Photosynthetic compensation by the reproductive structures in the spring ephemeral *Gagea lutea*

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Abstract Growth and reproduction of spring ephemerals inhabiting deciduous forests progress simultaneously during a short period from snowmelt to canopy closure. To clarify the mechanism to mitigate the cost of reproduction, contributions of foliar and non-foliar photosynthetic products to seed production were examined in a spring ephemeral *Gagea lutea*. Leaf growth, foliar and non-foliar photosynthetic activities, and total assimilated products were compared among reproductive-intact, floral-bud removal and vegetative plants. Translocation of current photosynthetic products to individual organs was quantified by $^{13}CO_2$-trace experiment. Bulb growth was compared between hand-pollination and floral-bud removal treatments. Finally, seed set was compared between intact, leaf-clipping and bract-clipping treatments. Fruit-forming plants retained leaves longer than vegetative and floral-bud removal plants, but the assimilative contribution of extended leaf longevity was negligible. Carbon supply by bract photosynthesis was large enough for fruit development, while carbon supply by fruit photosynthesis was offset by the high respiration loss. Foliar photosynthetic products were largely transported to bulbs, while translocation to reproductive functions was negligible. Because the floral-bud removal increased the bulb growth, lack of reproduction could lead to more storage. The leaf-clipping had no effect on seed production, while the bract-clipping significantly reduced the seed production. Therefore, current photosynthesis of leafy bracts might be a major carbon source for fruit development. This self-compensative mechanism of reproductive structure enables the continuous reproductive activity in this species.

Keywords: Carbon fixation, Non-foliar photosynthesis, Reproductive compensation, Spring ephemerals.

Introduction

The production of flowers, fruits and seeds requires substantial amount of resources and this makes the plants manage the process of reproduction effectively (Banuelos and Obeso 2004). Such the gross material costs of reproduction often result in a decrease in allocation to non-reproductive functions (Wardlaw 1990), and occur at the expense of somatic investment that may cause a decline in survival and/or future reproduction, i.e. demographic cost of reproduction (Reznick 1985). Thus, the concept of cost of reproduction assumes that the gross cost of annual reproduction can translate into demographic cost (Obeso 2002). Many studies have reported the evidence of reproductive cost in both herbaceous (Obeso 1993; Primack and Stacy 1998; Gehring and Delph 2006) and woody plants (Newell 1991; Nicotra 1999), while others have found little evidence (Dudash and Fenster 1997; Ramsey 1997). Such a discrepancy can be explained by at least three compensative mechanisms for resource provision to seed production. Firstly, temporal variation in sink-source pathway may prevent a simple trade-off between resource allocation to current seed production and storage for future performance. Early-blooming forest herbs, for instance, commonly construct the aboveground structure using resources previously stored in the underground parts **(**Muller 1978; Routhier and Lapointe 2002), but this function changes afterwards to sink for future activities (Geber et al. 1997; Ida and Kudo 2008). Because the translocation of annual photosynthetic products may vary temporally (Ida and Kudo 2008), the relative contributions of current photosynthesis and stored resources to reproduction should also vary within a reproductive period (Ida and Kudo 2008; Kudo and Ida 2010).

Secondly, the resource demand of reproductive sink may elevate the assimilative capacity of source function (Watson and Casper 1984; Wardlaw 1990). If a carbon supply from photosynthetic organ is controlled by the demand of sink organs (Wardlaw 1990), a large part of carbohydrates may be supplied by the increased photosynthesis of adjacent leaves (Gifford and Evans 1981) or overall increase in photosynthetic ability (Wardlaw 1990; Lehtilä and Syrjänen 1995) although such up-

regulation of photosynthesis may not be universal (Watson and Casper 1984). Furthermore, the extension of leaf life-span during the reproductive period may contribute to assimilative capacity. For instance, larger reproductive cost in female individuals of dioecious trees than male individuals (Lloyd and Webb 1977; Obeso 2002) is compensated by longer leaf life-span and/or larger leaf size (Jonasson et al. 1997; Tozawa et al. 2009) in addition to the elevation of photosynthetic capacity.

Finally, the reproductive structures themselves may also boost resource uptake during reproduction. Significance of photosynthetic carbon gain by non-foliar organs, such as greenish flowers, developing fruits, green petals and stem tissues, has been reported (Blanke and Lenz 1989; Salopek-Sondi et al. 2000; Aschan and Pfanz 2003; Herrera 2005). These photosynthetic parts associated with inflorescences can reduce the gross cost of reproduction to some extent (Marcelis and Hofman-Eijer 1995; Antlfinger and Wendel 1997). However, because the net photosynthesis by reproductive structures may be canceled by their high respiration rate (Watson and Casper 1984; Obeso 2002), careful considerations are required to clarify the extent of compensative capacity of non-foliar structures.

Spring ephemerals inhabiting deciduous forests often have small number of leaves and very short leaf longevity. They are faced with a declining photosynthetic carbon gain because light availability consistently decreases from spring to early summer due to leaf expansion of canopy trees. Nevertheless, previous studies demonstrated that spring ephemerals commonly have high fruit production (Kudo et al. 2008; Bernett et al. 2009). Thus, they should have some mechanisms to ensure the high reproductive output under restricted growth period with limited number of leaves. This is partly related to high photosynthetic rates under high irradiance before canopy closure (Rothstein and Zak 2001; Kudo et al. 2008) and/or use of storage resource instead of current assimilation (Horibata et al. 2007; Kudo and Ida 2010). To clarify the reproductive strategies of carbon assimilation in spring ephemerals, however, intensive assessment of compensative mechanisms is required.

This study, therefore, evaluates what kind of compensative mechanisms for seed production exists and to what extent the annual cost of seed production is assured in a spring ephemeral, *Gagea lutea*, in a deciduous forest in northern Japan. Although a previous study on *G. lutea* suggests the importance of current photosynthetic products for seed production (Nishikawa 2009), the responses of seed production to defoliation treatments were inconsistent between years. Nevertheless, since reproductive individuals of this species always have a pair of leafy bracts on a flowering stem, it is anticipated that bracts may indicate an important compensatory strategy to foster carbon gain during reproduction. Based on carbon fixation and dry weight analysis, we aimed to clarify the demographic cost of reproduction and compensative strategies, and to quantify the resource for seed production by answering the following questions: (a) Does the resource provision to seed production translate into a trade-off between current reproduction and storage for future growth? (b) Do the plants elevate foliar photosynthetic capacity, such as the increments of size, longevity and/or photosynthetic rate, to satisfy the carbon demand for seed production? (c) Does the non-foliar (young fruits and bracts) photosynthesis substantially contribute to seed production?

