic assay following to the manufacturer's protocol with slight modifications. Following ethanol extraction, tissue pellets were re-suspended in 1 mL of sodium acetate (100 mM, pH 5.0) and starch was converted to glucose by the addition of 10 μ L each of α -amylase (2500 U mL^{-1}) and amyloglucosidase (3300 U mL^{-1}). Samples were centrifuged at 15,500 g_n for 5 min at room temperature, and 50 μ L of the supernatant were incubated with 1.5 mL of Megazyme GOPOD reagent. Glucose released from starch was then spectrophotometrically quantified at 510 nm. For carbohydrate analysis, two technical replicates were analyzed per biological replicate. Whole-fruit contents were calculated by multiplying the concentration based on FW by the FW. For comparison purposes, carbohydrate concentrations and contents were expressed as moles of carbon.

Assuming that photosynthates unloaded into the fruits, except for that consumed in respiration, was distributed between sucrose, glucose, fructose, starch, and other carbon compounds, the relative distribution of the new photosynthates was determined by dividing the accumulation rate of each component by that of total carbon contents. The total carbon content was calculated by multiplying the DW by the amount of carbon per unit gram of DW (0.45 g C g^{-1} DW), which was an average value calculated from literature data of various species (Cakpo et al., 2020). The content of the other carbon compounds was calculated by subtracting the contents of the four aforementioned carbohydrates from the total carbon content.

3.2.6. *Fruit growth analysis*

As reported by Roch et al. (2020), the time courses of FW and DW were, after log transformation, fitted to double sigmoid functions by the least square methods using Broyden-

Fletcher-Goldfarb-Shanno algorithm implemented by the optim function of the R stats package (R Core Team, 2023). These fitting parameters were used to calculate the absolute growth rate (AGR) and relative growth rate (RGR) based on FW and DW. AGR was obtained as the first derivative of the double sigmoid functions. RGR was calculate by dividing AGR by FW or DW.

3.2.7. Calculation of photosynthate unloading rate

The unloading rate was calculated based on the fruit carbon balance as follows (Eq. 3.3):

$$Unload = \frac{dCarbon}{dt} + R \quad (3.3)$$

where *Unload* is the unloading rate (mmol d⁻¹), *Carbon* is the total carbon content (mmol), and *R* is the respiration rate (mmol d⁻¹). *Carbon* was obtained by multiplying the AGR based on DW by the amount of carbon per unit gram of DW (0.45 g C g⁻¹ DW, Cakpo et al., 2020). *R* was calculated based on the Q10 concept according to Luo et al. (2020a) as follows:

$$R = q_g AGR + q_m DW Q_{10}^{(T-20)/10} \quad (3.4)$$

where *q_g* (0.045 g C g⁻¹ DW, Zhu et al., 2019) is the growth respiration coefficient, *AGR* is the absolute growth rate based on DW, *q_m* is the maintenance respiration coefficient at 20 °C (0.000168 g C g⁻¹ DW h⁻¹, Zhu et al., 2019), *Q₁₀* is the temperature ratio of maintenance respiration (2.5, Menzel, 2023), and *T* is temperature (°C). In this study, *q_g* and *q_s* were taken from the literature on grape berries, while *Q₁₀* was obtained for the literature on strawberry fruits. *T* was the averaged air temperature during the experiments.

3.2.8. Statistical analyses

All statistical analyses were performed in R version 4.3.1 (R Core Team, 2023) in the RStudio environment (version 2023.06.0) (RStudio Team, 2023). In Figure 3.2–Figure 3.4, data were statistically expressed as means ± standard error (*n*=12–14). Where the error bars are not visible, the standard error was smaller than the plotting symbol.

3.3. Results

3.3.1. Environmental conditions

Daily averaged air temperature during the growth period gradually decreased, despite remaining above the heater threshold temperature (8 °C) (Figure 3.1A). Daily averaged relative humidity stayed within the range of 51% to 98% (Figure 3.1B). Daily cumulative PPFD exhibited significant fluctuations throughout the experimental period, with a maximum of approximately 12.6 mol m⁻² d⁻¹ and a minimum of 1.5 mol m⁻² d⁻¹ (Figure 3.1C).

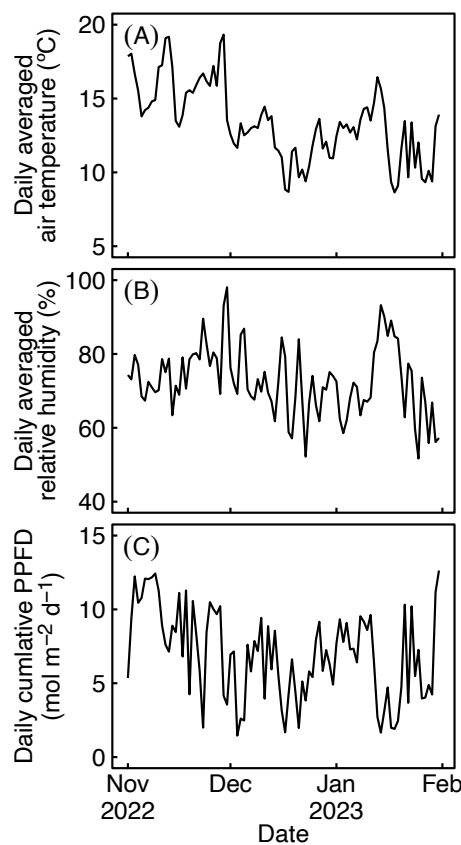


Figure 3.1 Changes in daily averaged air temperature (A) and relative humidity (B) and daily cumulative PPFD (C) measured in the greenhouse throughout the experimental period (November 11, 2022–January 31, 2023).

3.3.2. Fruit dimensions and volume

The three dimensions of fruit followed a double sigmoid curve with two rapid growth periods (5–20 DAA and 30–45 DAA) (Figure 3.2A). The length was always longer than the width and the thickness and continued to increase until 55 DAA, while the width and the thickness similarly increased and reached a plateau at 45 DAA. The volume immediately after anthesis (5 DAA) was approximately 2.3 cm³ (Figure 3.2B). Subsequently, the volume increased linearly until 30 DAA, and then it followed a single sigmoid curve, reaching approximately 37.5 cm³ at 55 DAA.

