

Seasonal patterns of carbon assimilation and allocation of a summer-green forest herb, *Parasenecio auriculata* (Senecioneae; Asteraceae)

Takashi Y. Ida · Gaku Kudo

Received: 21 September 2009 / Accepted: 26 February 2010 / Published online: 10 March 2010
© Springer Science+Business Media B.V. 2010

Abstract Summer-green herbs inhabiting deciduous forests often put out aerial shoots under bright conditions before tree-canopy closure and grow until late summer under the closed canopy. Some of them produce leaves continuously even after the initiation of canopy closure, indicating an exploitation of the low light period. The manner of carbon assimilation during bright and shade periods within a growth season should reflect the seasonal patterns of vegetative growth and reproductive allocation of individual species. We examined the seasonal patterns of assimilation, partitioning of photosynthate between reproduction and storage, and the budget of reproduction of a perennial understory herb, *Parasenecio auriculata*. Although photosynthetic rates per unit leaf area decreased with the seasonal reduction in light level, net assimilation at the whole-plant level was maintained at a high level even after canopy closure owing to the increase in the total leaf area. Stored resource in tubers contributed to the rapid development of aerial shoots in the early season, and annual tuber growth was completed before flowering. Instant photosynthetic products considerably contributed to the maintenance of flowers but not to fruit development because of low assimilation rate during

fruiting. These findings indicated that carbon assimilation during flowering contributes to sexual reproduction without influencing the development of storage organs. Stable carbon assimilation over summer by shade-acclimatized leaves enabled the maintenance of high productivity associated with high sexual reproduction.

Keywords Carbon translocation · Continuous leaf production · Deciduous forest · Light availability · Resource allocation · Summer-green herb

Introduction

Seasonal changes in light availability on the floor of temperate deciduous forests reflect the dynamics of leafing phenology of canopy trees. Short periods of high irradiance in the spring and autumn are important for the carbon assimilation of understory plants (Rothstein and Zak 2001; Ida and Kudo 2008). However, summer-green plants have to photosynthesize under shade conditions during the most of their growth season. Height growth of understory summer-green herbs is often accelerated as light availability decreases with the progress of canopy closure (Kawarasaki and Hori 2001; Tani and Kudo 2006). This is because the development of above-ground structures may be advantageous for efficient assimilation under decreasing light conditions

T. Y. Ida (✉) · G. Kudo
Graduate School of Environmental Science,
Hokkaido University, Kita 10 Nishi 5, Kita-ku,
Sapporo 060-0810, Japan
e-mail: id@ees.hokudai.ac.jp

(Lezberg et al. 1999; Tani and Kudo 2006). Although the construction and maintenance of large above-ground structures require large carbon investment, the accumulation of leaf area within plants should contribute to the efficient carbon gain for these plants.

The manner of leaf production greatly influences the carbon assimilation of plants. There are two types of leafing patterns (e.g., Kikuzawa 1983, 2003). One is simultaneous leaf production, in which most leaves appear within a short period and they are often maintained over the photosynthetic period. The other is continuous leaf production, in which a small number of leaves are produced successively during the growth season. The effectiveness of continuous leaf production may vary depending on the light situation. First, it may contribute to maintain high photosynthetic activity over growth season by recruiting new leaves (Kikuzawa 2003). Production of leaves with high photosynthetic ability and short longevity is especially efficient under constantly bright conditions because plants can photosynthesize using young active leaves without self-shading. In contrast, new leaf production may be advantageous for the regulation of photosynthetic acclimation to changing light conditions (Newell et al. 1993; Lovelock et al. 1994) if the modification of anatomical traits (such as large area of mesophyll cell surface to arrange chloroplasts) of already expanded leaves may entail additional costs (e.g., Oguchi et al. 2008). Finally, continuous production of long-lived leaves accumulates total leaf area within plants, which may contribute to maintain the assimilative ability of whole plants in some circumstance. Tani and Kudo (2006) revealed that a positive carbon balance was maintained in some summer-green herbs by the accumulation of total leaf area throughout canopy closure even when vertical leaf structure caused shading by upper leaves within plants (e.g., Yamada et al. 2000; Kikuzawa 2003). Thus, the ecological and physiological significances of continuous leaf production should vary depending on the light situation. However, little is known how the patterns of carbon assimilation and vegetative growth are related to the reproductive performance of plants.

Production of flowers and fruits is often accompanied by a reduction in growth and/or storage functions, which is defined as a direct cost of reproduction (Obeso 2002). The trade-off relationships may reflect the balance between fecundity in a

given year and subsequent survival and reproduction (Iwasa and Cohen 1989; De Jong and Klinkhamer 2005). For summer-green herbs, which maintain their leaves mostly under the closed canopy, the cost of reproduction may be a crucial factor influencing vegetative growth and storage functions if photosynthetic carbon gain is restricted by the low light level. In order to reduce the conflict, phenological separation of resource investment between reproduction and storage organs may be available (e.g., Garcia and Ehrlen 2002; Ida and Kudo 2008). For instance, fruit production in spring-blooming *Trillium* species occurred after the development of storage organs, and fruiting greatly depended on the photosynthetic products acquired before canopy closure that were stored in aerial stems, because carbon assimilation was limited in the late season (i.e., fruiting season) due to the low light level (Routhier and Lapointe 2002; Ida and Kudo 2008). If understory herbs can exploit the long shade period for carbon assimilation, then they may save the excess carbon for the subsequent reproduction under the closed canopy, resulting in a stable, high fruit/flower ratio.

Summer-green understory herbs that have a tall aboveground structure and continuous leaf production may exploit shade conditions effectively for assimilation owing to their accumulation of numerous shade-acclimatized leaves (Tani and Kudo 2006). This may result in a stable, large investment of current photosynthetic products in both flower and fruit productions. In order to test this prediction, we investigated the seasonal patterns of (1) growth (by dry mass), (2) carbon assimilation (by photosynthetic measurements), and (3) translocation of photosynthetic products (by ^{13}C tracing) in *Parasenecio auriculata* (Senecioneae; Asteraceae), a common perennial summer-green species inhabiting deciduous forests of northern Japan. This species shows a stable, high fruit to flower ratio (Kudo et al. 2008), tall aerial shoots (60–120 cm), and continuous production and accumulation of summer-green leaves. As the below-ground structure (tuber) is composed of 2-year segments, a large amount of resource stored in tubers should be replaced with current photosynthetic products. Thus, annual photosynthetic assimilation may greatly influence the allocation pattern between current reproduction and storage. We aimed to clarify the light harvesting strategy of this species in terms of growth schedule of aerial shoots and storage organ

and carbon budget for reproduction. Specifically, the following questions are asked: (1) Does the intensive leaf production and accumulation effectively contribute to the maintenance of net assimilation at the whole-plant level after canopy closure? (2) Does the development of tubers occur prior to or simultaneously with flowering and fruiting (i.e., the existence of phenological separation)?, (3) How high is the contribution of current photosynthesis to flower and fruit production (i.e., the carbon budget for reproduction)?

