Intraspecific Variation in Leaf Life Span for the Semi-evergreen Liana *Akebia trifoliata* is Caused by Both Seasonal and Aseasonal Factors in a Temperate Forest

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ABSTRACT: We investigated the leaf demography of a temperate woody liana, *Akebia trifoliata,* in a temperate forest in Japan. *Akebia* is semi-evergreen: some leaves are shed before winter, while others remain through the winter. Previous studies of semi-evergreen species found that variation in leaf life span was caused by variation in the timing of leaf emergence. Leaves that appeared just before winter over-wintered, while leaves appearing earlier were shed. However, it is unclear whether leaves of the same cohort (i.e., leaves that appear at the same time within a single site) show variation in life span under the effect of strong seasonality. To separate variation in life span among the leaves in each cohort from variation among cohorts, we propose a new method - the single leaf diagram, which shows the emergence and death of each leaf. Using single leaf diagrams, our study revealed that *Akebia* leaves within a cohort showed substantial variation in life span, with some over-wintering and some not. In addition, leaves on small ramets in the understory showed great variation in life span, while leaves on large ramets, which typically reach higher positions in the forest canopy, have shorter lives. As a result, small ramets were semi-evergreen, whereas large ramets were deciduous. The longer lives of leaves on small ramets can be interpreted as a shade-adaptive strategy in understory plants.

Key words: *Akebia trifoliata*, Leaf demography, Leaf lifespan, Leaf phenology, Liana, Phenotypic plasticity, Semi-evergreen

INTRODUCTION

Intraspecific variation in leaf lifespan has been reported both in the tropics (Ackerly and Bazzaz 1995, Reich et al. 2004, Vincent 2006) and in temperate zones (Jurik and Chabot 1986, Oikawa et al. 2004). It is generally believed that leaf life span is adjusted so as to maximize whole-plant photosynthesis (Franklin and Agren 2002, Hikosaka 2003, 2005, Kikuzawa and Lechowicz 2006, Oikawa et al. 2006, 2008). Models predict that when whole-plant photosynthesis is high, leaves are more frequently shed as the plant prioritizes investment in new leaves (Kikuzawa 1991, Hikosaka 2003), and this pattern is consistently observed across different biomes and taxa (Reich et al. 1999, Wright et al. 2004, Karst and Lechowicz 2007). Hence, intraspecific variation in leaf life span should be considered as an adaptation for carbon gain (Oikawa et al. 2004).

Some temperate species called semi-evergreens shed some of their leaves before unfavorable periods, but also keep a considerable number of leaves over unfavorable periods. Jurik and Chabot (1986) investigated the leaves of two species of wild strawberry and found that leaves that appear in spring are shed in autumn, while leaves

that appear later in the growing season are retained over the winter, resulting in a longer leaf life span. A similar pattern was reported in a Mediterranean bush species *Phlomis fruticosa* (Kyparissis et al. 1997), which sheds many leaves before the dry season in summer. Leaves that appear immediately after one dry season fall before the next dry season, while leaves that appear later remain over the next dry season, resulting in a longer leaf life span. Therefore, late appearance is linked to a longer life span. These studies indicate that the timing of emergence relative to the unfavorable period has a pronounced effect on leaf life span in temperate regions. This makes interpretation of other aspects of variation in leaf life span difficult in seasonal climates, because it is difficult to determine whether observed variation is caused by seasonal or aseasonal factors, or both. Adaptation for carbon gain is an important aseasonal factor that may affect leaf life span (Oikawa et al. 2004), but variation in leaf life span caused by aseasonal factors may only be easily detected in the tropics, where seasonality is absent (Reich et al. 2004, Vincent 2006). Previous studies of intraspecific variation in leaf life span have compared leaves appearing in different seasons (Oikawa et al. 2004), making the contribution of seasonal and aseasonal factors unclear.