Materials and methods

Study site and species

This study was conducted in a secondary deciduous forest within the campus of Hokkaido University, Sapporo of northern Japan (43° 04' 57" N, 141° 20' 22" E) during 2010-2011. Forest floor is usually covered with snow from early December to early April. Snowmelt occurred on 3 April and 5 April in 2010 and 2011, respectively. Air temperature and photosynthetic active radiation (PAR) at 2 m above the ground were automatically recorded in the forest at 1-min intervals using a combined data logger with a solar radiation and thermometer (HOBO weather station; Onset Co., MA, USA) from 2 April to 31 May 2010.

Gagea lutea Ker-Gaul. (Liliaceae) is a polycarpic perennial herbaceous species pollinated by bees. This species has a typical spring ephemeral lifecycle; blooming starts immediately after snowmelt at the same time of leaf expansion (in mid- to late April) and fruits mature about two weeks after anthesis. Aboveground parts usually die at the time of seed dispersal by canopy closure in late May. Thus, total growth season is less than two months. Vegetative (non-reproductive) individuals produce only one leaf, while one basal leaf and a pair of long and short leaf-like bracts on the scape are produced in reproductive plants (Fig. 1). Each reproductive shoot bears multiple flowers (3.4 ± 0.2 s.e., n = 40) on a single scape that bloom sequentially (Nishikawa 1998). The underground part (bulb) is a storage organ, which is the only tissue existing over years. In this study, we divided reproductive phase into following stages: 'flowering stage' from first to full opening flowers, 'early-fruiting stage' dominated by young fruits with <30% flowers, and 'late-fruiting stage' mixture of young and mature fruits but no flowers.

Photosynthetic activity

To assess the photosynthetic capacity of basal leaves, we selected 3-4 plants per reproductive status at four growth stages: pre-flowering stage on 16 April, flowering stage on 5 May, early-fruiting stage on 18 May, and late-fruiting stage on 30 May (only for reproductive-intact plants) in 2010. Furthermore, basal-leaf photosynthetic capacity of vegetative individuals (at 16 April, 5 May, and 18 May) and bud-removal individuals (at 5 and 18 May; mentioned later) was measured as same as reproductive intact plants. In addition, fruit photosynthesis of same reproductive-intact plants was measured at early- and late-fruiting stages in 2010. To assess the photosynthetic capacity of bracts, we selected three reproductive-intact plants at each of five growth stages: floral-bud stage on 14 April, early-flowering stage on 28 April, late-flowering stage on 11 May, early-fruiting stage on 20 May, and late-fruiting stage on 31 May in 2011. We measured light responses of photosynthetic rates per unit area using LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NB, USA). Ten light conditions (2000, 1500, 1000, 800, 500, 300, 100, 50, 10, and 0 μmol m^{-2} s^{-1}) of PAR were provided using a red-

blue LED light source at constant leaf temperature (15°C). Ambient CO_2 concentration and the humidity of incoming air during the measurement were maintained at 380 µl l^{-1} and 1.1 vapor pressure deficit (VPD, kPa), respectively. Net photosynthetic rate per area (P_{area}) can be described as a non-rectangular hyperbola of photon irradiance (I, µmol m^{-2} s^{-1}) as follows:

$$P_{area} = \frac{\alpha I + P_{\max} - \sqrt{(\alpha I + P_{\max})^2 - 4\alpha I \theta P_{\max}}}{2\theta} - R_d, \quad (1)$$

where P_{max} is the light-saturated photosynthetic rate per unit area (µmol m^{-2} s^{-1}), α is the initial slope (µmol m^{-2} s^{-1}), θ is the degree of curvature, and R_d is the dark respiration rate (µmol m^{-2} s^{-1}; Marshall and Biscoe 1980). Data obtained for individual plants were fitted to this equation by non-linear least-squares estimates of the parameters. Mean values of individual parameters obtained in each period were used as representative of photosynthetic capacity. Because the width of basal leaves and bracts was smaller than the chamber size (2 cm × 3 cm), we corrected photosynthetic parameters by the replacement of chamber area by actual leaf area that was included in the chamber.

Carbon fixation capacity

To assess the effects of reproductive activity on assimilation capacity, we compared total carbon fixations by basal leaves and bracts among different reproductive status (reproductive-intact, bud-removal, and vegetative plants). Soon after shoot emergence (8 April 2010), 40 plants with floral buds and 20 plants without floral buds but having similar-sized leaves to the former (vegetative plants) were arbitrarily selected and marked with numbered tags. After recording the number of floral buds, all buds were removed from the 20 of reproductive plants (bud-removal plants). All buds of the remaining 20 plants (reproductive-intact plants) were retained and supplemental pollination was conducted. Pollen donors for a supplemental pollination were selected from multiple reproductive plants >5 m apart from the recipient plants. We therefore compared the assimilation capacity among reproductive status. First, to assess the seasonal changes in photosynthetic area, the leaf and bract sizes (length, L and width, W) of all plants were measured with a digital calliper at 5-day intervals

except on rainy days throughout the growth period. This measurement involved only the green area that was photosynthetically active. Basal leaf and bract area (A) was estimated as $A = 0.83 \times L \times W$ ($R^2 = 0.968$, $n = 5$). Also, surface area of harvested fruits was measured with the image analysis software (Image J version 1.34; National Institutes of Health, Bethesda, MD, USA). Furthermore, leaf senescence pattern of each plant was recorded to quantify the leaf longevity.

We determined the total carbon fixation by basal leaves, bracts, and fruits throughout the season from the data of photosynthetic area, photosynthetic parameters, and light availability. The 1-min PAR data measured at the study site was converted to hourly mean values before the estimation. Net photosynthetic rates per unit area per hour (μmol m^{-2} s^{-1}) were estimated based on the light availability and photosynthetic parameters obtained at each growth stage (four, five, and two stages for leaves, bracts, and fruits, respectively) explained above. Hence, daily CO_2 fixation rate (mmol m^{-2} day^{-1}) was expressed as the sum of hourly photosynthetic rates, calculated using the equation (1) with hourly mean PAR and photosynthetic parameters of each reproductive status in each period. In this calculation, daily transitions of photosynthetic area were estimated as a proportional increase or decrease between days of measurements. Then, by summing the daily CO_2 fixation rate multiplied by photosynthetic area during the growth period (average leaf longevity of each reproductive status), total carbon fixation (mg C) by basal leaves, bracts, and fruits were obtained. Carbon concentration in plant tissue of *G. lutea* was 45% of dry mass (NS, *unpublished data*), and the conversion efficiency of energy required to transform glucose or sucrose into the different cell components is supposed to be about 70% in general plant tissues (e.g. Poorter and Villar 1997). Thus, total biomass gain caused by the photosynthetic carbon fixation (C-fixed) was calculated as C-fixed × 1/0.45 × 0.7.