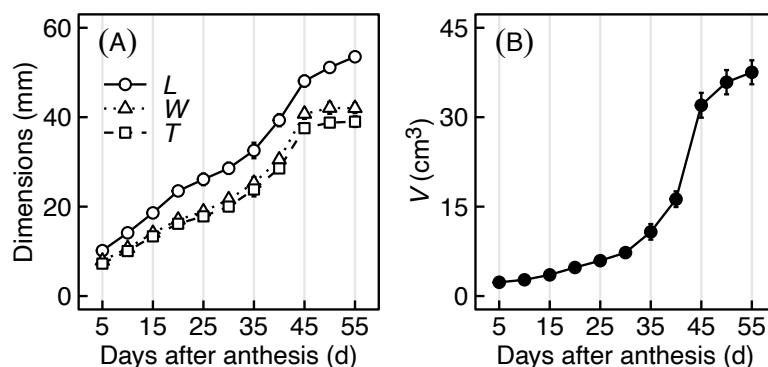


Figure 3.2 Changes in fruit dimensions based on length (*L*; opened circles), width (*W*; opened triangles), and thickness (*T*; opened squares) (A) and fruit volume (B). Error bars represent the standard error of the mean values ($n = 12-14$).

3.3.3. Fruit weight and water content

The changes in FW and DW showed similar pattern to the volume (Figure 3.3A). FW began to show a gradual linear increase from approximately 0.2 g at 5 DAA, and then rapidly increased to approximately 35 g, following a single sigmoid curve between 30 and 55 DAA. DW was approximately 0.04 g at 5 DAA, and then increased to approximately 4.4 g, following a single sigmoid curve after the linear increase. FWC increased from approximately 78.5% to 90.0%, peaking at 40 DAA, and then decreasing to approximately 87.3% (Figure 3.3B).

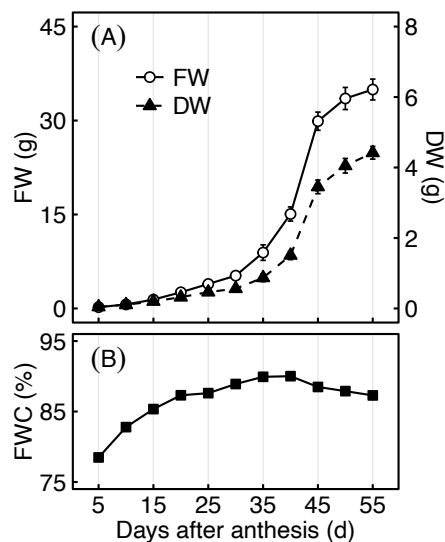


Figure 3.3 Changes in fruit fresh weight (FW; opened circles), dry weight (DW; closed triangles) (A), and fruit water content (FWC) (B). Error bars represent the standard error of the mean values ($n=12-14$).

3.3.4. Fruit carbohydrate concentration and contents

Sucrose concentration remained almost constant between 5 DAA and 30 DAA with only an increase of approximately $4 \mu\text{mol C g}^{-1}$ FW but increased to $1400 \mu\text{mol C g}^{-1}$ FW, following a single sigmoid curve, from 30 DAA (Figure 3.4A). Contrastingly, glucose concentration did not exhibit a rapid increase, and it linearly increased until 35 DAA and then increased slightly faster to $830 \mu\text{mol C g}^{-1}$ FW. Fructose concentration increased to $810 \mu\text{mol C g}^{-1}$ FW, following a similar pattern to that of glucose. The starch concentration at 5 DAA was approximately $280 \mu\text{mol C g}^{-1}$ FW, similar to that of the other three carbohydrates, but then exponentially decreased and was below the detection limit after 45 DAA.

Sucrose, glucose, and fructose contents increased following a single sigmoid curve, but starch contents remained almost constant at $0.1 \text{ mmol C per fruit}$ until 45 DAA, reaching values below the detection limit after this point (Figure 3.4B).

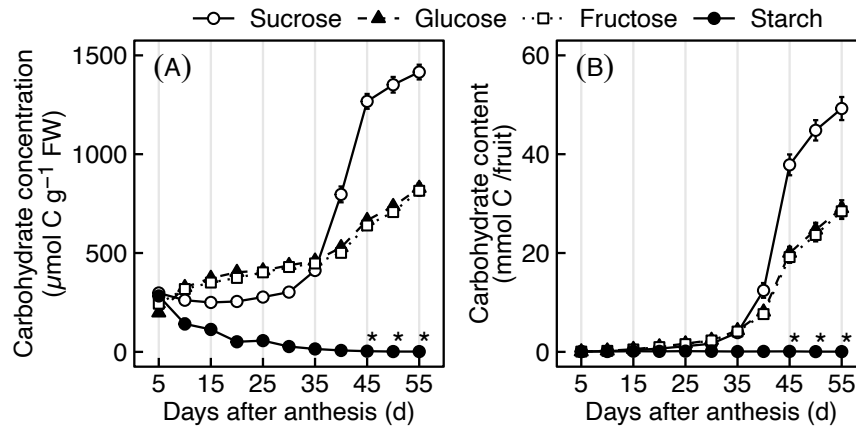


Figure 3.4 Changes in concentrations (A) and contents (B) of sucrose (open circles), glucose (closed triangles), fructose (open squares), and starch (closed circles). Error bars represent the standard error of the mean values ($n=12-14$). Values marked with an asterisk were below the detection limit.

3.3.5. Accumulation rate and relative distribution ratio of photosynthate

Both the accumulation rate of sucrose, glucose, fructose, starch, and other carbon compounds (Figure 3.5A) and the relative distribution of new photosynthates between them (Figure 3.5B) changed depending on fruit growth. At the early growth stage, other carbon compounds accumulated more notably than soluble carbohydrates and starch, with over 70% of the newly unloaded photosynthates (excluding those consumed in respiration) distributed to them. With fruit growth, accumulation of soluble carbohydrates increased, with over 50% of the new photosynthates distributed to them after 25 DAA. Although there were few changes in relative distributions between 25 DAA and 35 DAA and between 35 DAA and 50 DAA, accumulation rates varied. All except for starch were predominantly accumulated between 40 DAA and 45 DAA. At the end of growth, most of the new photosynthates into the fruits was distributed to soluble carbohydrates.

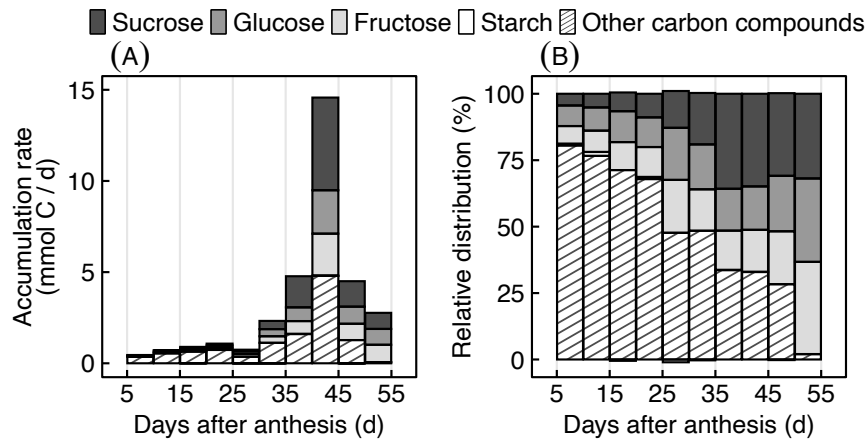


Figure 3.5 Changes in the accumulation rate of sucrose, glucose, fructose, starch, and other carbon compounds (A) and their relative distribution ratios to the newly unloaded photosynthates into the fruits (B).