To separate the effects of seasonal and aseasonal factors on leaf

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life span, we propose the use of single-leaf diagrams, a new method for recording the timing of emergence and death of each single leaf. With these diagrams, variation within and among cohorts can be distinguished. Variation among cohorts (e.g., Jurik and Chabot 1986, Kyparissis et al. 1997, Oikawa et al. 2004) can be interpreted as the result of both seasonal and aseasonal factors, whereas variation within a single cohort can be interpreted as evidence of a carbon-gain strategy that is independent of seasonality. Our new analysis will permit us to separate variation induced by seasonal and aseasonal factors, which may independently affect leaf life span.

The timing of leaf emergence is tightly linked to the pattern of shoot elongation. For some species, such as *Fagus crenata,* almost all leaves appear in a single flush at the same time of year, so all of the leaves belong to a single cohort. On the other hand, species like *Alnus hirsuta* produce new leaves at a steady rate until the end of the summer (Kikuzawa 1983, 1984, 1988). Many species, including *Akebia,* are intermediate between those two extreme types. To analyze different cohorts from a single plant, shoot elongation patterns must first be clarified.

Leaves from different-sized plants may have different carbon gain strategies and different lifespans, even when their leaves appear in the same season. In the tropics where strong seasonality is absent, Reich et al. (2004) reported a shorter average leaf life span for larger individuals within a single species. Larger plants that reach the canopy receive more light than smaller plants (Reich et al. 2004, Poorter 2007). The higher light environment in the canopy permits higher whole-plant photosynthesis, and carbon-gain models predict that leaf life span should be shorter when whole-plant carbon gain is high (Kikuzawa 1991, Hikosaka 2003). Therefore, size-dependent intraspecific variation in leaf life span can be interpreted as resulting from differences in carbon-gain strategies under different light regimes experienced through ontogeny (Reich et al. 2004). Kikuzawa (1984) investigated the semi-evergreen shrub *Euonymus alatus* in a cool temperate forest with heavy snowfall. Leaves on large vertical stems were all shed in autumn, while leaves on small prostrate stems over-wintered. As snow cover is known to protect over-wintering leaves from cold injury in other species (Sakai 1976, Kume and Tanaka 1996), snow cover may also enable survival of over-wintering leaves in that region (Kikuzawa 1984). However, if we interpret this variation in leaf life span as a carbon gain strategy in the understory (Kikuzawa 1984), then we should expect to also observe this pattern in temperate regions where snow cover is absent. We sought to test whether size-dependent variation in leaf life span, which has been reported in the tropics, also occurs in temperate forests where snow cover is absent or negligible, which would support the generality of size-dependent intraspecific variation in leaf life span as a carbon-gain strategy.

The woody liana *Akebia trifoliata* has two kinds of leaves, autumn-falling leaves and over-wintering leaves, in temperate forests where snow cover is low. We collected data to address the following two questions: (1) Is there variation of leaf life span within a single cohort, even across an unfavorable period? (2) Is leaf life span size-dependent in temperate regions, even those without substantial snow cover?

MATERIALS AND METHODS

Study Site

We conducted this study in a secondary, temperate mixed broad-leaved forest consisting of deciduous and evergreen trees in the Kamigamo Experimental Forest Station of Kyoto University in the city of Kyoto (35° 4' N, 135° 46' E, altitude 140 m). Mean annual temperature and precipitation at the study site from 1971 to 2000 were 14.6 ℃ and 1,581.8 mm, respectively. The mean daily low temperature from 1971 to 2000 was −0.9 ℃ in January , while the mean number of days with snow cover from 1993～2000 was 12.2 days, and the mean snow depth of those days was only 4.4 cm. In winter of 2003～2004, when the study was conducted, there were 7 days with snow, with an average depth of 1.2 cm (Kamigamo Experimental Forest Station, unpubl. data).

Study Species

Akebia trifoliata (Thunb.) Koidz. (Lardizabalaceae) is a woody liana species distributed in East Asia (Ohwi and Kitagawa 1992). *A. trifoliata* is commonly found in forest edges, gaps and in the forest understory in Japan.

Leaf Demography Census

In early March 2003, when no current-year leaves had yet emerged, we selected 115 ramets from various environments (e.g., open gaps and the understory) to monitor, of which 95 survived to the end of the study. Four ramets were too large to investigate entirely, so we selected one branch on each for monitoring.