$^{13}CO_2$ labeling

To clarify the allocation pattern of current photosynthetic carbon fixed by basal leaves to individual organs, ^{13}C-trace experiment was performed at flowering (1 May, $n = 9$) and late fruiting (28 May, $n = 4$) periods by supplying $^{13}CO_2$ to leaves for two days in 2010. ^{13}C levels for five individuals without

$^{13}CO_2$ exposure were also measured as a control in each period. For this experiment, leaves were enclosed in a 40 × 30-cm sealed nylon bag in which a cylinder containing 30 mL of lactic acid and two tubes containing 150 mg of 99.9% $Ba^{13}CO_2$ (Isotec Inc., Miamisburg, OH, USA) were present. The $^{13}CO_2$ labeling was carried out when there was sufficient sunlight to cause net CO_2 uptake, on two successive sunny days. Injections of $^{13}CO_2$ were made in early morning on the first and second days. Barium carbonate was added to lactic acid, releasing $^{13}CO_2$ into the bag. Plants were harvested two days after the labeling period. After harvesting, individual plants were separated into six organs (flower or fruit, stem, leaf, bract, bulb and root) and oven-dried at 70°C for 72 h. After weighing each organ, they were ground 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) was used to measure $\delta^{13}C$ (see Ida and Kudo 2008 for details). Because dry mass and ^{13}C contents were very small in roots, we excluded root data from the analysis. The distribution of excess ^{13}C via photosynthesis was expressed with the proportion of excess ^{13}C in each organ against total absorbed ^{13}C per plant.

Bulb growth between years

To assess the effects of reproductive on bulb growth, we compared bulb size between hand-pollination (maximum fruit set) and floral-bud removal (no flower and fruit production) treatments. Bulbs of 34 plants were unearthed on 20 November 2009 in the forest floor, then the length (L) and width (W) of individual bulbs were measured using a digital calliper (initial bulb size). Because the shape of bulbs was non-uniform (ellipsoid), the mean of the two broader widths was taken as the width, and the bulb volume was expressed as $4\pi \times W^2 \times L / 3$. After the measurement, they were planted in pots with numbered tags for identification. The pots were put in holes under the forest so that bulbs could continue to grow at the same depth as they normally grow and under natural climate conditions. Occasional wetting was performed to prevent evaporation. In early spring of 2010, plants having floral buds (n = 28) were divided into two groups. All flowers of the first group (n = 15) were

hand-pollinated to set maximum fruits, while floral buds of other group (n = 13) were eliminated to remove the resource provisioning to flowering and fruiting. Hand-pollination was performed by brushing the dehisced anthers from donors >5 m away across the clear stigma of the flowers using forceps until the surface of stigmas was completely covered with pollen. The floral-bud number was recorded before the bud removal, and the sizes of basal-leaf and bracts of every plant were measured during the fruiting season in mid-May (at maximum size) using a digital calliper. On 14 October 2010, individual bulb sizes were measured again (final bulb size). Then, final bulb volume was analysed with the consideration of reproductive activity and initial bulb size.

Annual reproductive output

To investigate the carbon source for seed production, 60 well-pollinated plants with three developing fruits were selected. The selected plants, identified with number tags, were divided into three groups of 20 individuals each (intact control, bract-clipping, and leaf-clipping). Clipping was performed just after the initiation of fruit development. On 7 June 2010, infructescences were harvested from all plants before the opening of capsules. Fruit, undeveloped ovule and mature seed numbers were counted. Then, effects of clipping on fruit-set rate (fruit/flower ratio) and seed-set rate per plant (seed/ovule ratio) were assessed.

Statistical analyses

We performed one-way analysis of variance (ANOVA) to compare leaf size and leaf carbon fixation among three treatments, respectively. Turkey's HSD test was used for post-hoc multiple comparisons. Relationships between flower number and leaf size, and between carbon fixation and leaf size were determined using Pearson's correlation coefficients. Leaf size and leaf carbon fixation were log-transformed before analysis to improve normality. Each of bract size, bract carbon fixation, and bulb size was compared between the reproductive-intact and bud-removal treatments using Student's t test without any data transformation. Relationships between flower number and bract size, and between

carbon fixation and bract size were determined using Pearson's correlation coefficients. Before conducting ANOVA or *t* test, normality of data distribution and homogeneity of variances were confirmed by Shapiro-Wilk test and Bartlett test, respectively. Leaf survival rate was compared among three treatments using the Cox proportional hazards regression model. To compare the photosynthetic activity among three treatments, P_{max} values of basal leaves were analyzed using a generalized linear model (GLM) postulated a gamma error distribution with log-link function in which treatment and growth stage (pre-flowering, flowering, early fruiting) were set as explanatory variables. Fruit-set rate (fruit/flower ratio) and seed-set rate (seed/ovule ratio) were compared among clipping treatments by GLM postulated a binomial error distribution with logit-link function in which intact was set as an intercept, and leaf and bract clipping as explanatory variables. An open source system, *R* version 2.12.1 (R Development Core Team 2011) was used for all statistical analyses.

Results

Leaf and bract growth

Basal leaves grew continuously until early-fruiting stage (mid-May), then the green area decreased with progress in leaf senescence from the tip, and died back completely by the end of May (Fig. 2a). Vegetative plants had significantly smaller leaves than reproductive-intact and bud-removal plants, while there was no significant difference between intact and bud-removal treatments (Table 1). There was a positive correlation between maximum leaf size and potential flower number ($r = 0.68$, $P < 0001$), indicating size-dependent reproductive investment.

Bracts were less than 20% of basal leaves at the maximum size, and bract size was rather stable during the growth period than basal leaf size (Fig. 2a). Similar to basal leaves, bract size did not differ between intact and bud-removal treatments (Table 1), and there was a positive correlation between maximum bract size and potential flower number ($r = 0.75$, $P < 0001$).

Basal leaves of both the bud-removal and vegetative plants senesced significantly earlier than leaves of reproductive-intact plants (Fig. 2b). This indicates that reproductive-intact plants had longer leaf life-span (54.7 ± 1.1 s.e. days) than bud-removal (49.6 ± 0.8 days) and vegetative plants (49.3 ± 0.8 days).