3.3.6. Fruit growth rate

Fruit growth rate was calculated based on daily changes in FW and DW estimated by parameter fitting. A double sigmoid curve was fitted well to both log-transformed FW and log-transformed DW (Figure 3.6). Both AGR and RGR exhibited similar patterns based on FW and DW (Figure 3.7). AGR began to gradually increase during early growth, increasing rapidly from 30 DAA, peaking at between 40 DAA and 45 DAA, and then exponentially decreasing until 55 DAA. RGR was high during early growth, decreasing until 30 DAA, then increasing again to the same level as that in the early stage, and peaking at around 40 DAA.

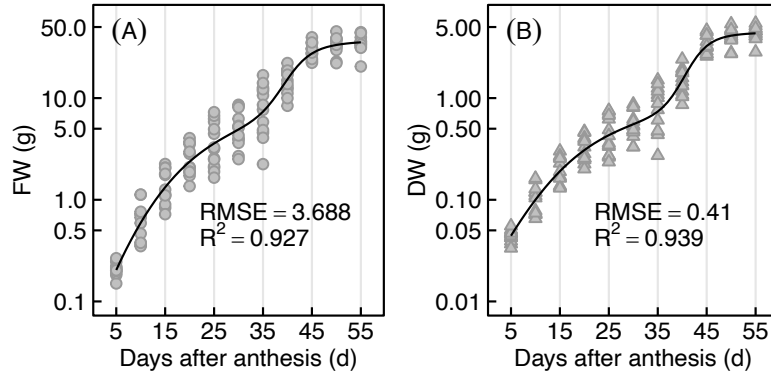


Figure 3.6 Measured data (circles and triangles) of fresh weight (FW; A) and dry weight (DW; B), and their corresponding fitted curves (lines) on a log scale. The root mean squared error (RMSE) and the coefficient of determination (R^2) are shown.

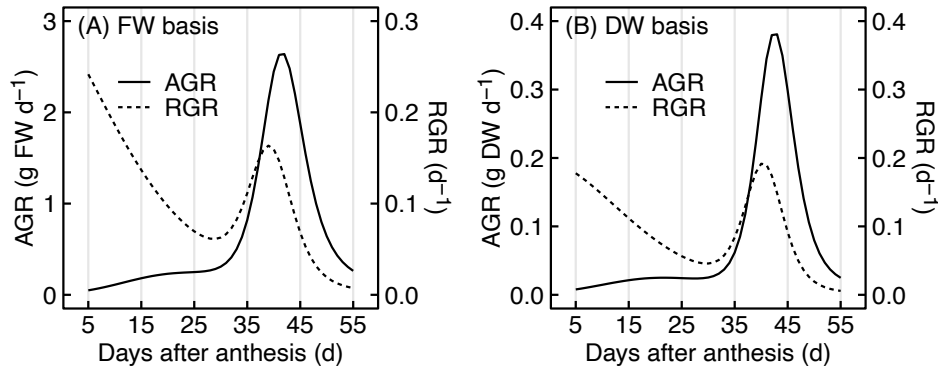


Figure 3.7 Changes in absolute growth rate (AGR; solid line) and relative growth rate (RGR; dotted line) based on FW (A) and DW (B).

3.3.7. Dynamics of photosynthate unloading

The photosynthate unloading rate was estimated based on the fruit carbon balance. While the respiration rate was estimated using the literature parameter obtained for other fruit species, it ranged from 0.03 to 1.8 mmol C d⁻¹, which was comparable to the values previously reported for strawberry fruit (Blanke, 2002). Similar to AGR in Figure 3.7, the unloading rate began to gradually increase during early growth, increasing rapidly from 30 DAA, peaking at between 40 DAA and 45 DAA, and then exponentially decreasing until 55 DAA (Figure 3.8).

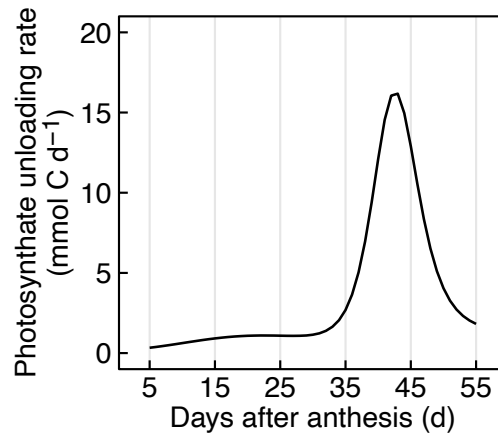


Figure 3.8 Changes in the unloading rate of photosynthates into fruit.

3.4. Discussion

3.4.1. Fruit growth pattern

Previous studies have demonstrated that the growth of strawberry fruits follows either a single sigmoid or double sigmoid curve in different cultivars (Perkins-Veazie & Huber, 1987; Menzel, 2023). Miura et al. (1990) and Li et al. (2012) reported a double sigmoid curve for fruit diameter in ‘Reiko’ and ‘Akihime’, respectively. Pei et al. (2020) identified a double sigmoid curve for fruit FW in ‘Hongyan’, while Symons et al. (2012) showed a single sigmoid curve for both diameter and FW in ‘Red Gauntlet’. In this study, a double sigmoid curve was shown for diameter (Figure 3.2) and a single sigmoid curve, followed by a linear increase, for volume (Figure 3.2), FW, and DW (Figure 3.3) in ‘Fukuoka S6’.

Fruit growth patterns are typically characterized by an initial phase of intense cell division and followed by a cell expansion phase. In strawberry, fruits grow slowly during the cell division phase for 15 to 20 DAA and then grow to their mature size during the cell expansion phase (Menzel, 2023). Pei et al. (2020) measured fruit FW, cell size, and cell number during growth in ‘Hongyan’ and found that they followed a double sigmoid curve after the cell division phase. The results of the present study, in which FW increased linearly until 30 DAA and then

followed a single sigmoid curve, suggested that in ‘Fukuoka S6’ fruit exhibiting potential growth, the cell division phase continued for approximately 30 days and then shifted to the cell expansion phase.