All leaves that emerged in 2003 were marked with plastic labels and ink-spots, and their survival was monitored. The ramets were monitored at 7- to 10-day intervals during the leaf-emergence period from March to April 2003, and subsequently at 8- to 44-day intervals until December 2004. The timing of leaf emergence was estimated as the mid-point between the date of a previous observation and the date when the new leaf was observed, and the timing of leaf fall was estimated as the mid-point between the observations before and after leaf fall. Leaf life span was calculated as the number of days between emergence and fall. The longevities of 14% of leaves could not be determined as they became unidentifiable

during the course of the study (through disappearance of labels in windstorms, etc.). Longevities were obtained for a total of 1,423 leaves that appeared in 2003.

Shoot Elongation Pattern

Three kinds of shoots were distinguished, following classification of Yagi and Kikuzawa (1999). Shoots with little shoot elongation were called "short shoots". "Long shoots" were shoots for which marked elongation was observed in the growing period. Finally, elongation of secondary shoots was also recorded.

Ramet Size

A. trifoliata is a liana. All of the large ramets were climbing on other trees, while small ramets were found near the ground. Many ramets were upright, but very small (typically less than 30 cm in height). According to our field observations, these small vertical ramets branched out from underground horizontal stems. Therefore, it was difficult to distinguish "standing" from "creeping" for small ramets. We defined ramet size as the maximum total number of leaves recorded on each ramet in a demography census in 2003. Ramet sizes were obtained for 91 ramets with of total 901 leaves, excluding the four very large ramets. We use ramet size as an indicator of plant size and developmental stage. All the statistics were calculated with SPSS 13.0J for Windows (SPSS Japan Inc, Tokyo, 2004).

RESULTS

Leaf Phenology

The timing of leaf appearance and fall are shown in Fig. 1. Most of the leaves (67.7%) appeared simultaneously on short shoots in March and April, while very few leaves (1.8%) emerged on short shoots later in the year. Leaves on the long shoots (24.7%) emerged successively from bud break until the shoot finished its elongation in spring or in summer, and a few leaves (5.8%) appeared on secondary growth shoots as late as early winter.

Mean dates of emergence of leaves on short and long shoots were 8 April and 25 April, respectively. The mean date of leaf emergence on secondary growth shoots was 10 August, much later than those of the former two types of leaf. As 94.2% of leaves emerged on short and long shoots, the dates of emergence for the total leaf population were concentrated in spring: 87.6% of leaves appeared in March or April, while 12.4% appeared in May or later.

Leaf Lifespans

Leaves appearing later has shorter lives ($P < 1.0 \times 10^{-17}$, Pearson's r = −0.231, $N = 1423$). However, 9.3% of leaves survived until the subsequent spring (i.e., until March 2004), remaining green like the leaves of evergreen species (Fig. 1). Note that this percentage of over-wintering leaves may not reflect that of the natural population, because we arbitrary chose different-sized *Akebia* ramets. Leaves that appeared in any season could fall in autumn or over-winter, and the few leaves that appeared late in the growing season did not necessarily over-winter. Variation in life span was observed not only for leaves on short shoots, but also for leaves on long and secondary shoots (Fig. 1).