Photosynthetic activity

The light-saturated photosynthetic rate per unit area (P_{max}) was high at the pre-flowering and flowering stage then, decreased rapidly during the fruiting period for all treatments (Fig. 3a). Result of GLM indicates that there was no significant difference in P_{max} among treatments but P_{max} significantly differed among growth stages (Table 2). Only the reproductive-intact plants survived and photosynthesized until late-fruiting period although the activity was very low (P_{max} was only 7% of flowering period). Dark respiration rate (R_d) was relatively stable throughout the growth period ranging from 0.9 to 1.8 μmol m^{-2} s^{-1}. Details of photosynthetic parameters are shown in Appendix 1.

Similar to basal leaves, bracts showed high P_{max} at the pre-flowering and flowering stages, then P_{max} constantly decreased during the fruiting period (Fig. 3b). Developing fruits showed relatively high P_{max} (6.4 μmol m^{-2} s^{-1}) comparable to bract P_{max} (6.2 μmol m^{-2} s^{-1}), but their R_d was much higher (2.7 μmol m^{-2} s^{-1}) than bract R_d (0.4 μmol m^{-2} s^{-1}) at the early fruiting stage (Appendix 2).

Carbon fixation

Seasonal transitions of daily CO_2 fixations of individual organs reflected the fluctuation of daily maximum PAR (Fig. 4). Basal leaves and bracts showed similar carbon fixation per unit area, remaining high in April but decreasing rapidly after mid-May with progress of canopy closure. In contrast, photosynthetic CO_2 fixation by fruits was almost zero or often negative throughout the fruit developing period.

Estimated carbon fixations by leaf and bract throughout the whole growth period indicated that total carbon fixation by basal leaves or bracts strongly depended on their size (r = 0.95, P < 0.0001

both for basal leaf and bract). Intact and bud-removal plants fixed significantly more carbon than the vegetative plants reflecting the difference in leaf size (Table 3). There was no significant difference in carbon fixation between intact and bud-removal plants irrespective of difference in leaf longevity. Total carbon fixation by bracts did not differ significantly between intact and bud-removal plants (Table 3). Carbon fixation by bracts occupied approximately 20% of total carbon fixation per plant.

The contribution of leaf photosynthetic products to mass increment (calculated as fixed-carbon × 1/0.45 × 0.7) was estimated as 413 mg, 353 mg, and 253 mg in the reproductive-intact, bud-removal, and vegetative plants, respectively. Also, the contribution of bract photosynthetic products to mass increment was 96 mg and 85 mg in the reproductive-intact and bud-removal plants, respectively. Because total fruits mass per plants in late May was 95 ± 8.6 mg in this population (unpublished data, Appendix 3), the photosynthetic products of bracts largely supported the mass increment of fruits. In contrast, total carbon fixation by the photosynthesis of fruits during the fruit-developing period was –0.09 mg C, corresponding to 0.14 mg mass reduction. Therefore, the fruit photosynthetic carbon gain was sufficient to largely support respiration but not sufficient for own mass development.

^{13}C allocation

The percentage of excess ^{13}C in each organ differed between flowering and fruiting periods (Fig. 5). At the flowering period, total excess ^{13}C per plant was 0.54 ± 0.09 mg. The ^{13}C percentage in bulbs (66%) was the highest, while that in reproductive organs was very low (<2%) except for reproductive stems (7%). This result indicates that most of the carbon assimilated by basal leaves at the flowering season was transferred to bulbs and translocation to reproductive organs was negligible. At the late fruiting period, however, total excess ^{13}C per plant was only 0.049 ± 0.017 mg and most ^{13}C (96%) remained in leaves with little translocation to bulbs (4%). Very low C-fixation ability in this period might reflect low photosynthetic activity.

Bulb growth between years

Mean bulb volume in 2009 did not differ between reproductive-intact and bud-removal plants (Table 4). In contrast, bulb volume was significantly larger in bud-removal plants after the experiment. Bulbs of the bud-removal plants increased 12% after one year, while intact plants maintained almost the same bulb volume after flower and fruit production. This indicates that seed production did not limit the vegetative growth but the absence of flower and fruit production could increase the vegetative growth.

Seed production

Plants with three flowers could set an average of 1.57 ± 0.16 fruits (52.2 ± 9.2% in fruit-set rate). Both clipping treatments did not influence the fruit-set rates. In contrast, the clipping treatments significantly affected the seed set per plant (Table 5); average seed-set rate was 14.5 ± 3.9% for the intact, 14.6 ± 3.3% for the leaf-clipping, and 8.9 ± 2.3% for the bract-clipping treatments. Bracts clipping significantly reduced seed production, while the leaf-clipping plants produced similar number of seeds as intact plants. This result indicates the significant contribution of bract photosynthesis to seed production.

Discussion

This study was designed to clarify whether a simple trade-off occurs between current reproduction and storage for future growth, whether the plants elevate foliar photosynthetic capacity to satisfy the carbon demand for seed production, and finally, to determine the contribution of non-foliar photosynthesis to seed production. Among these questions, first and second ones are not evident in *G. lutea*: a simple trade-off between current reproduction and storage for future growth seem to be absent (to be discussed later), and foliar photosynthesis does not contribute to seed production. In contrast, the last question was evident in that, the contribution of non-foliar photosynthesis strongly related to reproductive compensation in this species. Responses of seed production to the leaf- and bract-

clipping treatments demonstrated that photosynthetic products by bracts had substantial effect on reproductive output in *G. lutea* as reported in other plant species (Wullsehleger and Oosterhuis 1990; Hori and Tsuge 1993). It is known that green parts of reproductive structures occasionally have high assimilatory capacity comparable to leaves (reviewed in Aschan and Pfanz 2003). Dry weight analysis of the carbon fixation revealed that bract's photosynthesis was able to support fruit development, but photosynthetic carbon gain by fruits probably offset the high respiration loss. The bract-clipping treatment significantly reduced the seed production but not completely. Because the bract clipping was performed soon after flowering period, photosynthetic products by bracts during pre-flowering and flowering periods (almost half of growing season) might be stored in scape that can be used for subsequent fruit development (Lapointe 1998). Considering the similar seed-set rates between intact and leaf-clipping treatments, the current photosynthetic products by reproductive structures are assumed to be essential for seed production. Also in the ^{13}C-trace experiment, photosynthetic products by leaves were mainly transported to bulbs and the translocation to reproductive organs was negligible. These indicate that carbon resource for seed production are independent of current leaf photosynthesis at least when bracts are intact.