Materials and methods

Study site and species

This study was conducted during 2007–2008 in a deciduous forest of Nopporo Forest Park (43°20'N, 141°31'E) in Hokkaido, northern Japan. Leaf flush of the canopy trees usually initiates in mid-May and is completed by mid-June, and the understory is completely shaded from mid-June to mid-October (Fig. 1). We established a research site (20 × 20 m) under the canopy.

Parasenecio auriculata (DC) J. R. Grant [synonym, *Cacalia auriculata* var. *kamtschatica* (Maxim.) Matsum] is a polycarpic perennial herb inhabiting deciduous forests. Leaf emergence usually occurs in late-April to early-May, and leaf production continues throughout the growth season until the flowering

stage. Flowering initiates in mid-August and subsequent fruit development continues until early-October. Nectar-feeding bumblebees (mainly *Bombus hypocrita sapporoensis*) and butterflies are major pollinators of this species. Pollinator exclusion restricts seed production of *P. auriculata*, indicating that it is an obligate outcrosser (Kudo et al. 2008). Clonal propagation sometimes occurs in this species, and plants are distributed patchily on the forest floor. We selected a plant that had a single shoot from each patch to avoid choosing clonal or genetically close plants. Tubers of this species are divided into two tissues (Fig. 2); a thick basal tuber composed of a single-year segment and a newly produced tuber that develops into a next-year tuber and aerial shoot. A new subterranean primordium emerges at the shoot emergence stage, while the previous-year tuber usually has disappeared at this time (Fig. 2). Therefore, the tissues of the belowground parts are exchanged at the interval of two growth seasons.

Meteorological data

In order to investigate the seasonal changes in climate conditions of the forest floor, air temperature and irradiance at a height of 2 m were recorded automatically at 1-h intervals using a combined data logger with a luxmeter and thermometer (HOBO pendant logger CO-UA-002-08; Onset Computer, MA, USA) from April 20 to October 31, 2007. The luxmeter was calibrated with a quantum sensor (S-LIA-M003;

Fig. 1 Daily maximum photosynthetic photon flux density (PPFD; solid line), daily mean temperature (dashed line), and fitted sigmoid curve of daily maximum PPFD (dotted line) on the forest floor in 2007

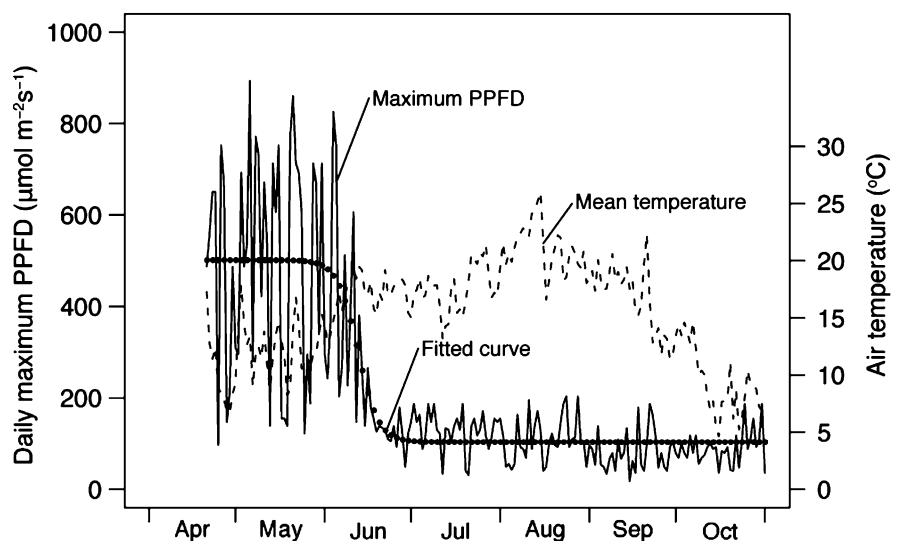
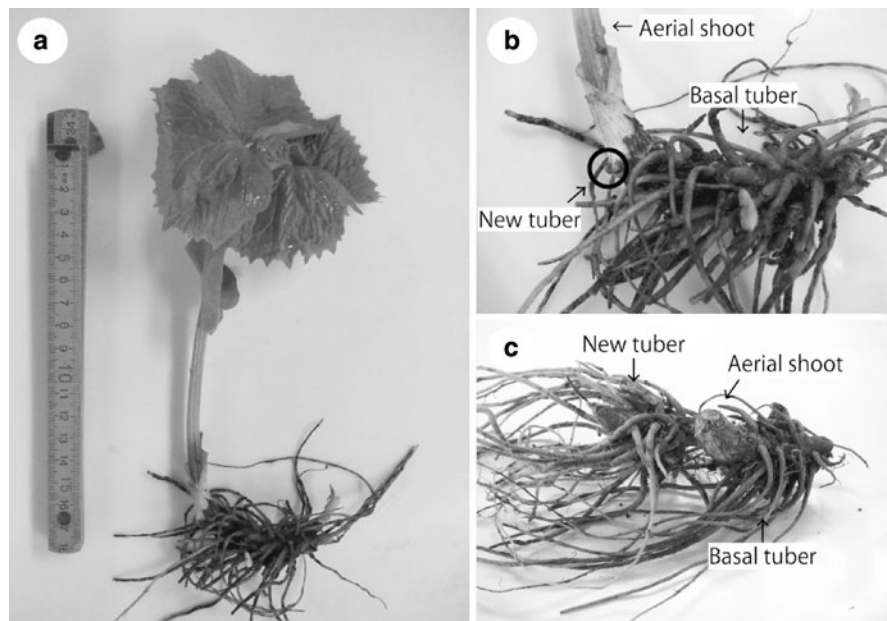


Fig. 2 Photographs of **a** *Parasenecio auriculata* at the time of shoot emergence in early spring; **b** belowground parts at shoot emergence; and **c** belowground parts at fruiting stage. Tubers are divided into 2-year segments. The primordium of new tuber is formed at the time of shoot emergence and develops over the growth season. Scale unit is cm in Fig. 2a



Onset Computer, MA), and recordings were made at a height of 2 m in the forest to convert lux into photosynthetic photon flux density (PPFD).

In order to measure the seasonal decline in light levels due to the progress of canopy closure, the light availability was fitted to a logistic curve with four parameters, in which the light level on day x (Julian date) can be written as:

$$\text{Light level } (x) = p_1 + p_2 / (1 + \exp(p_3(x - p_4))),$$

where p_1 is the mean daily maximum PPFD before canopy closure, p_2 is a negative value indicating the difference between asymptotic minimum and maximum irradiance (i.e., maximum PPFD before and after canopy closure), p_3 represents an inflection point of the curve, and p_4 represents the timing of the reduction in the curve. Daily values were fitted to the logistic curve using non-linear least-squares estimates of the parameters. Based on the fitted curve, initiation, intermediate point, and completion of canopy closure were defined as 5, 50, and 95% reduction from the maximum value (i.e., p_1), respectively.