3.4.2. Photosynthate accumulation and relative distribution ratio

In strawberry, photosynthates are unloaded into fruits as sucrose (Forney & Breen, 1985a), which is then converted into other carbohydrates or carbon compounds. As mentioned earlier, strawberry fruits primarily accumulate sucrose, glucose, and fructose, and their composition varies depending on cultivars and growth stages (Sturm et al., 2003; Fait et al., 2008). In this study, sucrose concentration was lower than glucose and fructose concentrations during the early growth stage, although it increased more rapidly and surpassed them during development (Figure 3.4A). These findings are consistent with those of previous studies conducted on other cultivars without fruit removal (e.g., Jia et al., 2013; Luo et al., 2020b). However, sucrose concentration declined more dramatically than glucose and fructose concentrations when carbohydrate levels decreased overall under insufficient sunlight conditions (Liu et al., 2020). Hence, the accumulation pattern of these three carbohydrates may be different depending on whether the fruit achieves its potential growth.

Starch concentrations continued to decrease from 5 DAA, reaching non-detectable levels at 45 DAA (Figure 3.4A). Starch concentrations were reported to decrease by half between 7 DAA and 14 DAA, being barely detectable after 31 DAA (Souleyre et al., 2004). Starch accumulation patterns in fruits exhibiting potential growth determined in this study match previously observed values. Contrary to expectation, starch content was practically constant throughout development (Figure 3.4B), suggesting that the decrease in starch concentration was due to a dilution caused by fruit volumetric growth rather than starch degradation. However, because it has been observed that starch granules in 70%–80% of the cells examined in ripe

fruits were lost (Knee et al., 1977; Souleyre et al., 2004), the detection accuracy in this study might have been insufficient to assess low starch concentrations.

Furthermore, this study found that the distribution of the newly unloaded photosynthates to other compounds was equal or greater than its distribution to the major carbon compounds in fruits (sucrose, glucose, fructose, starch) during most stages of growth. These other compounds notably accumulated between 40–45 DAA (Figure 3.5A), when fruit volume rapidly increased (Figure 3.2). These results suggest that the other carbon compounds were mainly cell wall materials such as cellulose (Taiz et al., 2015).

3.4.3. Sink strength

Sink strength is a determinant of potential photosynthate unloading and is proposed to depend on sink size and sink activity, as described above. In this study, sink strength was quantified as the AGR of a fruit exhibiting potential growth, sink size as fruit weight, and sink activity as the RGR. Until 30 DAA, both sink strength and sink size gradually increased, while sink activity greatly decreased (Figure 3.3 and Figure 3.7). Subsequently, sink strength and sink activity exhibited a peak, while sink size continued to increase. Although several studies have argued that sink activity, rather than sink size, impacts sink strength (Marcelis, 1996; Hidaka et al., 2019), the results of the present study suggest that the major determinant of sink strength was sink size during early fruit growth, changing to sink activity during late growth. While this study assessed sink size and sink activity by the practical approach (i.e., fruit weight and RGR), further elucidation of the mechanisms to determine sink strength would benefit from the analysis of cell number and physiological processes.

The AGR pattern was consistent with that of other fruit species whose FW followed a single sigmoid curve (Roch et al., 2020). The AGR dynamics suggested that sink strength was altered during fruit development, reaching a maximum between 40 DAA and 45 DAA. Hidaka

et al. (2019) implemented the carbon isotope tracer method to report that sink strength, represented by the photosynthate import rate into the fruit, was faster in white-colored fruits compared to green-colored fruits and fruits turning red. The color of strawberries changed from green to white in the early growth stage and then to red at maturity. In this study, strawberries were green at 5–30 DAA, white at 35–40 DAA, and turning red at 45 DAA. This slight discrepancy may be due to differences in the number and position of fruits on the inflorescence, which affect photosynthate partitioning among fruits. While this study followed the specific fruits exhibiting potential growth, Hidaka et al. (2019) compared different fruits on the inflorescence to assess changes in sink strength. In addition, growth conditions may have also affected the results, as they affect anthocyanin accumulation in fruits, and thus coloration (Ikeda et al., 2011; Zhang et al., 2018). Furthermore, the RGR based on both FW and DW showed a similar pattern to that based on fruit length reported by Miura et al. (1990). They also demonstrated that, although there were differences in peak position and value, the RGR based on the length of fruits exhibiting potential growth was similar to that of fruits impacted by photosynthate competition. Further studies with an increased focus on RGR peak position and value are required to evaluate the effects of carbon competition and environmental conditions on the sink activity of each fruit.

Additionally, sink strength and photosynthate competition can vary among inflorescences. Strawberry plants continually produce multiple inflorescences, causing the environmental conditions to which the plants are exposed during fruit growth change throughout the cultivation period. Environmental factors such as air temperature can affect sink activity which reflects physiological processes in fruits. These factors also influence photosynthesis and, consequently, the translocation to fruits. It has been reported that approximately 90% of the photosynthates supplied from source is distributed to fruits during the fruit growth period of the first inflorescence (Nishizawa & Hori, 1988). Because photosynthate competition among fruits and/or other sinks, as well as consequent photosynthate unloading, may change during long-

term strawberry production, future research on the difference in sink strength dynamics among inflorescences would be required.

3.5. Conclusion

This chapter was designed to evaluate the dynamics of photosynthate unloading and sink strength on the strawberry fruits exhibiting potential growth. Fruits under non-limiting conditions generated by flower removing exhibited a linear increase followed by a single sigmoid curve in both volume and weight. The relative distribution ratio of newly unloaded photosynthates to soluble carbohydrates was low during early growth but increased with fruit growth. The accumulation pattern of carbohydrates was different between sucrose and hexose (i.e., glucose and fructose); hexose accumulated faster than sucrose during early growth, and then sucrose accumulated markedly during development. The potential unloading rate and its determinant, sink strength, appear to be strongly influenced by sink size during early growth but by sink activity during late growth.

The dynamics of potential unloading, achieved under non-limiting conditions, are determined by sink strength; however, actual dynamics under field conditions are affected by the ability of sources, termed source strength, in addition to sink strength. In the next chapter, the responses of fruit growth to altered source-sink balance were investigated, and their effects on photosynthate unloading were analyzed.

Chapter 4

Response of fruit growth to altered balance between source and sink strength

4.1. Introduction

Plants allocate photosynthates translocated from sources into various sinks for growth, maintenance, and storage. Photosynthate supply from the source can exceed or fall below the photosynthate demand of sink depending on environmental conditions and plant capacity. The capacity of photosynthate supply is referred to as source strength, which is strictly defined by the loading rate but is generally represented by the photosynthetic rate. The photosynthate demand of the whole plant depends on the number of sinks and the sink strength of individual sinks. Sink strength is the capacity of sink to attract carbon and can be quantified as the growth rate under the conditions of non-limiting carbon supply as described in Chapter 3 (Ho, 1988; Marcelis, 1996). The balance between source and sink strength regulates photosynthate allocation and unloading, which in turn influences fruit growth (Osorio et al., 2014). When source strength is lower than cumulative sink strength, individual sinks compete for the supplied photosynthates, and their growth is inhibited by source strength (source-limited growth). When source strength is higher, all sinks are supplied with sufficient photosynthates to meet their demand, and their growth is limited by sink strength (sink-limited growth).

