Leaf lifetime photosynthetic rate and leaf demography in whole plants of Ipomoea pes-caprae growing with a low supply of calcium, a ‘non-mobile’ nutrient

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Abstract

The adaptive significance of leaf longevity has been established in relation to restrictive nutrients that can be retranslocated within the plant. However, the effect of deficiencies in ‘non-mobile’ nutrients on leaf lifespan and photosynthetic carbon gain is uncertain. Calcium is frequently given as an example of an essential nutrient with low phloem mobility that may alter the leaf senescence process. This study has been designed to estimate leaf lifespan, leaf production ($L_p$) and leaf death ($L_d$) rates, the age structure of leaves, and the decline in maximum photosynthetic rate ($A_{\text{max}}$) with age in plants of Ipomoea pes-caprae growing with a full supply of nutrients and with a low Ca supply. The Ca deficiency produced reductions in $L_p$ and leaf lifespan compared with control plants. In spite of the differences in the demographic parameters between treatments in control and low-Ca plants, the percentage of leaves of a given leaf age class is maintained in such a way that the number of leaves per plant continues to increase. No relationship was found between Ca supply and $A_{\text{max}}$. However, the decline in $A_{\text{max}}$ with leaf senescence was rather sudden in control plants compared with plants growing with a low Ca supply. The importance of simultaneously using the total leaf demographic census and the assimilation rate along with leaf lifespan data in order to understand the performance of whole plants under constrained conditions is discussed.

Key words: Calcium, carbon gain balance, gas exchange, Ipomoea pes-caprae, leaf age structure, leaf demography, leaf longevity, leaf senescence.

Introduction

Leaf photosynthetic capacity and longevity determine the total carbon return from a leaf during its lifetime and can influence fluxes of canopy carbon uptake (Chabot and Hicks, 1982; Kitajima et al., 1997; Dungan et al., 2003). Thus, knowing the leaf longevity, the effect of leaf age on photosynthetic capacity, and the leaf population of the whole shoot, it is possible to estimate the maximum CO$_2$ assimilated throughout the leaf lifespan and the long-term carbon gain of a whole plant (Nilsen et al., 1987; Kitajima et al., 1997, 2002). Despite the importance of knowing the distribution of leaf ages and the $A_{\text{max}}$ in each age class present in the plant in order to estimate its overall photosynthetic performance, there are few quantitative data regarding these parameters within a given species and/or in a whole plant (Kitajima et al., 1997; Dungan et al., 2003).

In general, leaf lifespan is considered as an evolutionary strategy to maximize the leaf and plant carbon gain under habitat constraints in such a way that a lower maximal initial photosynthetic rate of fully expanded leaves in species growing under low-resource conditions, compared with those with high-resource availability, is generally partially compensated by a longer leaf lifespan (Millard and Proe, 1991; Reich et al., 1992; Kitajima et al., 1997). Additionally, it has been proposed that older leaves function as sources of nutrients used in the production of new tissue, and so the demand for nutrients from growing
tissues may drive nutrient resorption and senescence processes (Shaver, 1981; Field and Mooney, 1986; Hikosaka et al., 1994; Aerts, 1996; Escudero and Mediavilla, 2003; Mediavilla and Escudero, 2003).

The importance of long leaf lifespan and high nutrient resorption efficiency as adaptations to a low nutrient supply has been established and is discussed in the literature, mainly in relation to the generally more restrictive nutrients under natural conditions, as is the case of N and P, which can be retranslocated within the plant (Millard and Proe, 1991; Reich et al., 1992; Mediavilla and Escudero, 2003). However, the relations between the effect of deficiency in non-mobile nutrients and the leaf lifespan and photosynthetic carbon gain of a whole plant still remain unclear. Non-mobile nutrients tend to increase with leaf age and are not available for use by the newer leaves with a higher photosynthetic rate. Consequently, under conditions of low non-mobile nutrient availability, high longevity may reduce plant productivity and nutrient use efficiency (Aphalo et al., 2002). Calcium is frequently given as an example of an essential ‘non-mobile’ nutrient as its mobility in the phloem is low, it is not cycled internally, and there is no remobilization associated with leaf ageing (Ramalho et al., 1995; McLaughlin and Wimmer, 1999; White and Broadley, 2003). Additionally, leaf Ca content influences photosynthetic rate and may alter the leaf senescence process (Pooaiah and Leopold, 1973; Chou and Kao, 1992; Ramalho et al., 1995; McLaughlin and Wimmer, 1999). Then, while a limited supply of N and P may favour a long leaf lifespan, a low Ca supply may favour an earlier leaf senescence process (Aphalo et al., 2002).