Growth pattern

In order to reveal the seasonal growth patterns of *P. auriculata*, the dry weight of each organ was measured for 10–15 plants at six points during the growth season (April 20, 2008; May 2, 2008; May 30,

2008; July 5, 2008; August 25, 2007, and September 24, 2007). Individual plants sampled were taken back to the laboratory and separated into five organs: flower or fruit, leaf, stem, basal tuber including roots, and new tuber including roots. Each sample was oven-dried at 70°C for 72 h and weighed. Leaf area was also measured using leaf samples for mass measurements to assess the size of the photosynthetic organs. Leaf samples were optically scanned into a computer, and leaf area (m²) was measured using image analysis software (Image J version 1.34, National Institutes of Health, Bethesda, MD, USA) by finding the optimal binary threshold for individual leaves. Before statistical analysis, data were log-transformed to improve normality. Each growth parameter was compared among six observation days by one-way factorial ANOVAs followed by Tukey's honest significant difference test.

Photosynthesis

In order to determine the annual productivity of plants, photosynthetic rates were measured on April 20, 2008, before the initiation of canopy closure (early-growth stage), August 25, 2007, at flowering stage, and September 24, 2007, at fruiting stage. Light responses of photosynthetic rates were measured for a top-positioned leaf in three individuals at each stage using a portable closed gas-exchange

system (LI6400; Li-Cor, Lincoln, NB, USA). This resulted in different cohorts of leaves being measured at each stage to reveal the seasonal changes of photosynthetic traits across different cohorts in different environments. Nine light conditions (1500, 1000, 800, 500, 300, 100, 50, 10, and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$) of photosynthetically active radiation (PAR) were provided using a red-blue LED light source at constant leaf temperature (20°C). Ambient CO_2 concentration and the humidity of incoming air during the measurement were maintained at 350 $\mu\text{l l}^{-1}$ and 1.1 vapor pressure deficit (VPD, hPa), respectively. The maximum photosynthetic rate (A_{max}), the initial slope of the light-response curve under low light ($\leq 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF) conditions (α) and dark respiration rate at 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (R_d) were obtained for individual plants. A_{max} was defined as the maximum value among nine light levels, and α was calculated by linear regression using values between 0 and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Before the statistical analysis, data were log-transformed to improve normality. We analyzed each photosynthetic parameter (A_{max} , α , and R_d) among growth stages using one-way factorial ANOVAs followed by Tukey's honest significant difference test.

Daily net assimilation was simulated under the assumption that photosynthetic rates were mainly determined by only light conditions, and that the effects of seasonal variations in the concentrations of ambient CO_2 and leaf temperature on total assimilation were small. This is because the light availability of the understory changes drastically from spring to summer, and it strongly determines the photosynthetic capacity for many understory herbs (e.g., Kudo et al. 2008). In order to assess the photosynthetic capacity corresponding to the light level, the net photosynthetic rate (A) as a function of photon irradiance (I , $\mu\text{mol m}^{-2} \text{s}^{-1}$) was described accurately using a non-rectangular hyperbola as follows;

$$A = \frac{\alpha' I + A'_{\text{max}} - \sqrt{(\alpha' I + A'_{\text{max}})^2 - 4\alpha' I \theta' A'_{\text{max}}}}{2\theta'} - R'_d,$$

where A'_{max} , α' , θ' , and R'_d indicate light-saturated photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), initial slope of the hyperbola ($\mu\text{mol m}^{-2} \text{s}^{-1}$), degree of curvature (dimensionless), and dark respiration rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), respectively (Marshall and Biscoe

1980). Data obtained for an average of plants at each stage were fitted to this equation using non-linear least-square estimates of the parameters. First, the hourly transition of light availability was estimated on each day based on the photon flux density at 1-h intervals (see “[Meteorological data](#)” section). In this estimation, the contribution of sunfleck to carbon assimilation was assumed to be small. Next, net photosynthetic rates per hour were estimated based on the light availability and photosynthetic parameters obtained at each growth stage (i.e., early growing, flowering, and fruiting stage). The net photosynthetic rates of individual leaves within a plant are assumed to be similar irrespective of the age difference between leaves. The whole growth season was divided into early season from leaf emergence (20 April) to the day when light level reduced to 50% (12 June), middle season from 13 June to the end of flowering (9 September), and late season for 10 September to fruit maturation (9 October). For each subdivided season, photosynthetic parameters obtained for early growing, flowering, and fruiting stages were applied, respectively, and the photosynthetic rate was calculated. Furthermore, we divided the foliage season into six periods to take into account the effect of leaf accumulation. These periods started on 20 April, 26 April, 16 May, 13 June, 1 August, and 10 September, respectively. We used the mean total leaf area observed six points (April 20, May 2, May 30, July 5, August 25, and September 24, see “[Growth pattern](#)” section) for individual periods. Thus, daily net assimilation per plant was calculated by the combination of hourly light availability, photosynthetic parameters divided into three periods, and total leaf area per plant divided into six periods throughout the growth season. There were two caveats in this estimation of daily net assimilation per plant, especially under shade conditions. The net assimilation per plant would be underestimated because sudden light improvement, such as sunfleck, was not detected in the 1-h interval measurements of light availability. In contrast, it would be overestimated because the photosynthetic rate of lower-positioned leaves may decrease because of the aging effect. We compared daily net assimilation among six periods using one-way factorial ANOVA followed by Tukey's honest significant difference test.

^{13}C tracing

Seasonal patterns of assimilative carbon translocation between the flowering stage (August 22–25, 2007) and the fruiting stage (September 22–24, 2007) were measured by supplying ^{13}C to each of 10 intact plants. We also measured the ^{13}C levels for 10 individuals as a control. On the days of labeling, whole leaves of individual plants were enclosed with a 40×30 cm sealed nylon bag (chamber), into which ^{13}C was injected. The ^{13}C was produced by mixing 150 mg of 99.9 at.% Ba ^{13}C (Isotec Inc., Miamisburg, OH, USA) with 30 ml of lactic acid per injection. The ^{13}C was injected twice on the first and second day at a concentration of CO_2 equivalent to that of normal air (about 360 ppm). Each chamber was removed after the second day labeling period. The ^{13}C -labeled plants were harvested at 2 days after labeling. After harvesting, individual plants were separated into five organs (flower or fruit, stem, leaf, basal tuber, and new tuber) and oven-dried at 70°C for 72 h. After weighing each organ, they were ground separately in a mortar.