Experimental manipulations of source and sink strength have been applied to investigate how source-sink balance affects the growth of sinks, particularly fruits. Under normal conditions, fruits generally exhibit source-limited growth (Heuvelink, 2005; Blanke, 2009). In various fruit species, enhanced photosynthesis through environmental control (e.g., supplemental lighting and CO₂ enrichment) increases fruit growth (Hidaka et al., 2013; Choi et al., 2015; Zhang et al., 2020). Additionally, it is reported that removing competitive sinks can

increase the growth rate and quality of the remaining fruits (Grossman & DeJong, 1995; Link, 2000). Conversely, sink-limited fruit growth is observed when plants exhibit high photosynthesis and/or have a small number of sinks (Pavel & Dejong, 1993; Matsuda et al., 2011). If fruit growth is limited by the sink strength, even with further increase in photosynthesis or removal of many more fruits, fruit growth does not increase. This suggests that sufficient photosynthates are supplied to allow all fruits to achieve their potential growth. However, under these conditions, down-regulation of photosynthesis may occur due to excess carbon accumulation in the source, consequently inhibiting fruit growth.

Environmental controls that increase photosynthesis and agronomic practices that decrease the number of sinks (i.e., fruit removal) can be expected to improve fruit growth and yield. However, unfortunately, most environmental controls require energy cost, and there is often a trade-off between the quality and number of fruits, both of which determined yield. To achieve highly profitable and stable production, it is essential to enhance photosynthesis with a minimum of energy resource to avoid source-limited growth and meet the demand for as many fruits as possible. Therefore, understanding the limiting factors for fruit growth is fundamental for identifying appropriate environmental controls and agronomic practices.

Strawberry (*Fragaria × ananassa* Duch.) is cultivated all over the world, and protected cultivation in greenhouse and various environmental control techniques toward enhancing photosynthesis are widely employed for high yield production (Hidaka et al., 2013). During the reproductive period, approximately 90% of carbon supplied from source is allocated to growing fruits (Nishizawa & Hori, 1988), suggesting that fruits are most strong sink (Forney & Breen, 1985b). The fruits grow on branched clusters called inflorescence. Sink strength of fruits is closely related to the position on the inflorescence (Forney & Breen, 1985c). Moreover, fruits within an inflorescence compete and photosynthates from sources are heterogeneously allocated and unloaded into each fruit in accordance with sink strength (Hidaka et al., 2019; Miyoshi et al., 2021). In strawberry, it is well established that enhanced photosynthesis

increases fruit growth (Hidaka et al., 2013; Yoneda et al., 2020). However, some studies have reported that fruit removal has an insignificant effect on fruit growth (Menzel, 2019). Based on the concept of source-sink balance, the former suggests source-limited growth, whereas the latter implies sink-limited growth. Most of these studies evaluated the effects of manipulating source or sink strength based on average fruit weight per plant, which may have been influenced by the positions within the inflorescence of remaining fruits after removal. They aimed to investigate the effects of source-sink balance on the yield rather than on fruit growth itself. Therefore, these conflicting results have not been discussed, and it remains inconclusive how fruit growth is limited by either source or sink strength.

The objective of this chapter was to investigate fruit growth in responses to altered source and sink strength, considering the effects of position within the inflorescence, to gain insight the regulatory mechanism of photosynthate unloading dynamics. Source and sink were manipulated through supplemental lighting and fruit removal, and the growth of the primary fruit was investigated.

4.2. Materials and methods

4.2.1. Plant materials and growth conditions

Similar to the previous chapters, the experiments were performed using strawberry plants (*Fragaria × ananassa* Duch. ‘Fukuoka S6’) grown under natural light in a greenhouse. On September 26, 2023, the nursery plants were transplanted into cultivation beds. The air temperature inside the greenhouse was kept at 8 to 27°C using a heater and natural ventilation through the roof and side windows. The other growth conditions are similar to those in Chapter 3.

4.2.2. Experimental treatments

There were two light treatments (Figure 4.1): natural light treatment (NL) and supplemental lighting treatment (SL). For SL, white LED sources (LLM0312A; Stanley Electric Co., Ltd., Japan) with peak wavelengths of 450 and 550 nm coupled to an external power supply (LLP0019A, Stanley Electric Co., Ltd., Japan) were installed at the height of 50 cm above the plant base at 30 cm intervals. The spectral distribution of the LED has been described in more detail by Hidaka et al. (2013). Supplemental lighting was applied 10 h daily (7:00 to 17:00) under the control of timer switches (TB171N, Panasonic Co., Ltd., Japan) from November 20, 2023. Within each light treatment, plants were pruned to either one (1F) or seven (7F) fruit(s) per inflorescence (Figure 4.2). In the 1F, a primary flower was retained, and subsequent flowers were removed before anthesis. In the 7F, a primary flower, two secondary flowers, and four tertiary flowers were retained, with others removed before anthesis. To simplify the evaluation of source and sink strength, the number of fully expanded leaves on each plant was maintained at approximately 8 throughout the experiments, and flowers and fruits were present only on the primary inflorescence.

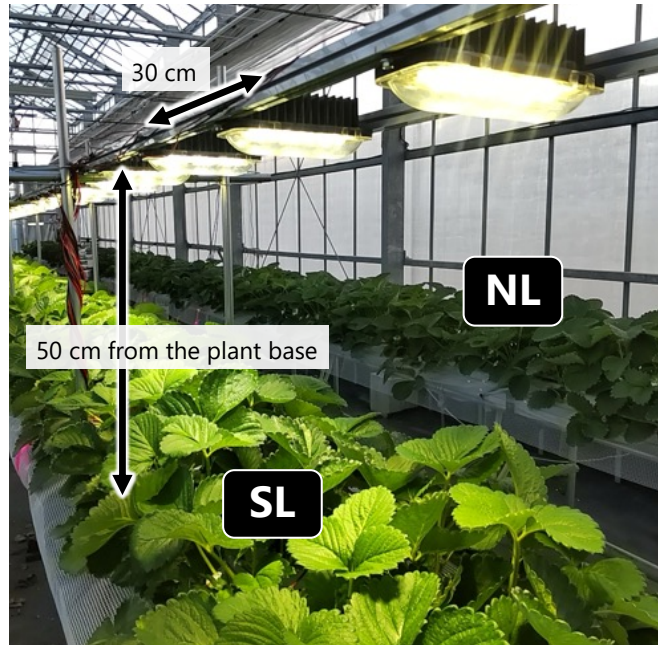


Figure 4.1 Photograph of two light treatments: natural light treatment (NL) and supplemental lighting treatment (SL).



Figure 4.2 Photographs of two pruning treatments: one fruit per inflorescence (1F) and seven fruits per inflorescence (7F).