This study was designed to estimate leaf demographic parameters and the decline in photosynthetic rate with age, as well as the maximum carbon assimilation throughout the leaf life in plants of Ipomoea pes-caprae (L.) R. Br. (Convolvulaceae) growing under two nutrient conditions: one with full supply of all essential nutrients and another with a low supply of a relatively non-mobile nutrient like Ca. I. pes-caprae is a clonal perennial herbaceous species with a pantropical distribution widespread in coastal areas along the beach and native to Florida (Wilson, 1977; Devall, 1992; Gilman, 1999). It grows in environments that are poor in N and P but not in cations, such as Ca$^{2+}$, K$^+$, Mg$^{2+}$, and Na$^+$ (Willis and Yemm, 1961; van der Valk, 1974; Harte and Pammenter, 1983; Ripley, 2001). Sources of these nutrients in natural conditions include swash deposition from the sea, salt spray, groundwater, mineralization, and meteorological inputs (Willis and Yemm, 1961; van der Valk, 1974; Ripley, 2001). The roots of I. pes-caprae grow to great depth and can be found up to 3 m deep, where the permanent water table is encountered (Ripley, 2001). This species has a rapid growth and leaf turnover (Wilson, 1977; Bach, 2000; Ripley, 2001), and there is minimal mutual shading between leaves due to the extended pattern of branch growth, allowing light effects on photosynthetic rate and nutrient content of leaves located at different plant depths in the canopy to be disregarded. It is hypothesized that under low Ca conditions, leaf longevity is reduced, decreasing photosynthetic carbon assimilation during the leaf’s lifetime and, finally, the total carbon gain of the plant. To prove this hypothesis, it is first necessary to answer the following questions: (i) How does Ca availability influence the decline in carbon gain with leaf ageing? (ii) How does low Ca supply change the leaf demography in the whole plant? and (iii) How does the combination of the previously mentioned factors affect the long-term carbon budget of a leaf and the total CO$_2$ assimilated by the whole plant?

**Materials and methods**

*Plant material and growth conditions*

Plants of *I. pes-caprae* were collected in the field at Tucacas (10°48’ N, 68°19’ W), Estado Falcon, Venezuela, and transferred to a glasshouse. Twenty-eight apical vegetative segments of uniform size, with four to seven leaves, were transplanted into individual 5.01 pots filled with washed sand. The vegetative segments were watered as needed with *Peat-Lite Special Peters*® WaterSoluble Fertilizers (Scotts Company, Marysville, OH, USA) plus an additional supply of Ca(NO$_3$)$_2$ to warrant full nutrient availability. The *Peat-Lite Special* solution contains N, P, and K in proportions of 20:10:20 and lower proportions of other macro- and micronutrients (B, Cu, Fe, Mn, Mo, and Zn) but does not contain Ca. This nutrient solution, diluted in tap water, contained (mol m$^{-3}$) the following: NO$_3^-$, 1.20; NH$_4^+$, 0.80; P, 0.21; K$^+$, 0.64; Ca$^{2+}$, 2.75; Mg$^{2+}$, 0.01; Fe, 0.003; Mn$^{2+}$, 0.003; B, 0.0004; Zn$^{2+}$, 0.0002; Cu$^{2+}$, 0.0002; and Mo$^{5+}$, 0.0002. In order to obtain a complete nutrient solution for the control plants, Ca(NO$_3$)$_2$ (2 mol m$^{-3}$) was added.