The combined system of an elemental analyzer (Flush EA; Thermo Fisher Scientific, Bremen, Germany) and an isotope ratio mass spectrometer (Delta V Plus; Thermo Fisher Scientific, Bremen, Germany) was used to measure $\delta^{13}\text{C}$. Approximately, 1-mg sample of plant tissues was packaged in a tin container, and carbon isotopic composition was analyzed. Excess ^{13}C was calculated according to the procedure by Simard et al. (1997). Each $\delta^{13}\text{C}$ value was converted into the absolute isotope ratio of the sample with the carbon isotope ratio of the international VPDB standard. The total ^{13}C content of sample was calculated from the ^{13}C abundance and total carbon weight of each organ. The excess ^{13}C was represented as the difference between the ^{13}C content of labeled samples and unlabeled control samples. Therefore, excess mg ^{13}C in each organ was calculated as a product of excess mg ^{13}C (per mg of sample) and the biomass of each organ. Individual carbon translocation (Excess ^{13}C , %) was expressed as a percentage of the excess mg ^{13}C of each organ against excess mg ^{13}C of the whole plant. Further details of the calculation of excess ^{13}C are described in Ida and Kudo (2008).

Before the statistical analysis, data were arcsine-transformed to improve normality. The ^{13}C

percentages in each organ were compared between the flowering and fruiting stages by *t* test. An open source system, R version 2.9.2 was used for all statistical analyses.

Results

Understory environment

The estimated daily maximum PPFD was $501 \mu\text{mol m}^{-2} \text{s}^{-1}$ before tree-canopy closure (Fig. 1). Initiation of canopy closure occurred on 30 May and canopy closure completed on June 21, 2007. Under the closed canopy, PPFD remained at $103 \mu\text{mol m}^{-2} \text{s}^{-1}$. Mean air temperature was 12.5°C in May and increased gradually to 21.0°C in August. Then, it decreased from mid-September to October (9.5°C).

Growth pattern

Aerial shoot growth in mass had been completed in early-July, and leaf and stem masses were maintained during the flowering period (Fig. 3). As total leaf mass and area decreased from the flowering to fruiting periods, the maximum leaf size was sustained under closed canopy before fruiting. The only exception was flower mass, in which floral bud formation occurred in mid- to late-June and developed under the closed canopy.

In the belowground parts, 2-year-old tubers had been completely exhausted by the time of shoot emergence in late-April, in which only 1-year-old tubers (i.e., basal tuber in Fig. 2) existed. The mass of the basal tubers in late-April (0.623 g) was only 27% of that in late season (2.315 g). Since the mass of new tubers (1.988 g) was similar to that of basal tubers in the late season, the mass of basal tubers just before shoot emergence (early-April) was estimated to be much larger than that in late-April. This indicated that respiration during the dormant period (i.e., winter consumption) and initial shoot production partly depended on stored material in basal tubers in addition to the full contribution of 2-year-old tubers. The mass of the basal tubers recovered rapidly by the initiation of canopy closure, and it was then maintained throughout the foliage period. New tubers

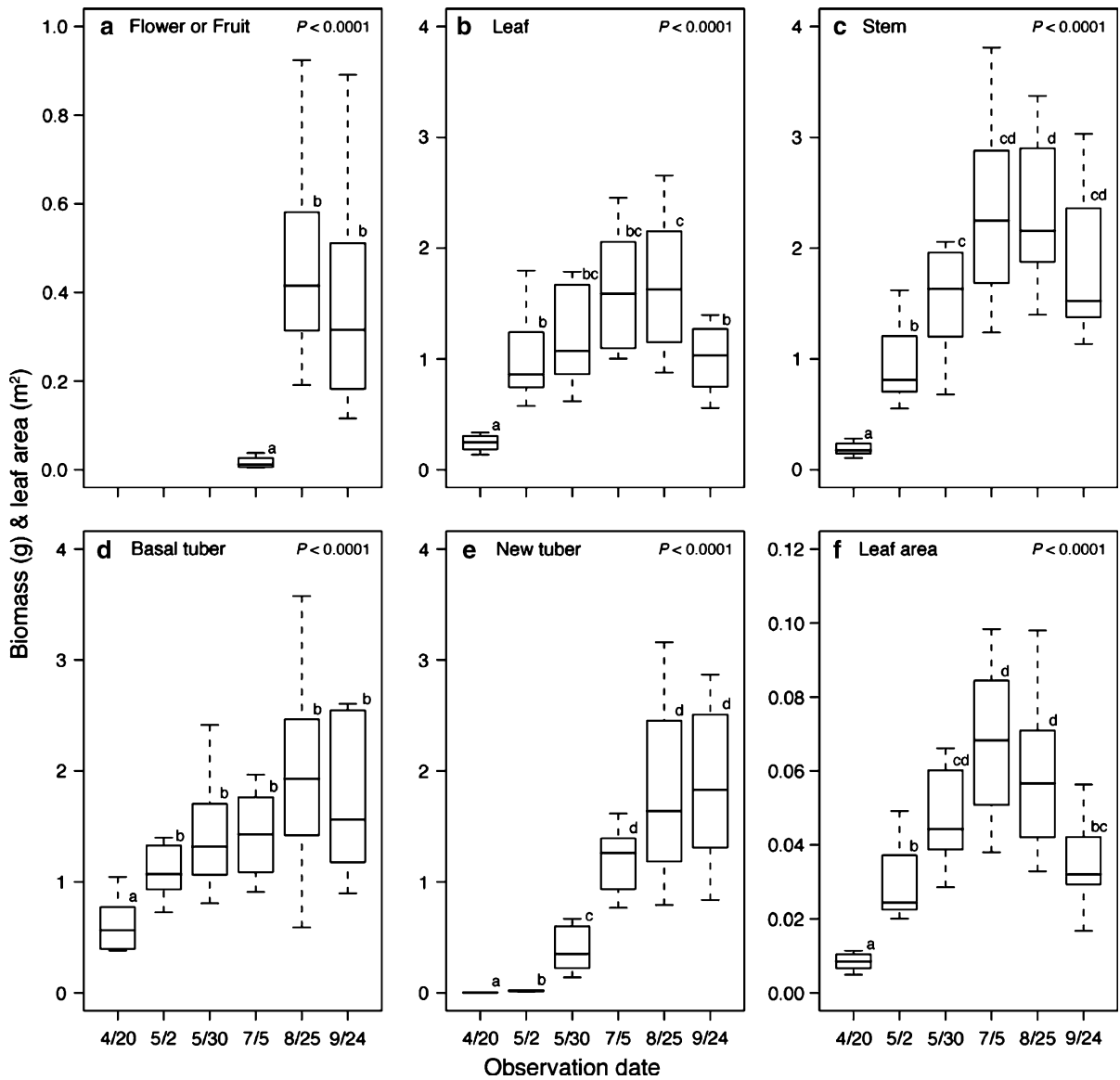


Fig. 3 Seasonal patterns of **a** flower or fruit mass, **b** leaf mass, **c** stem mass, **d** basal tuber mass, **e** new tuber mass, and **f** total leaf area. Belowground parts (basal tuber and new tuber) contained tuber and root. Box-and-whiskers plot represents 75th, 50th, and 25th percentiles, *top whisker* ranges from the

75th to 90th percentile, and the *bottom whisker* from 25th to 10th percentile. *P* values by one-way ANOVA are indicated. Different letters above symbols indicate a significant difference: $P < 0.05$ by Tukey's honest significant difference test following one-way ANOVA

started to develop after the replenishment of basal tubers during June and July under the closed canopy. As the masses of aerial shoots and belowground parts did not increase from the flowering to fruiting stages, annual growth had been completed by the flowering stage in terms of mass.