4.2.3. Measurement of environmental conditions

Photosynthetic photon flux density (PPFD) of each light treatment was measured using light quantum meters (PAR-02D; Prede Co., Ltd., Tokyo, Japan) that were located at a height of 25 cm above the base of plants (near the top of the canopy). In addition, air temperature and relative humidity, CO₂ concentration, and wind speed were measured and recorded at the same

time intervals using the same sensors and data logger as described in Chapters 1 and 3. Daily cumulative PPFD and daily averaged air temperature were calculated in R version 4.3.1 (R Core Team 2023).

4.2.4. Fruit harvest and measurements of fruit growth characteristics

In each treatment (the combination of light treatment and fruit removal), 29–30 primary flowers that bloomed between 8 and 20 Nov 2023, were tagged at anthesis, and the fruit growth stage was identified as the number of days after anthesis (DAA). Fruits were harvested at 5 different growth stages: 5, 15, 25, 35, and 45 DAA. For each stage, 5–6 fruits were collected, and each fruit served as a biological replicate, i.e., 5–6 replications per treatment were investigated.

Measurements of fruit growth characteristics (volume, fresh and dry weight, and soluble sugars—sucrose, glucose, and fructose—and starch concentrations) were conducted using the same methods described in Chapter 3.

4.2.5. Statistical analysis

A two-way analysis of variance (ANOVA) was applied to assess the influences of source strength (light treatments), sink strength (fruit removal), and their interactions of fruit volume, fresh and dry weight, and soluble sugars concentration at 45 DAA. All statistical analyses were performed in R version 4.3.1 (R Core Team, 2023) in the RStudio environment (version 2023.06.0) (RStudio Team, 2023).

4.3. Results

4.3.1. Environmental conditions

PPFD increased by approximately $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ due to supplemental lighting (Figure 4.3a), and daily cumulative PPFD was approximately $5.9 \text{ mol m}^{-2} \text{d}^{-1}$ in NL treatments and $27.5 \text{ mol m}^{-2} \text{d}^{-1}$ in SL treatments (Figure 4.3b). Daily averaged air temperature in the greenhouse gradually decreased during the experimental period, remaining in the range of 8– 22°C (Figure 4.3c).

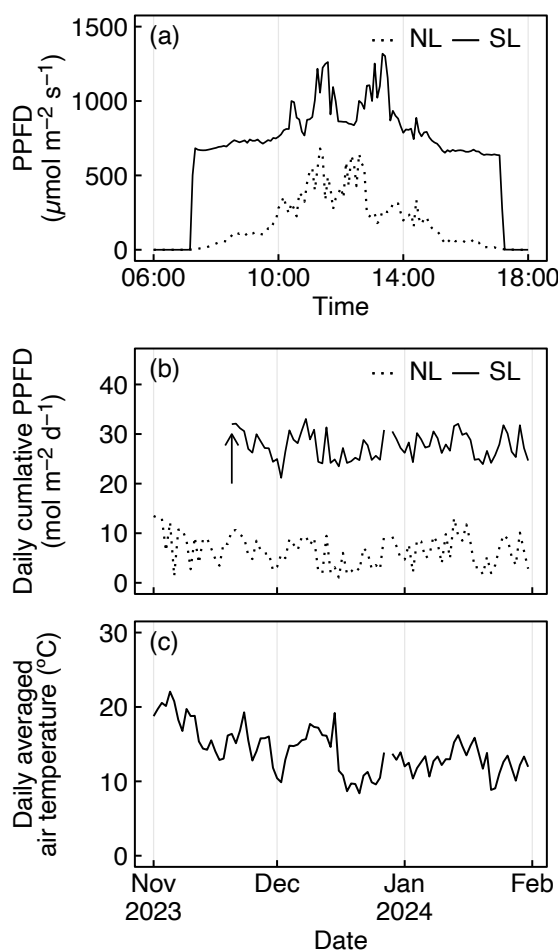


Figure 4.3 Diurnal changes in PPFD on a typical day (December 30, 2023) (a) and daily cumulative PPFD (b) in two light treatments: natural light treatment (NL, dotted line) and supplemental lighting treatment (SL, solid line) and daily averaged air temperature (c) measured in the greenhouse throughout the experimental period (November 1, 2023–January 31, 2024).

Vertical arrow in b represent start of supplemental lighting.

4.3.2. Fruit growth

To investigate the effects of source and sink strength on fruit growth, the volume, fresh weight, dry weight, and carbohydrate concentration of the primary fruit were measured in four treatments: natural light treatment with one fruit per inflorescence (NL1F); natural light treatment with seven fruits per inflorescence (NL7F); supplemental lighting treatment with one fruit per inflorescence (SL1F); supplemental lighting treatment with seven fruits per inflorescence (SL7F). The volume, fresh weight, and dry weight gradually increased until 25 DAA and then rapidly increased across all treatments (Figure 4.4). Fruits in SL treatments showed a tendency for more rapid growth than in NL treatments from 25 DAA onward. Additionally, while the volume and fresh weight in NL7F increased more than in NL1F between 35 and 45 DAA, dry weight showed a similar change in both treatments. Sucrose concentration slightly decreased between 5 and 15 DAA, followed by a gradual increase until 25 DAA and a rapid increase thereafter across all treatments (Figure 4.5a). The sucrose concentration in NL1F was higher than in NL7F from 35 DAA, but concentrations in SL1F and SL7F showed similar values. Glucose and fructose concentrations increased more linearly than sucrose concentrations and showed similar values to each other (Figure 4.5b, c). In contrast to sucrose, while the concentrations in NL1F and NL7F showed similar values, those in SL1F were higher than in SL7F at 35 DAA; however, they were similar between the two treatments at 45 DAA. Starch concentration exponentially decreased from 5 DAA and remained below the detection limit from 15 DAA (Figure 4.5d). The volume, fresh weight, dry weight, and concentrations of sucrose, glucose, and fructose at 45 DAA significantly increased under supplemental lighting; however, the effects of fruit removal and their interaction were statistically non-significant (Figure 4.4 and Figure 4.5).