Photosynthesis by reproductive structure has been found in every reproductive part (Aschan and Pfanz 2003), and it may occupy a significant fraction of total carbon and energy provision for reproduction (Bazzaz et al. 1979; Hori and Tsuge 1993; Marcelis and Hofman-Eijer 1995; Antlfinger and Wendel 1997). Previous review papers (Watson and Casper 1984; Obeso 2002) reported that the contribution of non-foliar photosynthesis to reproduction varied in a broad range (from < 1% to > 60%). If resources provided by belowground storage organs contribute to current reproduction to some extent, however, contribution of non-foliar photosynthesis might be overestimated. In contrast, contribution of reproductive structures to seed production in *G. lutea* was much larger and enough to supply the resources for total fruit development. Because previously stored resources in the old tissue of bulbs have almost been exhausted by the end of flowering period in (Fig. 1b), furthermore, resource supply from the storage organs for fruit development may be insignificant if resource

transportation from the current tissue of bulbs to fruits does not occur (we discuss this possibility later). Thus, the photosynthesis by own reproductive structures largely supports the carbon economy in seed production independent of vegetative structures in this species.

Annual bulb growth was enhanced by the suppression of fruit production by the floral-bud removal. Traditionally, the cost of reproduction hypothesis (Obeso 2002) expects that a decline in resource translocation to storage function with increasing current reproductive activity should be interpreted as a demographic cost of seed production. Specifically, cost of reproduction is defined as losses in the potential future reproductive events, mortality, growth, and/or vegetative propagation by current reproduction. In contrast, the gross cost of seed production in *G. lutea* was largely supported by own reproductive structures, indicating the functional separation between annual seed production and bulb growth as a storage function. In other words, photosynthetic carbon translocation from leaf to new bulb was assured irrespective of reproductive investment. Therefore, increment in bulb growth in bud-removal plants might be attained by a carryover of resource, which was prepared to compensate for current seed production but was saved from expenditure. Since the bud-removal plants retained bracts as long as intact plants (Fig. 2a), photosynthetic products by bracts should have been transported to other organs than fruits in the bud-removal plants. Thus, the compensative mechanism by own reproductive structures can mitigate a linkage between gross cost of seed production and demographic cost.

Although spring ephemerals commonly show high fruit production (Kudo et al. 2008), carbon source for fruit production varies among species, such as foliar photosynthesis early in the season (Ida and Kudo 2008), stored carbohydrates in stems (Lapointe 1998), photosynthesis by fruits (Horibata et al. 2007), and stored resources in old tissues (Kudo and Ida 2010). The present study newly revealed that seed production in *G. lutea* is largely supported by bract photosynthesis independent of foliar photosynthesis. The separation of carbon sources between fruit production and bulb growth may be determined by the morphological structure of bulbs (Fig. 1b). In the middle of growth period, bulb is composed of ‘previous tissue’ that has been almost exhausted and ‘current

tissue' in which current photosynthetic carbohydrates have been stored. A reproductive shoot is connected to the previous tissue, while a basal leaf is to the current tissue. This promotes the functional separation between reproductive and vegetative structure, i.e. foliar photosynthetic products are directly stored for next season, while fruit development is self supported within the reproductive stem that is isolated from the current foliar photosynthesis. This functional separation confirms the conclusion that non-foliar photosynthesis may mitigate the cost of reproduction even after full fruit production by hand-pollination.

It is obvious that a long-term monitoring of same populations is needed to evaluate the demographic cost of reproduction (e.g. Primack and Stacy 1998), but the present study, at least, proved that seed production in one year does not always reduce the subsequent reproductive activities if some compensative mechanism exists. The photosynthesis of reproductive structure is a major carbon source for seed production in *G. lutea* that may enable the continuous sexual reproduction from year to year.

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Table 1. Maximum leaf size and bract size (cm^2) among treatments. Mean ± s.e., $n = 20$. Results of one-way ANOVA and *t*-test are also shown.

Reproductive intact	Bud-removal	Vegetative	Statistical scores
Leaf size			
23.2 ± 1.7^a	20.4 ± 1.2^a	15.9 ± 0.8^b	$F_{2, 56} = 8.92$, $P < 0.001$
Bract size			
4.0 ± 0.3	3.7 ± 0.3		$t_{38} = 0.83$, $P = 0.41$

a, *b*: Tukey's honest significant difference test ($P < 0.05$)

Table 2. Result of GLM for P_{max} among treatments (reproductive intact, bud-removal, vegetative) and growth stages (pre-flowering, flowering, early fruting).

Variables	Coeffecient	s.e.	*t* value	*P* value
Intercept*	3.066	0.107	28.55	<0.0001
Treatment: bud-removal	–0.018	0.126	–0.14	0.89
Treatment: vegetative	–0.171	0.107	–1.59	0.13
Stagg: flowering	0.187	0.126	1.48	0.15
Stage: fruiting	–0.977	0.126	–7.76	<0.0001

* Intercept (Treatmet: reproductive intact, Stage: pre-flowering)

Table 3. Estimated total carbon fixation (mg C) by leaf and bract per plant of reproductive intact, bud-removal and vegetative plants throughout the growth period in 2010. Mean ± s.e., $n = 20$. Results of one-way ANOVA and *t*-test are also shown.

Reproductive intact	Bud-removal	Vegetative	Statistical scores
Leaf			
265.4 ± 18.7^{a}	226.9 ± 12.9^{a}	162.8 ± 8.7^{b}	$F_{2,57} = 14.8$, $P < 0.001$
Bract			
61.5 ± 5.0	54.9 ± 4.0		$t_{38} = 1.03$, $P = 0.31$

a, *b*: Tukey's honest significant difference test following one-way ANOVA ($P < 0.05$)

Table 4. Comparison of bulb size (cm^3) betwen reproductive intact and bud-removal treatments before and after experiment using Student *t*-test. Mean ± s.e.

Reproductive intact	Bud-removal	Statistical scores
Initial bulb size		
1.74 ± 0.11	1.81 ± 0.2	$t_{27} = 0.34$, $P = 0.73$
Final bulb size		
1.73 ± 0.11	2.14 ± 0.16	$t_{27} = 2.18$, $P = 0.03$

Table 5. Result of GLM for fruit-set and seed-set rates among leaf clipping treatments (intact, leaf-removal, bract-removal) .