Photosynthesis

ANOVAs for the maximum photosynthetic rate (A_{max}) and dark respiration (R_d) revealed significant differences among growth stages, while the initial slopes (α) did not differ (Table 1). Plants at the early-growth

Table 1 Results of one-way ANOVAs for photosynthetic parameters among growth stages (early growing, flowering, and fruiting stages)

Variables	Stage		
	Early growing	Flowering	Fruiting
A_{\max} ($F_{2,6} = 13.87$, $P < 0.01$)	8.70 ^a ± 0.63	4.29 ^b ± 0.29	3.05 ^b ± 0.79
α ($F_{2,6} = 3.03$, $P > 0.1$)	0.032 ^a ± 0.001	0.032 ^a ± 0.003	0.022 ^a ± 0.005
R_d ($F_{2,6} = 52.39$, $P < 0.001$)	1.74 ^a ± 0.25	0.22 ^b ± 0.01	0.19 ^b ± 0.05

Before analyses, data were log-transformed to improve normality. Mean ± 1SE. Sample size is 3 at each stage

* Means within a row followed by the same letter are not significantly different according to Tukey's honest significant difference test following one-way ANOVA ($P < 0.05$)

stage showed 103% higher A_{\max} and 682% higher R_d than those at the flowering stage, and these differences were significant ($P < 0.05$ and $P < 0.001$, respectively). In contrast, there were no significant differences in every parameter between the flowering and fruiting stages ($P > 0.17$). These results indicated that the photosynthetic rates of *P. auriculata* vary in response to the seasonal changes in light level.

Estimated daily net assimilation per plant significantly varied among six periods within the growth season ($F_{5,167} = 19.91$, $P < 0.0001$; Table 2). There was a bimodal pattern with peaks in the second period (before canopy closure) and the fourth period (before flowering under the closed canopy). Before canopy closure, daily net assimilation increased with the increase in the total leaf size (Fig. 3). The subsequent decrease in daily net assimilation reflected the decrease in light availability due to the progress of canopy closure. The daily net assimilation under closed canopy attained the second peak owing to leaf accumulation, and it then decreased after the

Table 2 Estimated daily net photosynthetic carbon gain per plant throughout the growth season

Period	Carbon gain ($\mu\text{mol CO}_2$ per plant; Mean ± 1SE)
1st period (20 Apr–25 Apr)	0.134 ^{abc} ± 0.061 (6)
2nd period (26 Apr–15 May)	0.294 ^{ab} ± 0.042 (20)
3rd period (16 May–12 Jun)	0.164 ^c ± 0.055 (28)
4th period (13 Jun–31 Jul)	0.535 ^d ± 0.024 (49)
5th period (1 Aug–9 Sep)	0.342 ^b ± 0.023 (40)
6th period (10 Sep–9 Oct)	0.109 ^{ac} ± 0.011 (30)

For phenological situations of individual stages, see Fig. 4

* Means followed by the same letter are not significantly different according to Tukey's honest significant difference test following one-way ANOVA ($P < 0.05$)

initiation of flowering. Note that the daily net assimilation in the third period may be underestimated because photosynthetic parameters in the first period were used, in which dark respiration rates were considerably higher (Table 1).

The cumulative daily net assimilation increased from the canopy closure (mid-June) to the flowering stages, while it increased only slightly during the fruiting period (Fig. 4). The contributions of individual growth periods (i.e., from first to sixth periods in Table 2) to total assimilation were 1.5, 10.1, 8.5, 48.6, 25.2, and 6.0%, respectively. Therefore, plants gained most of their carbon resource while under the closed canopy before fruiting when their leaf area attained its maximize size (Fig. 3f).

Isotope analysis

The translocation of absorbed ^{13}C to leaves and basal tubers was similar between flowering and fruiting stages ($P > 0.05$), but those to other organs varied significantly (Fig. 5). The excess ^{13}C in flowers decreased drastically (80% change, $P < 0.01$) at the fruiting stage. In contrast, excess ^{13}C at the fruiting stage obviously increased in stems (95% change, $P < 0.05$) and new tubers (201% change, $P < 0.01$). These results indicated that the contribution of instant photosynthetic carbon to reproduction decrease drastically from the flowering to fruiting stage.

Discussion

Light utilization pattern

Photosynthetic characteristics changed drastically before and after canopy closure. Maximum

Fig. 4 Estimation of cumulative daily net assimilation per plant. *Horizontal line* represents foliage period, and *arrows* represent flowering and fruiting periods. The *closed square* and *circle* on the line indicate the time of bud formation and initiation of seed dispersal, respectively. *Shaded area* indicates canopy-closing period. *Close triangles* indicate that the initiation of individual growth stage (see Table 2)