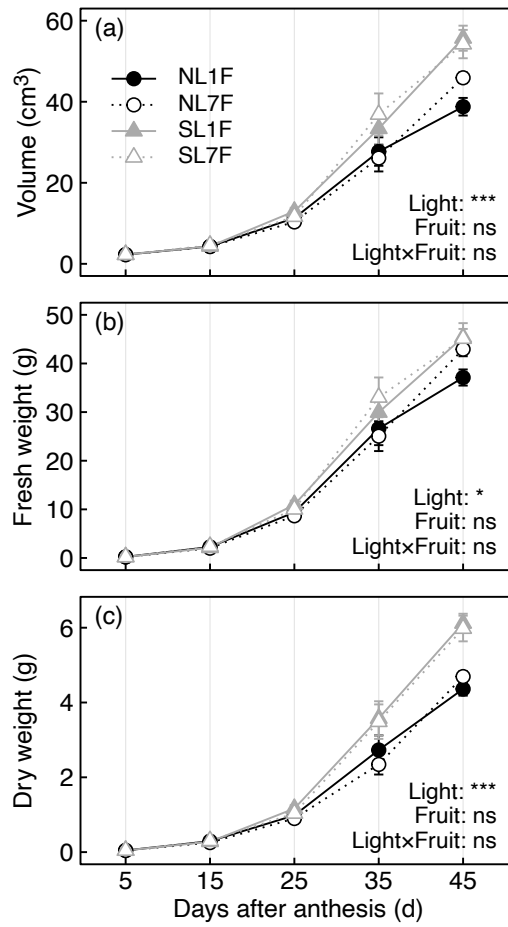


Figure 4.4 Changes in fruit volume (a), fresh weight (b), and dry weight (c) in four treatments: natural light treatment with one fruit per inflorescence (NL1F, closed circle); natural light treatment with seven fruits per inflorescence (NL7F, opened circle); supplemental lighting treatment with one fruit per inflorescence (SL1F, closed triangle); supplemental lighting treatment with seven fruits per inflorescence (SL7F, opened triangle). Error bars represent the standard error of the mean values ($n = 5-6$). The result of two-way ANOVA with light treatments (Light) and fruit removal (Fruit) as independent variables and their interaction (Light $\times$ Fruit) for each dependent variable is shown in each panel. * $p < 0.05$, *** $p < 0.001$; ns = not significant.

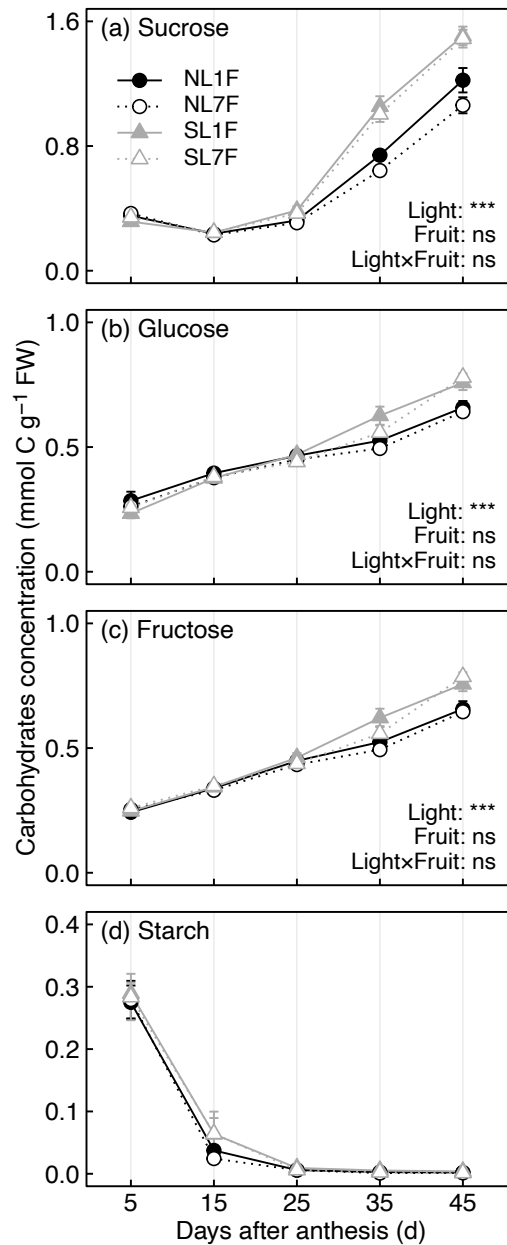


Figure 4.5 Changes in concentrations of sucrose (a), glucose (b), fructose (c), and starch (d) in four treatments: natural light treatment with one fruit per inflorescence (NL1F, closed circle); natural light treatment with seven fruits per inflorescence (NL7F, opened circle); supplemental lighting treatment with one fruit per inflorescence (SL1F, closed triangle); supplemental lighting treatment with seven fruits per inflorescence (SL7F, opened triangle). Error bars represent the standard error of the mean values ($n = 5-6$). The result of two-way ANOVA with light treatments (Light) and fruit removal (Fruit) as independent variables and their interaction (Light $\times$ Fruit) for each dependent variable is shown in each panel: * $p < 0.05$, *** $p < 0.001$; ns = not significant.

4.4. Discussion

4.4.1. *Effects of supplemental lighting on source strength*

In this study, source strength is defined as the photosynthetic rate of a whole plant. During the experiments, because the number of leaves was arranged to around eight on each plant, the source strength would mainly depend on a single leaf photosynthetic rate. For the same cultivar strawberry used in this study, the light saturation point for the single-leaf photosynthetic rate has been reported to be at PPFD of 400–600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Hidaka et al., 2013). In the SL treatments, PPFD near the top of the canopy generally exceeded the light saturation point (Figure 4.3a), meaning that the photosynthetic rate was close to the maximum rate throughout the day. Moreover, supplemental lighting significantly accelerated fruit growth either 1F or 7F treatments (Figure 4.4 and Figure 4.5). These results indicate that source strength was increased by supplemental lighting.

4.4.2. *Source-limited vs sink-limited*

Comparison between NL1F and SL1F showed a significant increase in fruit growth due to enhanced source strength, suggesting fruit in NL1F was limited by source strength; however, comparison between NL1F and NL7F showed no difference in fruit growth despite fruit removal, indicating fruit in NL1F was limited by sink strength. This study evaluated the effect of source-sink balance on fruit growth itself using only the primary fruits, unlike previous studies (e.g., Menzel, 2019) that used various position fruits within the inflorescence; however, similar conflicting behavior was observed. There are several possible explanations for these results.

First, source-sink balance during the developmental period of the primary fruit may not be affected by inferior position fruits on the inflorescence. Strawberry has basically a dichasial cyme inflorescence, in which two fruits are produced on two sides of the earlier produced fruit.

Due to this structure, there is a hierarchy of fruits with growth rate and size decreasing in inferior positions (Forney & Breen, 1985c). Moreover, it is reported that sink strength of strawberry fruit was low during early fruit growth and rapidly increases during late growth (Nakai et al., 2024). In this study, secondary flowers and tertiary flowers in NL7F and SL7F bloomed approximately 5–8 days and 13–22 days after the anthesis of the primary flower, respectively (data not shown). The hierarchy and differences in growth periods within an inflorescence could result in extremely low sink strength of the inferior position fruits, leading to no significant impact on the cumulative sink strength of a whole plant and, consequently, on the growth of primary fruits. Therefore, it is likely that the increases in fruit growth were due solely to enhanced source strength, indicating that the growth of NL1F was limited by source. The finding that the number of fruits had no significant effect on fruit growth contrasts with previous studies on tomato, which reported that fruit removal increased the growth of the remaining fruits (Heuvelink, 2005; Matsuda et al., 2011). Tomato inflorescences are basically classified as monochasial cyme. The inflorescence structure would be linked to the arrangement of the vascular system and consequently the translocation pathways, which may influence the differences in carbon competition among fruits between strawberry and tomato.