Variables	Coeffecient	s.e.	*z* value	*P* value
Fruit-set rate				
Intercept (intact)	-4.49×10^{-16}	0.258	-1.74×10^{-15}	1.00
Leaf-removal	0.365	0.368	0.95	0.36
Bract-removal	–0.067	0.365	–0.18	0.85
Seed-set rate				
Intercept (intact)	–1.835	0.078	–23.36	< 0.0001
Leaf-removal	0.044	0.111	0.40	0.69
Bract-removal	–0.570	0.127	–4.50	<0.0001

Figure legends

Fig. 1. (a) Whole plant structure of *Gagea lutea* at early fruiting stage. (b) Section of a bulb at early fruiting stage. Reproductive stem is connected to previous tissue that has been exhausted, while leaf is connected to developing current tissue.

Fig. 2. (a) Seasonal changes in the photosynthetically active (green) area of basal leaves and bracts in reproductive intact, floral-bud removal and vegetative plants throughout the growth season. (b) Survival rates of basal leaves in intact, bud-removal and vegetative plants under natural conditions. *** P <0.0001, z = 4.20 and 4.35 for bud-removal and vegetative plants, respectively by Cox proportional hazard regression comparing with intact plants.

Fig. 3. (a) Seasonal changes in P_{max} of basal leaves in intact, bud-removal, and vegetative plants at the pre-flowering, flowering, early-fruiting, and late-fruiting stages in 2010. (b) Seasonal changes in P_{max} of bracts in 2011 at the pre-flowering, early-flowering, late-flowering, early-fruiting, and late-fruiting stages. Vertical bars indicate standard errors (s.e.).

Fig. 4. Seasonal transitions of daily CO_2 fixation of leaf, bract and fruit estimated from actual light intensity and photosynthetic parameters of individual organs.

Fig. 5. Distribution of excess ^{13}C (%) fixed by basal leaves to individual organ at flowering (n = 9) and late fruiting (n = 4) stages. Vertical bars indicate s.e.

Figure 1

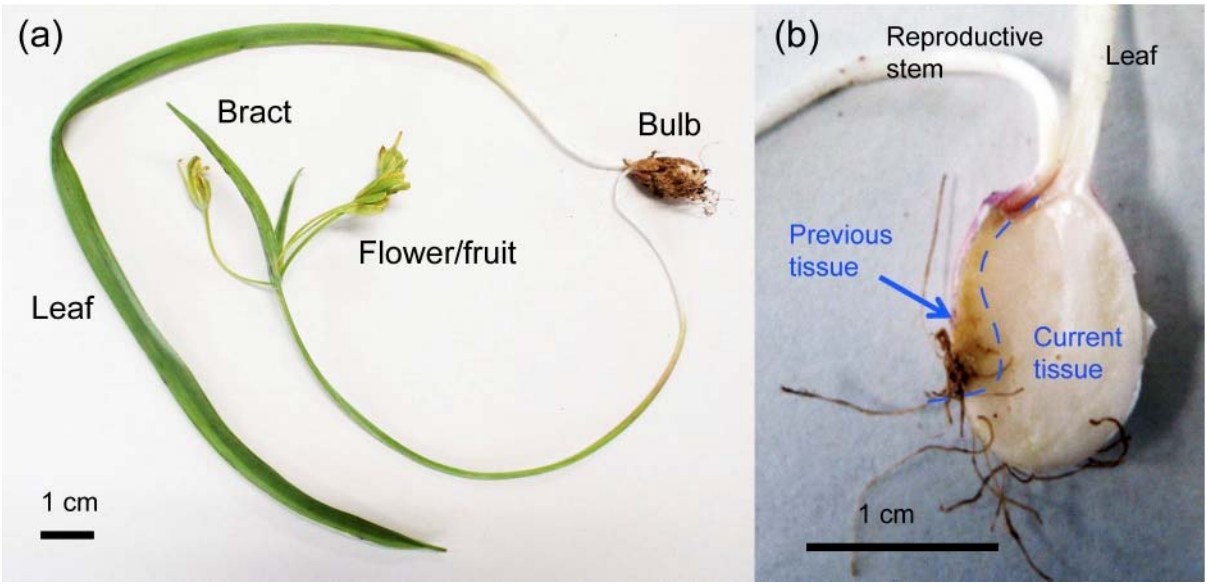

(a)
Bract
Bulb
Flower/fruit
Leaf
1 cm
(b)
Reproductive stem
Leaf
Previous tissue
Current tissue
1 cm

Figure 2

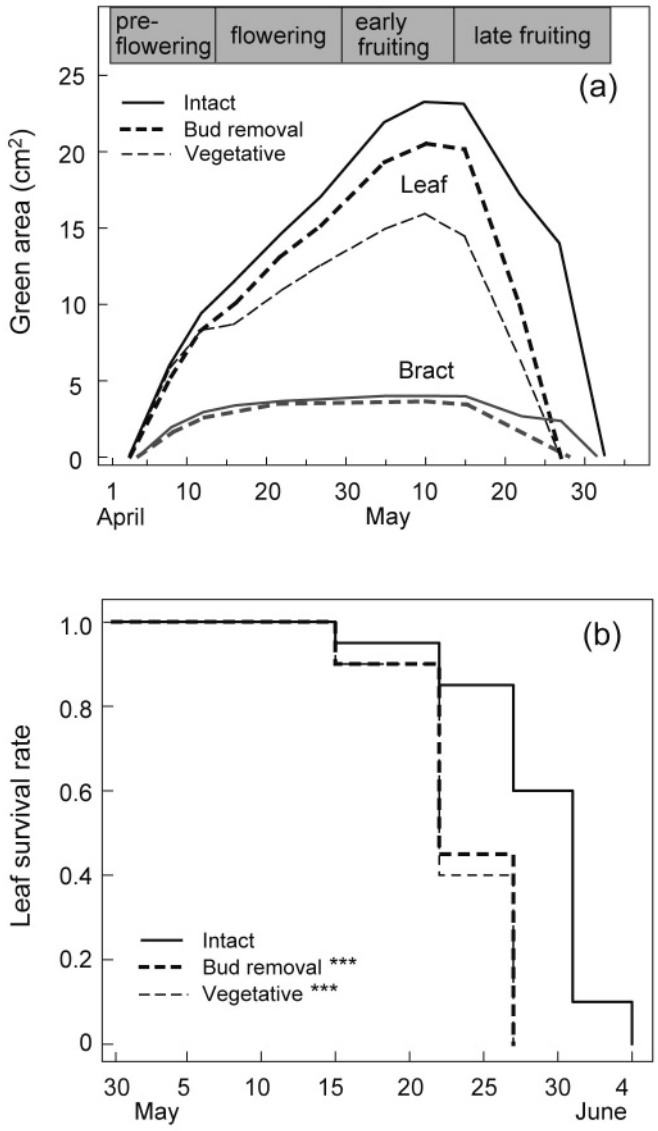

pre-flowering
flowering
early fruiting
late fruiting
(a)
Intact
Bud removal
Vegetative
Leaf
Bract
Green area (cm²)
25
20
15
10
5
0
1
April
10
20
30
10
May
20
30
(b)
Leaf survival rate
1.0
0.8
0.6
0.4
0.2
0
Intact
Bud removal ***
Vegetative ***
30
May
5
10
15
20
25
30
4
June