Before measurements were taken, apical segments were grown for 3 weeks to allow recovery from transplantation and initial root formation. All plants survived this 3-week period. The plants were maintained in these conditions for 10 months until they had a large biomass, with the number of leaves ~80 and roots that fully explored the soil volume. Then 8–10 plants were randomly selected for each of the two nutrient treatments: control and low Ca. The control nutrient solution contained 2.21 and 2.73 mol m$^{-3}$ and the low-Ca solution 2.05 and 0.44 mol m$^{-3}$ of N and Ca, respectively. The small amount of Ca in the latter solution corresponded to that present in tap water and probably to traces present in the soluble fertilizers. In the glasshouse, the maximum level of photosynthetically active radiation was 1510±370 µmol m$^{-2}$ s$^{-1}$ and the photoperiod was $\approx$12 h throughout. The average air temperatures were 27 °C at daytime and 18 °C at night. Relative humidity was 49–97%.

*Assessment of the effects of short- and long-term plant growth on leaf demography*

An initial experiment was carried out in order to determine the possible changes in leaf demography associated with plant growth during the first 10 months after transplanting. Thus, leaf demographic parameters were measured in plants growing for short- and long-term (3 weeks and 10 months, respectively) periods after transplanting, with plenty of water and a full nutrient supply. After the 3-week or 10-month growth periods, plants were pruned and had an initial number of leaves of 4–10. Pruning allowed elimination of the possible effect of the initial number of leaves present in the plant on the new leaf production rate. In both periods, all leaves from 28 plants were marked to differentiate them from the newly sprouted leaves. Subsequently, for a period of 70 d, the numbers of born and dead leaves per plant within 7-d intervals were counted. A leaf was considered born when the two
halves unfolded, which happened ~7 d after the first evidence of leaf appearance, whereas a leaf was considered to be dead when it had fallen. With these data, leaf production and leaf death rates per plant were calculated. At the same time, new leaves encountered at each subsequent census (at 7-d intervals) were marked and treated as different-aged cohorts. Eleven cohorts were checked during 84–126 d and censused every 7 d until death. The census of leaf cohorts allows estimation of the survival probability and the leaf lifespan. All the cohorts within the same plant were considered as replicates of the same sequential event, and the data were averaged. Leaf production (Lp) and leaf death (Ld) rates after a period of 70 d were calculated as follows:

\[ L_p = \frac{b}{t} \] and \[ L_d = \frac{d}{t} \]

where \( b \) and \( d \) are the cumulative number of born and dead leaves, respectively, per period of \( t \) days. Leaf demographic data were used to calculate the leaf half-life (\( t_{50} \)), the time elapsed until 50% of the leaf population died, and the leaf turnover rate, which represents the frequency of leaf loss. Leaf half-life was obtained from an estimated survival curve, and leaf turnover rate was calculated as \( 1/t_{50} \). The structure of leaf age in whole plants was calculated using a static leaf life table obtained for plants growing for a period of 70 d (11 age classes). Using a stationary leaf life table, a cross-section allowed the number of deaths, the survival rate, and the number of leaves alive in each leaf age group to be estimated.

**Calcium effects**

When the previously described initial experiment had been performed, plants showed a sustained shoot growth. Thereafter, the effect of Ca was evaluated 18 months after transplanting. Eighteen plants were randomly separated into two groups: one group of eight plants was maintained with full nutrient supply (control plants) and a second group of 10 plants was grown in a nutrient solution limited in Ca. After 20 d, when the plants had produced enough leaves in each nutrient treatment, all plants were pruned so as to bear four to six leaves. Pruning allows for the elimination of the effect of the initial number of leaves per plant and of the Ca previously accumulated in the leaves.

**Nutrient solution composition.** Total N and Ca concentrations in the nutrient solution used in both treatments were measured. N was measured by the micro-Kjeldahl method after sulphuric acid digestion. The digest was distilled in the presence of NaOH and collected in boric acid and then titrated with standard acid (Williams, 1984). Total Ca concentrations were determined by means of an atomic absorption spectrophotometer (Model AA6; Varian Techtron, Walnut Creek, CA, USA).