Second, sink strength may increase with supplemental lighting. Generally, when there is no significant difference between plants with one fruit per inflorescence and those with more fruits, it can be assumed that the fruits achieve their potential growth and the growth rate indicates the sink strength. In this study, comparison between 1F and 7F revealed no differences in fruit growth under both NL and SL, suggesting that the fruits achieved their potential growth limited by sink strength under each light treatment. Furthermore, based on this assumption, it can be inferred that sink strength in SL was higher than that in NL. Sink strength comprise of sink size as the physical restraint and sink activity as the physiological restraint (Ho, 1988). When the sink size such as the cell number is genetically predefined and unchangeable, sink strength depends on sink activity, which reflects phloem unloading, intercellular transport,

metabolism, and compartmentation (Yamaki, 2010). Ji et al. (2020) reported that key genes related to these processes were upregulated by far-red radiation, resulting in an increase in sink strength. Although the present study did not use far-red radiation, the enhanced sink activity might have been induced by supplemental lighting.

Third, it is possible that source strength was influenced by sink strength in this study. An imbalance between source and sink strength can lead to feedback regulation of photosynthesis through excessive accumulation of photosynthates (Paul & Foyer, 2001). Accumulated photosynthates in source leaves, stored as non-structural carbohydrates, are known to suppress the expression of photosynthetic gene involved in Calvin–Benson cycle and stomatal opening (Stitt et al., 2010). The down-regulation of photosynthesis has been reported to occur under enhanced CO₂ concentration conditions, which enhances source strength (Ainsworth et al., 2004). Similarly, low sink strength has been reported to decrease photosynthesis (Iglesias et al., 2002). While the degree of down-regulation in strawberries remains unclear, they accumulate a large amount of non-structural carbohydrates in leaves during the day shown in Chapter 1 and 2, which may influence photosynthesis. In this study, as the focus was solely on fruit growth, the possible interference of the downregulation of photosynthesis cannot be ruled out. Thus, the interaction between source and sink might flexibly modulate fruit growth in response to manipulations of source and sink strength.

4.5. Conclusion

In this chapter, the limitations of fruit growth by source and sink strength were investigated. Unlike previous studies that assessed the effects of source-sink balance on average fruit weight, I focused on the impact on the growth of the primary fruit. Nevertheless, the results were complex and could be interpreted as either source-limited or sink-limited growth. These findings suggest that the position of fruit within the inflorescence, the environmental response

of sink strength, and the interaction between source and sink may influence fruit growth. Further research is required to identify the limiting factors for fruit growth in response to altered source-sink balance; however, the present study contributes to a fundamental understanding of the regulatory mechanism of photosynthate unloading.

General conclusion

In fruit crops such as strawberry, photosynthate translocation from leaves (source) to the harvestable target organs (sink) directly affect crop productivity. For realizing the high quality and high yield production, it is keenly desired to evaluate the translocation dynamics and to optimize translocation by environmental controls based on it. This study aimed to evaluate the dynamics of photosynthate translocation and explore the regulatory mechanisms in strawberries under fluctuating environmental conditions.

Firstly, through three different day/night cycles, the dynamics of photosynthate loading were evaluated based on the leaf carbon balance. During the daytime, when photosynthesis is the primary source of available photosynthates and sucrose concentration exceeded $150 \text{ mmol C m}^{-2}$, these are allocated to sucrose and starch. An increase in sucrose concentration enhanced the loading rate. At night, when photosynthates are supplied by the sucrose and starch stored during the day, and the loading rate was lower compared to the daytime. These results suggest that in the daytime, the coordination of photosynthate availability for loading was affected by photosynthate allocation between sucrose and starch, whereas, at night, it was affected by leaf photosynthate storage levels at dusk. Overall, the loading dynamics in strawberry leaves under field conditions were elucidated, advancing the understanding of the role of photosynthate allocation in the regulatory of loading. Secondary, the photosynthate allocation between sucrose and starch was further analyzed to explore the regulatory mechanisms of loading. To clarify the inherent characteristics of the allocation in strawberry leaves, plants grown under identical day/night cycles were used, and the effect of different photosynthetic activities on leaf carbohydrates dynamics under controlled light conditions was investigated. The results suggest that when photosynthetic activity is low and the sucrose concentration does not reach the leaf storage capacity, photosynthates are preferentially allocated to sucrose over starch; however, when photosynthetic activity is high and the sucrose concentration exceeds the storage capacity,

photosynthates are allocated to starch. The results contribute to further understand the regulatory mechanisms of photosynthate loading. Thirdly, the dynamics of potential photosynthate unloading, unaffected by competition among fruits, was analyzed based on sink strength, which govern the dynamics. During the early stages of fruit growth, the unloading rate and sink strength were low, peaked around 40 days after anthesis (DAA), and subsequently declined in the later stages of growth. The determinants of sink strength shifted during fruit growth: sink size played a predominant role in the early growth stage, while sink activity became more critical in the later stage. Finally, the effects of the altered balance between source and sink strength on fruit growth and carbohydrate accumulation were analyzed to explore the regulatory mechanisms of unloading. Changes in source strength through supplemental lighting had a greater effect on growth and carbohydrate accumulation in the primary fruits than changes in sink strength through fruit removal. These results suggest complexity in the regulatory mechanisms of unloading, with the possibility that it may be associated with the hierarchy in strawberry inflorescences, environmental response of sink strength, and/or the source-sink interaction.

In this thesis, the focus has been on highlighting photosynthate loading and unloading, with each analyzed separately. However, further research that integrates them as a set of translocation processes is needed to provide more definitive evidence. Directly measuring translocation dynamics could provide more definitive data. I used the evaluation based on the carbon balance to assess translocation dynamic due to its usability under field conditions, but it does not allow for observation of actual photosynthate movement. Although the applicability is limited, stable or radioactive isotopes are a powerful tool for directly capturing these dynamics (Bögelein et al., 2019; Mincke et al., 2021; Miyoshi et al., 2021). Additionally, mathematical modeling enables the integration of separately obtained data, such as presented in this thesis, allowing comparison with continuous data (Baldazzi et al., 2013). It would be also useful for adapting data collected under controlled conditions to field studies. Nonetheless,

I believe my research has provided advance made in the understanding of the translocation dynamics and their regulatory mechanism in strawberries and thus will contribute to the development of cultivation systems to improve fruit crop yield and quality in the future.

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