Ramet Size and Leaf Lifespan

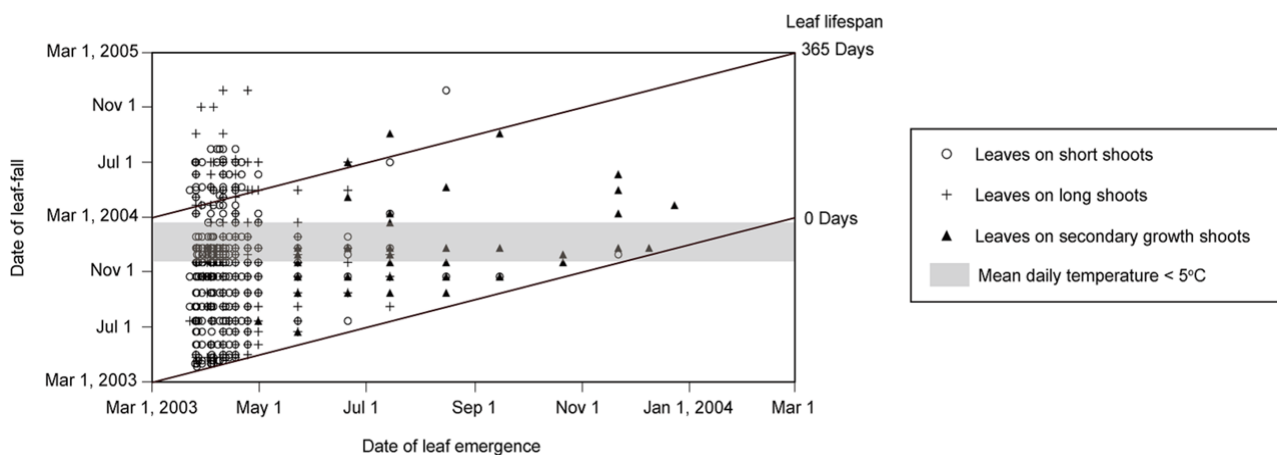For leaves that appeared in March or April as well as those

Fig. 1. A single leaf diagram showing the date of leaf appearance, the date of leaf fall and the resulting life span for each leaf. Each symbol indicates the timing of emergence and fall for a single leaf ($N = 1,423$). The two oblique lines are isoclines for leaf life span of 0 and 365 days, respectively. Open circles: leaves on short shoots. Crosses: leaves on long shoots. Closed triangles: leaves on secondary shoots. The gray box indicates winter (i.e., days on which the mean temperature was less than 5 ℃ at the site).

appearing in May or later, leaves on larger ramets had shorter lives (Fig. 2). In contrast, leaves on small ramets showed substantial variation in life span and often over-wintered. This relationship between ramet size and leaf life span was observed among leaves on short shoots ($N = 553$, $R^2 = 0.113$, $P < 1.0 \times 10^{-15}$), long shoots ($N = 277$, $R^2 = 0.041$, $P < 0.001$) and secondary shoots ($N = 71$, $R^2 = 0.105$, $P < 0.01$).

DISCUSSION

In contrast with previous reports on semi-evergreen species (Jurik and Chabot 1986, Kyparissis et al. 1997), the over-wintering leaves of *A. trifoliata* were not necessarily late-appearing. Rather, this semi-evergreen plant showed substantial variation in the life span of leaves within a single cohort. Leaf life span was affected by two independent factors. First, 90.7% of leaves appearing in any season fell before the beginning of the winter like the leaves of deciduous trees. As a result, leaves that appeared in later seasons had a shorter mean life span. This is an effect of seasonality; winter imposes a limit upon leaf life span in temperate regions, as has been discussed elsewhere (e.g., Reich et al. 2004, Vincent 2006). However, our single-leaf diagram (Fig. 1) allowed us to detect additional variation in life span among leaves that appeared simultaneously. In particular, the life span of leaves on smaller ramets near the ground is often longer than one year (Fig. 2). As a result, small ramets were semi-evergreen while large ramets were deciduous at the site.

Our field observations suggested that larger *Akebia* ramets often grow into or over the forest canopy. Their leaves are therefore more likely to experience well-lit conditions than leaves on smaller ramets, which were found in the understory. Deciduous species in general have a higher photosynthetic rate and shorter leaf life span than evergreens, while evergreens are more shade-tolerant (Chabot and Hicks 1982). Similarly, within a single species, an individual in a well-lit place has a higher photosynthetic rate (Noda et al. 2004) than a shaded individual. A cost-benefit analysis showed that having a higher photosynthetic rate leads to a high leaf-turnover rate, which in turn leads to a shorter leaf life span (Kikuzawa 1991, Hikosaka 2003, 2005). Accordingly, a shorter mean leaf life span has been reported for individuals in well-lit environments relative to shaded conspecifics (Seiwa and Kikuzawa 1991, Ackerly and Bazzaz 1995, Hikosaka 2005, Vincent 2006). While the same pattern has been reported in regions with heavy snow (Kikuzawa 1984), the effect of snow-cover was negligible at our study site. Therefore, size-dependent intraspecific variation in leaf lifespan can be interpreted as a carbon gain strategy, irrespective of snow cover.