**Maximum carbon assimilation in whole plants.** Maximum CO\(_2\) assimilation per unit time, in each of the 11 age classes of leaves from a plant, was estimated as the \( A_{\text{max}} \) corresponding to a particular age multiplied by the total leaf area of this age class. The estimate was carried out using the structure of leaf age at 77 d of growth and the \( A_{\text{max}} \) value for an instantaneous moment of the day when the leaf photosynthetic rate reaches a maximum. The estimated total maximum CO\(_2\) assimilation per leaf age was calculated assuming that the leaf area kept constant along the leaf age, and that the CO\(_2\) assimilation continued as long as leaves reach their physiological maxima at 21–28 d old, 7 d after a leaf unfolds, it reaches the maximum area.

**Statistical analysis**

The statistical significances of area per leaf, Chl\((a+b)\), and LSM between treatments, were tested using Student’s \( t \) test. Differences between gas exchange parameters and percentage of leaves present...
in each age class within each treatment were tested using a one-way analysis of variance test. Bonferroni and Dunnnett’s T3 tests were performed a posteriori when homogeneous or non-homogeneous variance, respectively, was found in the data. The numbers of born and dead leaves per plant were estimated by non-linear regression of the number of leaves as a function of time. All statistical analyses were performed with SPSS 12.0 (SPSS Inc., Chicago, IL, USA). Leaf lifespan between treatments was analysed with Regression Wizard (SigmaPlot 11.0; SPSS Inc.). Leaf survival probability curves were adjusted by non-linear regression using sigmoid three-parameter \( y = y_0 + a/(1 + e^{-(x-x_0)/b}) \) and five-parameter \( y = y_0 + a/[1 + e^{-(x-x_0)/b}]c \) curves for short- and long-term plant growth and Ca effect experiments, respectively. A significance value of \( P < 0.05 \) was used throughout. Details of the statistical procedures followed Sokal and Rohlf (1995).

Results

Effects of short- and long-term plant growth on leaf demography

The initial experiment to determine the possible changes in leaf demography associated with plant growth showed differences depending on the time elapsed after transplantation. The leaf production \((L_p)\) and leaf death \((L_d)\) rates differed between plants with a short (3 weeks) and a long (10 months) growth period after transplanting (Fig. 1A; Table 1). Both \(L_p\) and \(L_d\) increase with time after transplanting (Table 1). Ipomoea pes-caprae plants, 10 months old and previously pruned, had a cumulative leaf net gain of \(-49\) leaves during 70 d, whereas those at 3 weeks old had a cumulative gain of 34 leaves over the same period. Consequently, the final number of leaves per plant was 22% higher in 10-month plants compared with that of 3-week plants. Leaf half-life \((t_{50})\) was 94 d and 60 d for plants grown for 3 weeks and 10 months, respectively (Fig. 1B). Higher \(L_p\) and \(L_d\) in combination with lower \(t_{50}\) in plants grown for 10 months resulted in a faster leaf turnover rate when compared with those grown for 3 weeks (Table 1). In addition, as a consequence of high \(L_p\) and \(L_d\) after a 10-month growth period, the age structure of a population of leaves using a static leaf life table shows a constant increasing number of younger leaves (Fig. 2A, B).

Fig. 1. (A) Cumulative number of new (circles) and dead (triangles) leaves and (B) survival probability as a function of time in leaves of Ipomoea pes-caprae cultivated for 3 weeks (white) and 10 months (grey) after transplantation of apical segments. Plants were growing with a full nutrient supply and had previously been pruned. The initial number of leaves was four to seven. In (A), the number of leaves that appeared and shed per plant was counted within a 7-d interval for a period of 70 d; in (B), the leaves that appeared within a 7-d interval were grouped in a cohort, and 11 cohorts per plant were included. Survival probability as a function of time was estimated for a period of 84–126 d. Means for 28 plants per period are shown. Bars represent SEM.