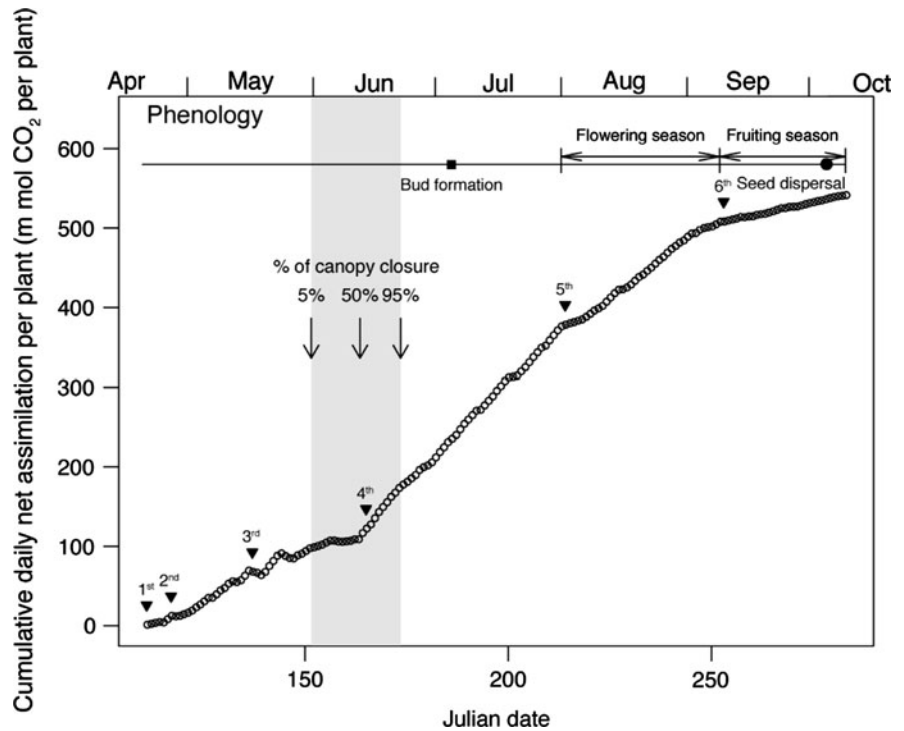
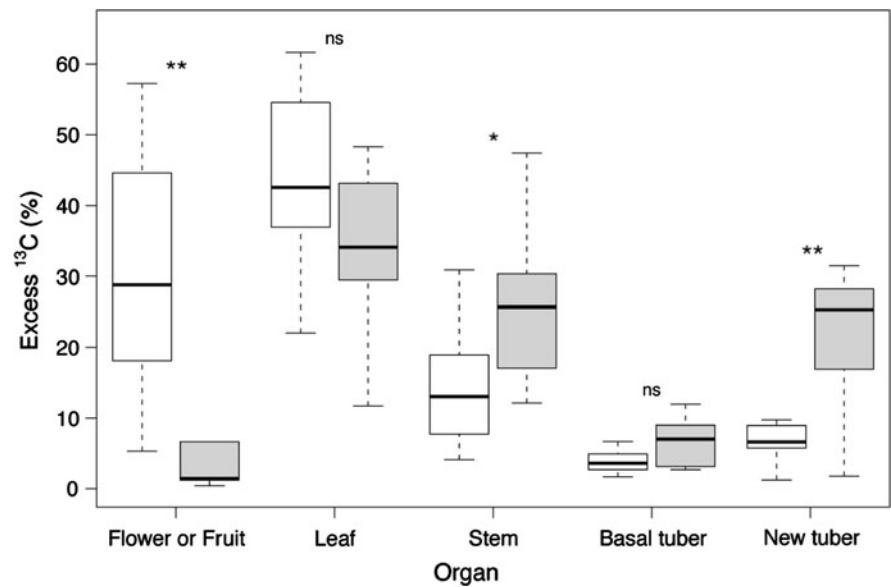


Fig. 5 Percentages of excess ¹³C in individual organs at flowering (white boxes) and fruiting stage (gray boxes). Comparison between flowering and fruiting stages was conducted by *t* test for excess ¹³C of each organ. Before the analyses, data were arcsin transformed to improve normality. ^{ns} *P* > 0.05; * *P* < 0.05; ** *P* < 0.001



photosynthetic rate and dark respiration rate decreased in parallel with the reduction in light availability, although the initial slope of the light curve was not influenced. The responses to light level indicated a high ability of photosynthetic acclimation in *P. auriculata*. Despite a sharp reduction in irradiance from spring to summer, the net assimilation per

plant was maintained at a high level over the summer season. Before canopy closure, the low level of leaf accumulation may limit assimilation at the whole-plant level even under high irradiation (Larcher 2003). Although some summer-green species possess high photosynthetic capacity in early season, their maximum photosynthetic rates are usually lower than

that of spring ephemerals that are specialized for high irradiance conditions (Rothstein and Zak 2001; Kudo et al. 2008).

Effective carbon gain at the whole-plant level in *P. auriculata* was attained by continuous leaf production. Spring-produced leaves often senesced by late-June (Ida TY personal observation), indicating the difficulty in achieving positive carbon balance under shade conditions due to the high respiration rate. Therefore, continuous leaf production under decreasing light availability may contribute to the regulation of leaf characteristics reflecting the light environment. Accumulation of leaves that acclimatized to shade conditions may offset the lower photosynthetic rate per unit area under the closed canopy. A similar trend was detected in some summer-green herbs with tall aboveground structures inhabiting deciduous forests (Tani and Kudo 2006), in which species with continuous leaf production maintained a positive carbon balance under progressing canopy closure, while species with simultaneous leaf production concentrated their assimilation during the short bright period before canopy closure. In *P. auriculata*, approximately 80% of total carbon assimilation was carried out under shade conditions after canopy closure. Canopy closure is inevitably a major limiting factor for growth and reproduction of understory plants in terms of carbon gain (Knapp et al. 1989). There are various ways to cope with this seasonal shade stress for understory plants. Spring ephemerals evade the period of low light level by a rapid completion of growth before canopy closure (e.g., Taylor and Pearcy 1976; Routhier and Lapointe 2002). Summer-deciduous, semi-evergreen, and some summer-green species exploit the brief periods of high irradiance in spring and/or autumn (Rothstein and Zak 2001). In contrast, *P. auriculata* exploits the long low-light period by effective assimilation owing to the accumulation of shade-acclimatized leaves in addition to the use of the short high-light period.

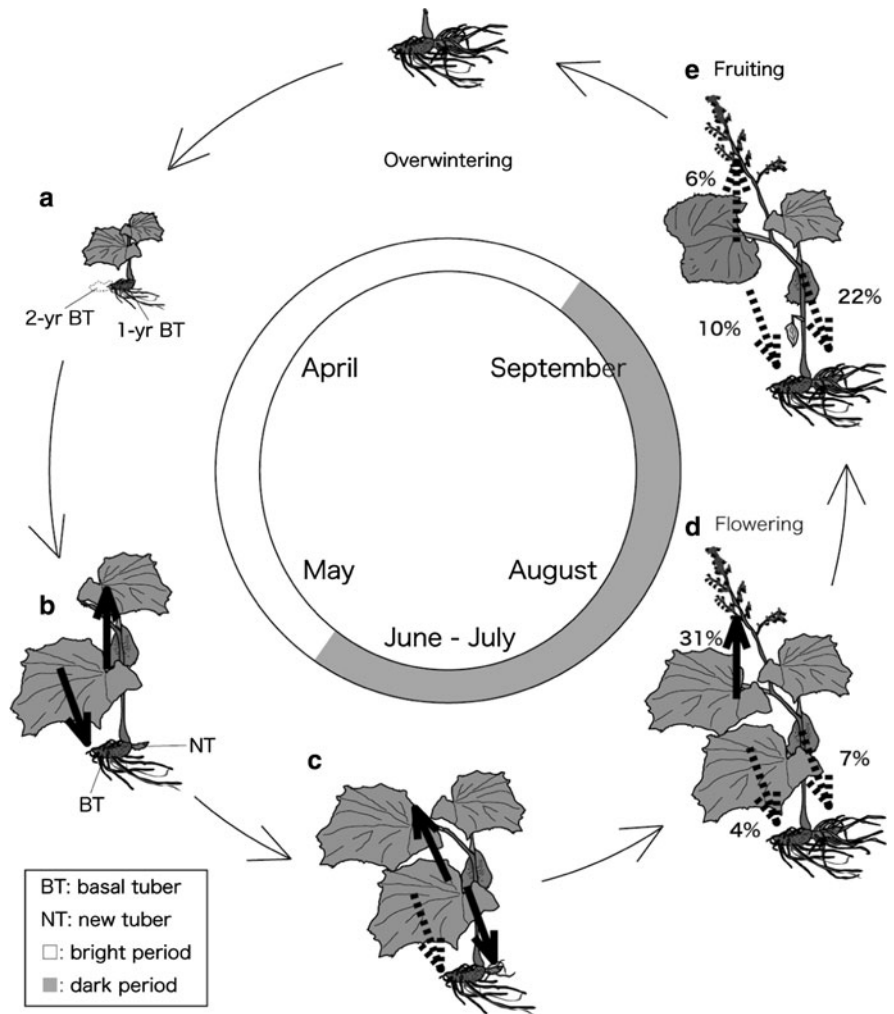
Growth schedule of aboveground and belowground parts