Figure 3

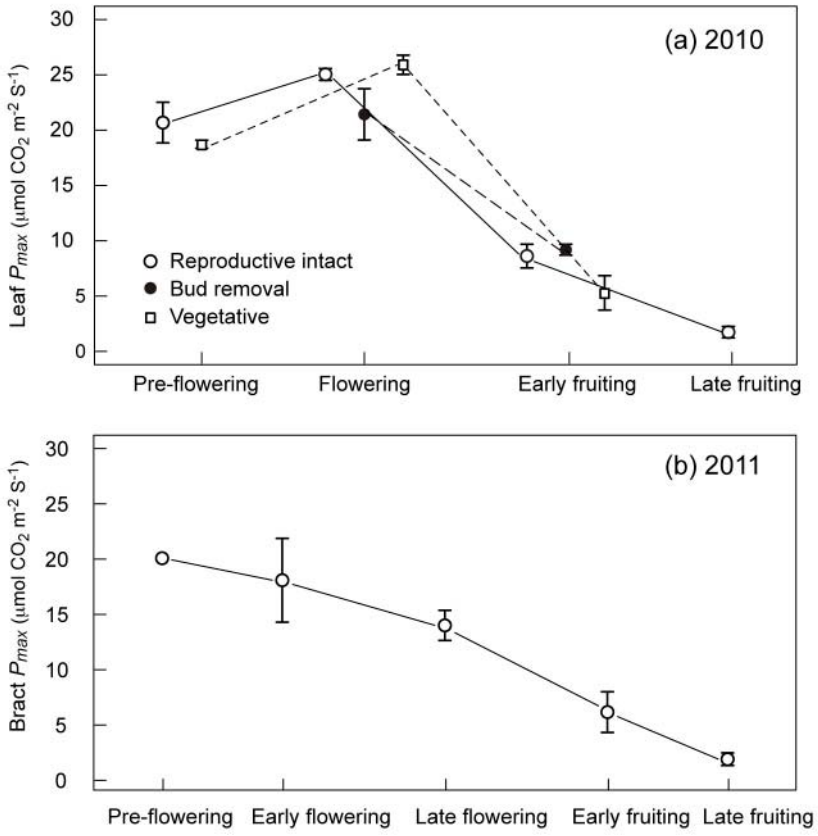

(a) 2010
Leaf P_{max} (µmol CO_2 m^{-2} S^{-1})
30
25
20
15
10
5
0
Reproductive intact
Bud removal
Vegetative
Pre-flowering
Flowering
Early fruiting
Late fruiting
(b) 2011
Bract P_{max} (µmol CO_2 m^{-2} S^{-1})
30
25
20
15
10
5
0
Pre-flowering
Early flowering
Late flowering
Early fruiting
Late fruiting