Table 1. Leaf production \((L_p)\) and leaf death \((L_d)\) rates, leaf half-life \((t_{50})\), and leaf turnover rate in plants of Ipomoea pes-caprae

| Short and long growth period | Effect of Ca |
|-----------------------------|-------------|
|                            | Control     | Low Ca      |
| \(L_p\) (Leaf d\(^{-1}\))   | 0.40 ± 0.13 | 1.94 ± 0.60 |
| \(L_d\) (Leaf d\(^{-1}\))   | 8.7 x 10\(^{-2}\) ± 3.1 x 10\(^{-2}\) | 54 x 10\(^{-2}\) ± 19.6 x 10\(^{-2}\) |
| \(t_{50}\) (d)               | 94          | 69          |
| Leaf turnover rate (d\(^{-1}\)) | 0.011      | 0.014       |
Calcium effects

Leaf composition. Differences in N and Ca in the nutrient solution between treatments were enough to affect some leaf characteristics but produced few changes in their chemical composition (Table 2). Low Ca concentration in the nutrient solution affected the area per leaf and the LSM of young fully expanded leaves 14–35 d old. Leaves growing with low Ca were, on average, 48% smaller than those of control plants (33.0 ± 4.9 and 63.2 ± 12.9 cm², respectively), while LSM was 25% higher in the low-Ca treatment compared with control plants (82.6 ± 8.0 and 66.2 ± 10.3 g m⁻², respectively). The Chl(a + b) content per leaf area was similar in both treatments (3.6 ± 0.4 and 3.5 ± 0.3 mg m⁻²).

Throughout leaf life, N content per leaf mass was higher in control plants than in plants growing with low Ca, but differences in Ca content per leaf area between both treatments were low (Table 2). However, as LSM was lower in controls than in low-Ca plants, the Ca content tends to be slightly higher in control plants when expressed in units of dry mass. Leaf N per unit leaf mass was high in the earliest stage of the lifespan and declined in the later stage in both treatments (Table 2). After leaf maturation, leaf chronological age and N content were negatively correlated in both treatments (r² = 0.85). Maximum N content was reached in leaves 7 and 21 d old, and the decrease from young to senescent leaves was 75% and 51% in control and low-Ca plants, respectively (Table 2). In contrast to N, Ca content increased as the leaf became older in both treatments. Leaf Ca content increased 9 and 12 times in leaves 7–63 d old in control and low-Ca plants, respectively (Table 2).

Leaf demography. When the effect of low Ca in the nutrient solution on leaf demography was evaluated, it was found that Lₚ was 58 ± 14 and 31 ± 4 leaf month⁻¹ in control and low-Ca plants, respectively, while Lₐ was similar in both treatments (Table 1; Fig. 3A). With time, plants growing with limited Ca accumulated lower numbers of total leaves per plant, although the net leaf gain was still positive (Fig. 3A). In addition, low-Ca plants have a 19-d shorter tₕ₀ than leaves of control plants (Table 1; Fig. 3B). As a consequence of the differences in Lₚ and tₕ₀ between both treatments, I. pes-caprae mean turnover rates were 42% higher in plants under low-Ca conditions (Table 1).
In both treatments, the age structure of a population of leaves shows a pyramidal shape caused by rising numbers of younger leaves (Fig. 2C, D). However, the low-Ca treatment stationary diagram shows a mean percentage of leaves at 7–14 d that is slightly lower than that of mature leaves at 21–35 d (10.7 versus 11.2%, respectively; Fig. 2C, D).

Gas exchange and nutrients. In *I. pes-caprae*, the maximum photosynthetic rate increased with leaf age, reaching a maximum in leaves 28 d old and declining thereafter with leaf senescence (Fig. 4A). The effect of leaf ageing on the maximum CO₂ assimilation rate ($A_{\text{max}}$) through leaf lifespan was similar in the two nutrient conditions and values overlap (Fig. 4A). The highest $A_{\text{max}}$ values were recorded in leaves 28 d old in both treatments, and in Ca-limited plants, $A_{\text{max}}$ was only 2% lower than in control plants; its decline with leaf senescence was faster in control plants (Fig. 4A).

In control plants, $A_{\text{max}}$ decreased by 68% from the fully expanded 28-d-old leaves to the last leaf age measured (77 d). However, in plants growing in a low-Ca solution, $A_{\text{max}}$ at shedding was still positive (72% of that at 28 d old) in spite of the short leaf lifespan. There was a substantial variation in the slopes and intercepts of the $A_{\text{max}}$–leaf age relationships between treatments; the slopes change when the data points for young leaves that have not reached their physiological maxima were not included. Slopes were $-0.12$ and $-0.09 \, \mu\text{mol m}^{-2} \text{s}^{-1}$ and the x intercepts were 147 and 171 d in control and low-Ca plants, respectively.