Parasenecio auriculata adjusted their carbon investment in each organ and carbon sources for growth in different annual growth stages (Fig. 6). Such temporal dynamics of resource allocation using a storage system composed of 2-year segments may reflect the

effective utilization of bright and shade light conditions before and after canopy closure. The timing of shoot emergence should affect subsequent photosynthetic carbon gain under decreasing light conditions, and the rapid establishment of aerial shoots is important for understory plants to utilize efficiently the short bright period before canopy closure. Rapid shoot establishment may depend on stored resources (e.g., Rothstein and Zak 2001; Ida and Kudo 2008). After shoot establishment, subsequent growth may be determined by the quantitative and qualitative structures of foliage parts reflecting the light harvesting strategy of individual species, which is formed by leaf production and turnover rate as mentioned before (Kikuzawa 2003). For instance, continuous leaf production is found in *Cardamine leucantha*, which is a common early-summer blooming species in our site, but the leaf-turnover rate of this species is high resulting in stable leaf number per plant during the growth period (Ida and Kudo 2009). Since rapid leaf turnover does not result in an increase in total leaf area, the exploitation of light-rich gaps may be important for *C. leucantha* to increase the productivity by young leaves with high photosynthetic ability. Therefore, seed production in this species is highly susceptible to light conditions after canopy closure (Ida and Kudo 2009). In contrast, the continuous leaf production with slow leaf turnover in *P. auriculata* was advantageous for the utilization of low light conditions under the closed canopy. Thus, the relationship between leaf production pattern and longevity clearly reflects the light harvesting strategy of individual species, even among species with a summer-green leaf habit.

Basal tubers recovered their mass by early in the season, and new tubers subsequently completed their development by the flowering stage (Fig. 6c). At the flowering stage, allocation of instant photosynthetic products to belowground parts was reduced, while carbon investment to flowers was large (Fig. 6d). At the fruiting stage, carbon allocation to belowground parts increased again (Fig. 5), although significant mass investment was not observed due to low assimilation activity (Fig. 6e). Several studies have demonstrated that stored resources in belowground parts facilitate continuous leaf production under shade conditions (Messier and Kimmins 1991; Huffman et al. 1994). As the mass of belowground parts increased continuously during early growth

Fig. 6 Schema of annual growth of *Parasenecio auriculata*: (a) shoot emergence in spring, (b) early-growing stage before canopy closure, (c) early-growing stage under the closed canopy, (d) flowering stage, and (e) fruiting stage. Light availability at the forest floor is low from mid-May to October due to canopy closure. Bold solid and dashed arrows indicate the major and minor movements of photosynthetic carbon, respectively. Values in the flowering (d) and fruiting (e) stages indicate the percentages of excess ^{13}C in individual organs under natural light conditions



season, the function of tubers as a source of shoot growth might be limited only in the initial stage. The temporal separation between basal-tuber and new-tuber growths may enable rapid the importance of shoot establishment in early spring, which could affect the subsequent shoot growth and photosynthetic carbon gain.

Budget for reproduction

The cost of flower production and maintenance largely depends on current photosynthetic products because 31% of absorbed ^{13}C was translocated to flowers at the flowering stage. In our previous studies on several understory herbs, the translocation of photosynthetic products to flowers was less than 1% in *Trillium apetalon* (Ida and Kudo 2008), 5.5% in

C. leucantha, 10.4% in *Smilacina japonica* (Ida and Kudo 2009), and 17.3% in *Corydalis ambigua* (Kudo and Ida 2010). Thus, the contribution of instant photosynthetic products to flowers is considerably larger in *P. auriculata*. In contrast, ^{13}C allocation to fruits was very small (6.1%). The contribution of instant photosynthetic products to fruit development may be small because the net assimilation rate was low. Nevertheless, fruit set of *P. auriculata* is usually high under natural conditions (Kudo et al. 2008) indicating that resource limitation was not a problem. This may be because temporarily stored resources are used for fruit development. For instance, seed production in understory *Trillium* species strongly depends on the photosynthetic products gained before canopy closure, which are stored in stems as a temporary resource reservoir (Lapointe 1998; Ida and

Kudo 2008). *P. auriculata* may also use photosynthetic products stored in aboveground parts, such as stems, for fruit production. This may result in low contribution of instant photosynthetic products to sexual reproduction during fruit development.

Reproduction when the plant size is large in the late season may reduce the cost of reproduction in *P. auriculata*. A delay in reproduction may be favored if resource stored during the vegetative season is used as a budget for reproduction (e.g., Chiariello and Roughgarden 1984; Reekie 1997). Previous studies demonstrated that the trade-offs between seed production and vegetative growth can be masked by size-dependent resource allocation to sexual reproduction (Reznick et al. 2000; Ehrlen and van Groenendael 2001). This is because larger plants may have an advantage in harvesting light resource more efficiently, which can moderate the trade-off relationship between reproduction and growth. Resource investment in reproduction is not large in this species because fruits occupied only 11% of the mass of the aerial shoot at the fruiting stage. Furthermore, it may be easy for *P. auriculata* that have attained a certain mass size at the reproductive stage to secure temporarily stored resources for sexual reproduction during the late growth season without a direct trade-off between reproduction and other functions. The exploitation of the stable low-light level after canopy closure may contribute to the balance between the assimilation ability of whole plants and the cost of reproduction, resulting in high fruiting ability without a reduction in future growth.

In summary, extension of the pre-flowering period combined with increasing leaf accumulation resulted in the maintenance of high assimilation ability under the closed canopy, which may lead to stably high fruit-set and subsequent seed-set ratios in *P. auriculata*. Relatively, small fruit size and temporal separation of resource investment between reproduction and storage functions may reduce the cost of reproduction. Such an efficient utilization of photosynthetic products may be a strategy of summer-green plants for the successful exploitation of the long shade period and stable reproduction under the closed canopy.