Importantly, while we observed that larger plants had a shorter leaf life span, several researchers (Kikuzawa and Ackerly 1999, Seiwa 1999) have reported a positive correlation between plant size and leaf life span. Kikuzawa and Ackerly (1999) developed a model that predicted that increasing plant size imposes a larger "supporting cost", which requires a longer payback time and longer leaf life span. The effect of this "supporting cost" may be an important determinant of leaf life span if different-sized individuals experience little difference in light regimes. However, plants in natural forests often
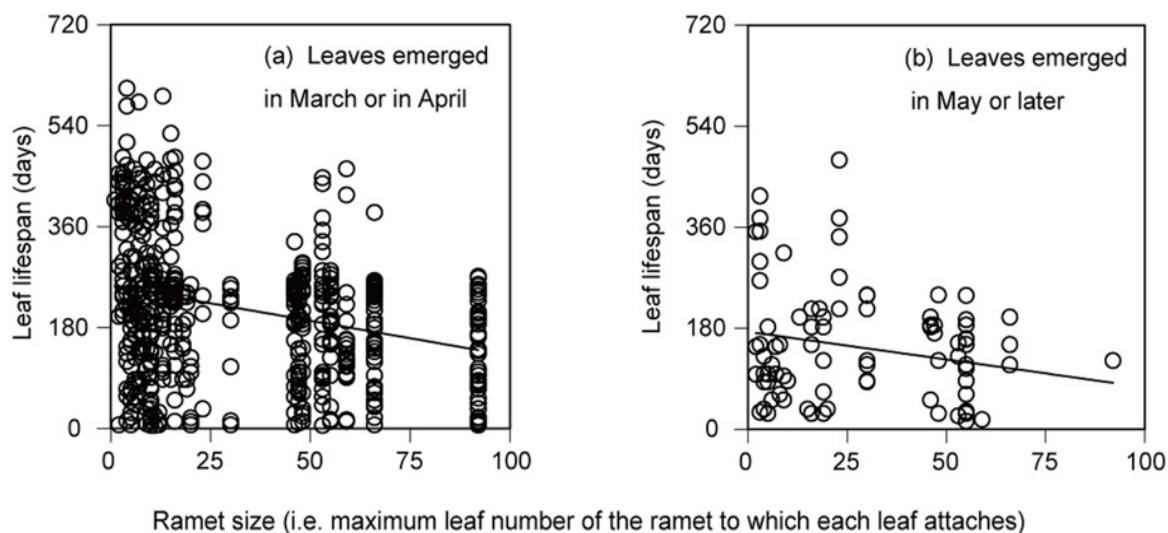

Fig. 2. Relationship between ramet size (maximum leaf number on each ramet in 2003) and leaf life span. Each circle indicates one leaf. For each leaf, size of the ramet to which that leaf is attached and life span of that leaf is plotted. Solid lines indicate significant linear regressions. (a) Leaves that emerged in March or in April: leaf lifespan = −1.24 Ramet size + 254.5, $R^2 = 0.095$, $P < 1.0 \times 10^{-17}$, $N = 768$. (b) Leaves that emerged in May or later: leaf lifespan = −0.985 Ramet size + 173.5, $R^2 = 0.0522$, $P < 0.01$, $N = 133$.

experience different light regimes through ontogeny (Reich et al. 2004, Poorter 2007). The relationship between leaf life span and plant size in forests seems to be affected by both "supporting cost" and light regimes.

We conclude that even though the timing of leaf emergence affects the life span of leaves of temperate species, there is also substantial size-dependent variation among leaves within a single cohort for *A. trifoliata*, as has been reported in the tropics.

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