Table 2 shows gas exchange parameters at different leaf ages in the two nutrient treatments; the tendencies along leaf life are not clear, but, in general, stomatal conductance ($g_s$) and transpiration rate ($E$) tend to increase in leaves 7–14 d old and then decrease with ageing. Stomatal conductance causes a proportional decrease in $E$ ($r^2=0.92$). In general, $E$ was higher throughout leaf lifespan in plants under low Ca than in the controls (Table 2).

The relative decrease in $g_s$ with leaf ageing was higher than that occurring in $A_{\text{max}}$ (Fig. 4A; Table 2). Therefore, internal-to-ambient CO₂ concentration ratio ($C_{i}/C_{a}$) in plants of *I. pes-caprae* tends to decrease with leaf ageing in both treatments but tends to be higher in low-Ca plants, implying that short-term water efficiency was reduced in comparison to control plants. WUE with leaf ageing did not show large differences between treatments in leaves 7–35 d old; however, above this age, WUE was higher in control than in low-Ca plants (Fig. 4B). In both treatments, the decrease in $A_{\text{max}}$ with leaf ageing took place together with a decrease in leaf N content (Fig. 4A; Table 2). After leaves reach their physiological maxima (21–28 d old), $A_{\text{max}}$ relates linearly to N content ($r^2=0.60$), resulting in relatively constant PNUE during leaf senescence (Table 2).

**Maximum carbon assimilation in whole plants.** In control plants, there were considerably more leaves at all leaf age classes than in plants growing with low Ca (Fig. 5A). A higher number of leaves in combination with larger leaves in control plants resulted in a significantly higher total leaf
area. Consequently, the maximum CO\textsubscript{2} assimilation per unit time in all leaf age classes present in a whole plant was also significantly higher in control compared with low-Ca plants (Fig. 5B). Maximum CO\textsubscript{2} assimilated at all leaf ages was highly correlated with the total leaf area ($r^2 = 0.93$) in such a way that the regression between the difference among treatments in leaf area vs. maximum CO\textsubscript{2} assimilated was 1:1 (Fig. 5B, inset). In both treatments, the highest proportion of CO\textsubscript{2} assimilated by a whole plant was provided by leaves 14–28 d old, progressively decreasing thereafter (Fig. 5B).

**Discussion**

*Effects of short- and long-term plant growth on leaf demography*

Differences in the leaf demographic parameters between plants growing for periods of 3 weeks or 10 months suggest that leaf demography may vary with the development stage of the plants (Table 1; Fig. 1). As the experiment began 3 weeks after the apical vegetative segments had been transplanted, they probably had not produced enough roots to sustain shoot growth; if the roots grow actively, the resources invested in the production of new leaves may decrease. The low leaf production rate ($L_p$) and leaf turnover rate during the initial phases of growth may be compensated by high leaf longevity in order to maximize the CO\textsubscript{2} assimilation through leaf lifespan. After 10 months of growth, the production of leaves is more active and, consequently, the number of standing leaves and the leaf turnover rate increase (Fig. 1A, B; Table 1). Thus, in the long term, the plants respond with an increase in the proportion of young leaves with a high photosynthetic rate and, probably, with a reduction in the cost associated with the maintenance of older leaves.

Differences in the stationary age structure of a population of leaves grown for 3 weeks or 10 months suggest that while the leaf production rate in the plant is not stabilized, growth period and age of the plants need to be considered when comparing leaf demographic results. Similarly, Kikuzawa et al. (2008) distinguished two phases during plant canopy development, an initial expanding phase that is characterized by an increase in new leaf production without loss of older leaves and, eventually, a phase where older leaves begin to senesce so that dynamic stability is reached when $L_p$ and $L_d$ are balanced and the number of leaves remains constant.