Acknowledgments We thank Atsuko Sugimoto and Yumi Hoshino for their assistance with stable isotope analysis, Tatsuya Saito and Asuka Koyama for their help in the field

survey, and two anonymous reviewers for their valuable suggestions. This work was partly supported by a JSPS Research Fellowships of Japan Society or the Promotion of Science for Young Scientists and Grants-in-Aid from the Japanese Society for the Promotion of Science for Scientific Research (No. 16370007 and 1840501007).

References

- Chiariello N, Roughgarden J (1984) Storage allocation in seasonal races of an annual plant: optimal versus actual allocation. *Ecology* 65:1290–1301
- De Jong TJ, Klinkhamer PGL (2005) Evolutionary ecology of plant reproductive strategies. Cambridge University Press, Cambridge
- Ehrlen J, Van Groenendael J (2001) Storage and the delayed costs of reproduction in the understorey perennial *Lathyrus vernus*. *J Ecol* 89:237–246. doi:10.1046/j.1365-2745.2001.00546.x
- Garcia MB, Ehrlen J (2002) Reproductive effort and herbivory timing in a perennial herb: fitness components at the individual and population levels. *Am J Bot* 89:1295–1302
- Huffman DW, Tappeiner JC, Zasada JC (1994) Regeneration of salal (*Gaultheria shallon*) in the central coast range forests of Oregon. *Can J Bot* 72:39–51
- Ida TY, Kudo G (2008) Timing of canopy closure influences carbon translocation and seed production of an understorey herb, *Trillium apetalon* (Trilliaceae). *Ann Bot* 101:435–446. doi:10.1093/aob/mcm296
- Ida TY, Kudo G (2009) Comparisons of light harvesting and resource allocation strategies between two rhizomatous herbaceous species inhabiting deciduous forests. *J Plant Res* 122:171–181. doi:10.1007/s10265-008-0212-6
- Iwasa Y, Cohen D (1989) Optimal growth schedule of a perennial plant. *Am Nat* 133:480–505. doi:10.1086/284931
- Kawarasaki S, Hori Y (2001) Flowering phenology of understorey herbaceous species in a cool temperate deciduous forest in Ogawa Forest Reserve, Central Japan. *J Plant Res* 114:19–23. doi:10.1007/PL00013964
- Kikuzawa K (1983) Leaf survival of woody plants in deciduous broad-leaved forests. 1. Tall trees. *Can J Bot* 61:2133–2139
- Kikuzawa K (2003) Phenological and morphological adaptations to the light environment in two woody and two herbaceous plant species. *Funct Ecol* 17:29–38. doi:10.1046/j.1365-2435.2003.00707.x
- Knapp AK, Smith WK, Young DR (1989) Importance of intermittent shade to the ecophysiology of subalpine herbs. *Funct Ecol* 3:753–758
- Kudo G, Ida TY (2010) Carbon source for reproduction in a spring ephemeral herb, *Corydalis ambigua* (Papaveraceae). *Funct Ecol* 24:62–69. doi:10.1111/j.1365-2435.2009.01601.x
- Kudo G, Ida TY, Tani T (2008) Linkages between phenology, pollination, photosynthesis, and reproduction in deciduous forest understorey plants. *Ecology* 89:321–331. doi:10.1890/06-2131.1
- Lapointe L (1998) Fruit development in *Trillium*: dependence on stem carbohydrate reserves. *Plant Physiol* 117:183–188

- Larcher W (2003) Carbon utilization and dry matter production. In: Larcher W (ed) *Physiological plant ecology*. Springer, New York, pp 69–173
- Lezberg AL, Antos JA, Halpern CB (1999) Belowground traits of herbaceous species in young coniferous forests of the Olympic Peninsula, Washington. *Can J Bot* 77:936–943
- Lovelock CE, Jebb M, Osmond CB (1994) Photoinhibition and recovery in tropical plant species: response to disturbance. *Oecologia* 97:297–307. doi:10.1007/BF00317318
- Marshall B, Biscoe PV (1980) A model for C₃ leaves describing the dependence of net photosynthesis on irradiance. *J Exp Bot* 31:29–39
- Messier C, Kimmins JP (1991) Aboveground and belowground vegetation recovery in recently clear-cut and burned sites dominated by *Gaultheria shallon* in coastal British Columbia. *Forest Ecol Manag* 46:275–294
- Newell EA, McDonald EP, Strain BR, Denslow JS (1993) Photosynthetic responses of *Miconia* species to canopy openings in a lowland tropical rain forest. *Oecologia* 94:49–56. doi:10.1007/BF00317300
- Obeso JR (2002) The costs of reproduction in plants. *New Phytol* 155:321–348. doi:10.1046/j.1469-8137.2002.00477.x
- Oguchi R, Hikosaka K, Hiura T, Hirose T (2008) Costs and benefits of photosynthetic light acclimation by tree seedlings in response to gap formation. *Oecologia* 155:665–675. doi:10.1007/s00442-007-0951-4
- Reekie EG (1997) Trade-offs between reproduction and growth influence time of reproduction. In: Bazzaz FA, Grace J (eds) *Plant resource allocation*. Academic press, San Diego, pp 191–209
- Reznick D, Nunney L, Tessier A (2000) Big houses, big cars, superfleas and the costs of reproduction. *Trends Ecol Evol* 15:421–425
- Rothstein DE, Zak DR (2001) Photosynthetic adaptation and acclimation to exploit seasonal periods of direct irradiance in three temperate, deciduous forest herbs. *Funct Ecol* 15:722–731. doi:10.1046/j.0269-8463.2001.00584.x
- Routhier MC, Lapointe L (2002) Impact of tree leaf phenology on growth rates and reproduction in the spring flowering species *Trillium erectum* (Liliaceae). *Am J Bot* 89:500–505
- Simard SW, Durall DM, Jones MD (1997) Carbon allocation and carbon transfer between *Betula papyrifera* and *Pseudotsuga menziesii* seedlings using a ¹³C pulse labeling method. *Plant Soil* 191:41–55. doi:10.1023/A:1004205727882
- Tani T, Kudo G (2006) Seasonal pattern of leaf production and its effects on assimilation in giant summer-green herbs in deciduous forests in northern Japan. *Can J Bot* 84:87–98. doi:10.1139/B05-145
- Taylor RJ, Pearcy RW (1976) Seasonal patterns of the CO₂ exchange characteristics of understory plants from a deciduous forest. *Can J Bot* 54:1094–1103
- Yamada T, Okuda T, Abdullah M, Awang M, Furukawa A (2000) The leaf development process and its significance for reducing self-shading of a tropical pioneer tree species. *Oecologia* 125:476–482. doi:10.1007/s004420000473