Figure 4

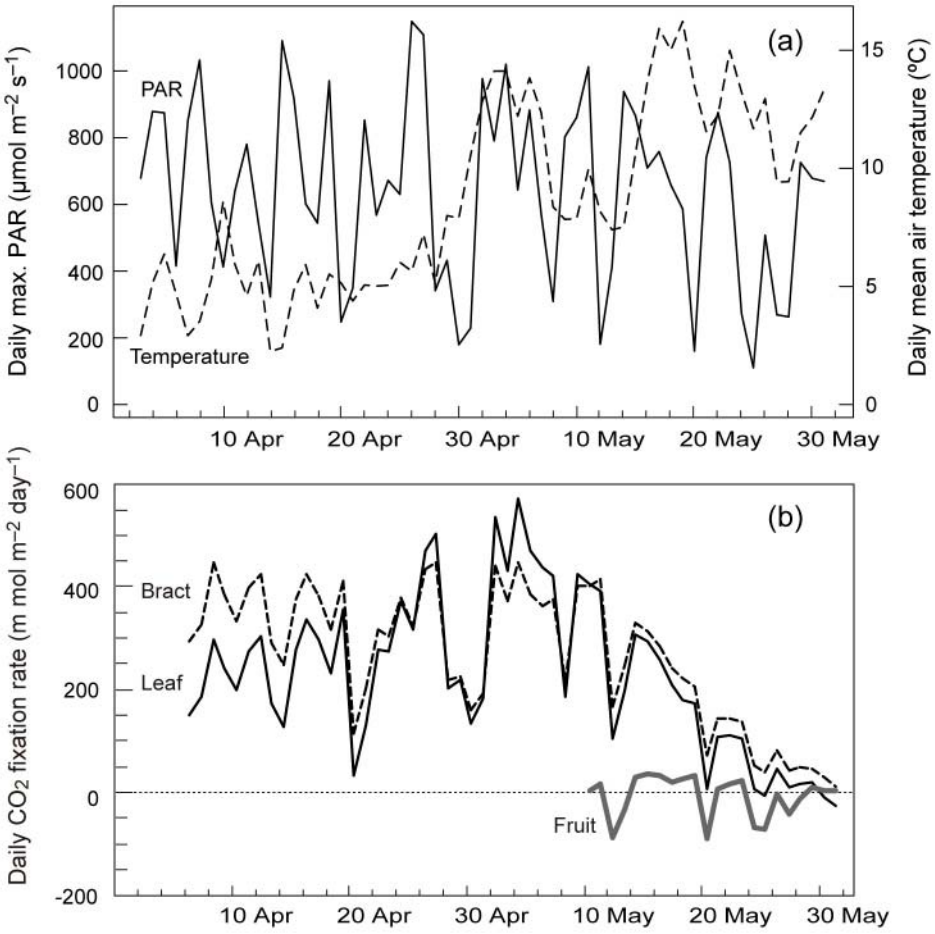
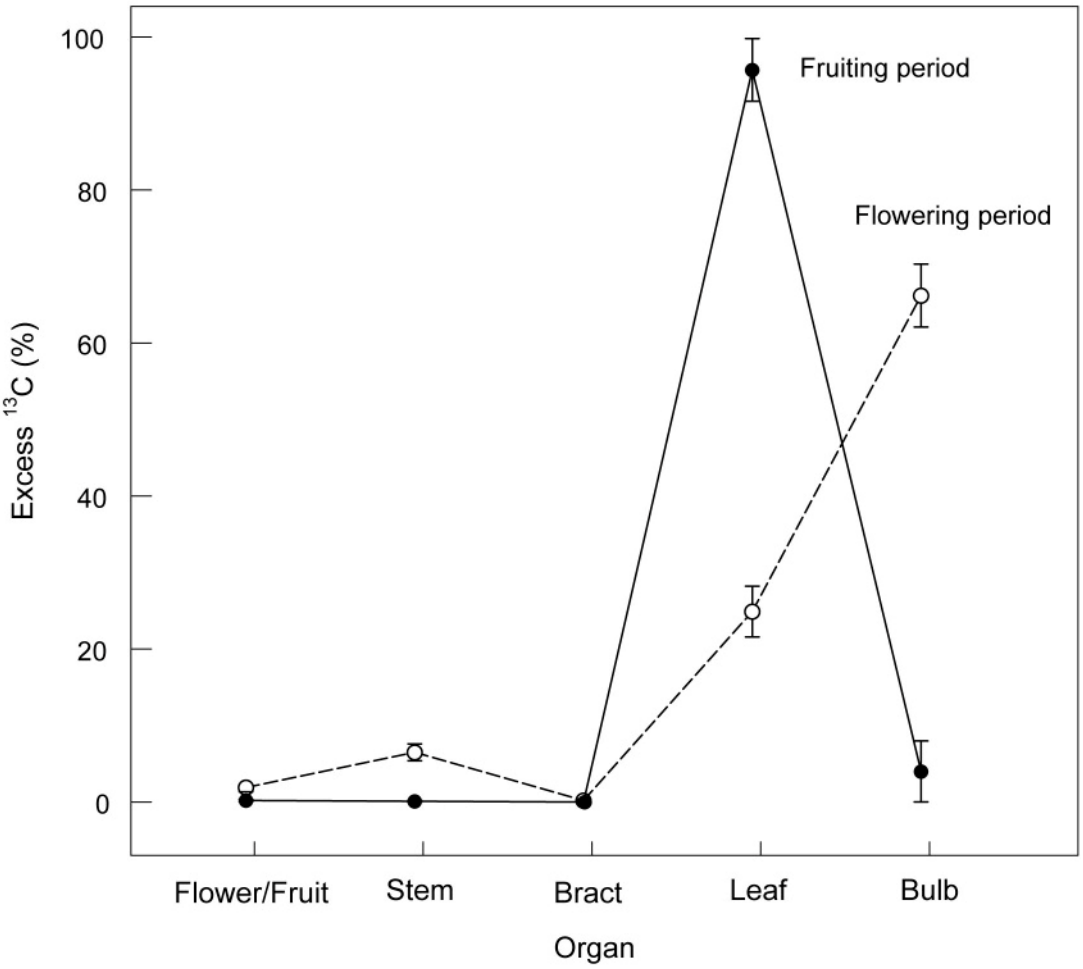

(a)
PAR
Temperature
Daily max. PAR (µmol m−2 s−1)
Daily mean air temperature (ºC)
1000
800
600
400
200
0
15
10
5
0
10 Apr
20 Apr
30 Apr
10 May
20 May
30 May
(b)
Bract
Leaf
Fruit
Daily CO2 fixation rate (m mol m−2 day−1)
600
400
200
0
-200
10 Apr
20 Apr
30 Apr
10 May
20 May
30 May

Figure 5


100
80
60
40
20
0
Excess ^{13}C (%)
Fruiting period
Flowering period
Flower/Fruit
Stem
Bract
Leaf
Bulb
Organ

Appendix 1. Photosynthetic parameters of *Gagea lutea* estimated for basal leaves at individual growth stages and reproductive treatments (reproductive intact, vegetative, and floral-bud removal plants) in 2010.

Treatment	Intact				Bud removal			
Photosynthetic parameter	α	P_{max}	R_d	θ	α	P_{max}	R_d	θ
Pre-flowering (16 April)								
Reproductive	0.051	20.68	1.81	0.54				
Vegetative	0.053	18.73	1.72	0.30				
Flowering (5 May)								
Reproductive	0.065	25.05	1.00	0.40	0.053	21.43	1.00	0.68
Vegetative	0.060	25.87	0.99	0.64				
Early fruiting (18 May)								
Reproductive	0.045	8.62	1.17	0.68	0.038	9.17	0.81	0.85
Vegetative	0.047	5.29	1.21	0.29				
Late fruiting (30 May)								
Reproductive	0.049	1.74	0.94	0.25				

Appendix 2. Photosynthetic parameters of *Gagea lutea* estimated for reproductive organs (fruit and bract) at individual growth stages in 2010 (for fruits) and 2011 (for bracts)

Organ	Bract				Fruit			
Photosynthetic parameters	α	P_{max}	R_d	θ	α	P_{max}	R_d	θ
Pre-flowering (14 April)								
	0.073	20.08	1.06	0.26				
Early flowering (28 April)								
	0.068	18.09	0.89	0.26				
Late flowering (11 May)								
	0.070	14.01	0.86	0.74				
Early fruiting (20 May)					(18 May)			
	0.057	6.17	0.41	0.31	0.043	6.44	2.67	0.82
Late fruiting (31 May)					(30 May)			
	0.062	1.91	0.92	0.86	0.145	5.56	2.49	0.09

Appendix 3. Dry weight allocation to individual organs at flowering (1 May) and fruiting (28 May) stages in *Gagea lutea*. Mean ± s.e. Unpublished data (Sunmonu, Ida and Kudo).

	Leaf	Flower/Fruit	Bract	Stem	Bulb	Root	Total
Flowering stage (n = 14)							
Dry wt (mg)	94.6 ± 9.3	41.0 ± 4.8	20.3 ± 3.0	55.3 ± 8.3	146.6 ± 15.4	8.8 ± 1.2	366.5 ± 36.3
Percent	25.8	11.2	5.5	15.1	40.0	2.4	
Fruiting stage (n = 12)							
Dry wt (mg)	149.0 ± 14.7	95.0 ± 8.6	21.1 ± 3.2	72.5 ± 10.0	518.4 ± 50.9	5.0 ± 0.9	861.0 ± 77.4
Percent	17.3	11.0	2.5	8.4	60.2	0.6	