**Calcium effects**

*Leaf composition.* N and Ca are key nutrients that influence a number of important leaf traits, and plants vary in the use and allocation of these nutrients (Loneragan et al., 1968; Poovaiah and Leopold, 1973; Horst et al., 1992; McLaughlin and Wimmer, 1999). While N is highly mobile in the phloem and is remobilized throughout the life of an individual leaf, once Ca is deposited in organs such as the leaves, it is redistributed with difficulty or not at all and hence has been regarded as ‘phloem immobile’ (Hocking, 1980; Jeschke and Pate, 1991). Plants growing under low-Ca conditions have small leaves with higher LSM. Previous studies also report that the final size of leaves is reduced by...
Ca restriction (Terry and Huston, 1975; Stromme et al., 1994). Lower leaf size may be a consequence of both reduced cell elongation and division, due to restriction of the internal Ca concentration, which affects cell extension in conjunction with auxins and the progression through mitosis (Burstrom, 1968; Marschner and Richter, 1974; Hertel, 1983; Hepler and Wayne, 1985; Gislerød, 1999). Additionally, high LSM in low-Ca plants may be related to carbohydrate accumulation within the leaf as a reduction in foliar Ca tends to diminish photosynthate translocation from foliage (Gossett et al., 1977). Chl(a+b) content per unit of mass was 20% lower in low-Ca plants compared with control. This agrees with the fact that low Ca in the external solution decreases chlorophyll accumulation (Swamy and Suguma, 1992; Ramalho et al., 1995; Reiss and Beale, 1995; Milivojevic and Stojanovic, 2003) and causes damage of chloroplast lamellae (Repka et al., 1971). However, the difference in Chl(a+b) content between treatments disappears when it is expressed per leaf area. The absence of differences in Chl(a+b) content per leaf area between both treatments is associated with the Ca effects on leaf size. In agreement with this, Terry and Huston (1975) found in Beta vulgaris that Chl(a+b) content per leaf area decreases only slightly in plants with Ca deficiency as a consequence of a limited leaf expansion, while chlorophyll synthesis is maintained at high rates.

The data suggest that the strategy of I. pes-caprae growing under low-Ca conditions is to maintain an adequate Ca concentration per leaf area through the reduction in leaf expansion. Ca is accumulated with leaf ageing, confirming the fact that the mobility of Ca is low and there is limited remobilization associated with leaf ageing (Stebbins and Dewey, 1972; Hepler and Wayne, 1985). Similarly, leaf Ca concentrations increased with leaf age in I. pes-caprae, indicating the net import of this macronutrient (Ripley, 2001). By contrast, in both treatments applied, leaf N...
content per leaf mass decreased with leaf age. A reduction in N content with leaf age has commonly been found in other species as well (Field and Mooney, 1986; Sobrado, 1992; Kitajima et al., 1997). In both treatments, at least 50% of the N present in young leaves was translocated, but N resorption was 25% higher in control plants. In this study, the N concentration in the nutrient solution was very similar in the two treatments, suggesting that other factors different from soil nutrient contents determine the comparatively lower N resorption in plants growing with a low Ca supply.

Leaf demography. In addition to the effects of N and Ca on leaf traits, both nutrients may influence total leaf area and leaf demography (Millard and Proe, 1991; Ramalho et al., 1995). The deficiency of Ca produced a reduction in $L_p$ and an increase in leaf turnover rate compared with control plants, leading to a reduction in the total leaf area. In addition, in plants of Ipomoea pes-caprae with a restrained Ca supply in the nutrient solution, the leaf senescence was accelerated. Similarly, Ramalho et al. (1995) found in Coffea arabica that Ca deficiency negatively affects the total leaf area and duration. Ca has a role in delaying plant senescence, deferring the peroxidation of membrane lipids and the net degradation of proteins and chlorophyll (Marinos, 1962; Poovaiah and Leopold, 1973; Chou and Kao, 1992; Ramalho et al., 1995; McLaughlin and Wimmer, 1999). Several authors have suggested that ageing represents a redistribution of resources rather than actual deterioration (Field and Mooney, 1986; Kitajima et al., 1997). However, in leaves of Ipomoea pes-caprae growing in low-Ca conditions, Ca showed limited remobilization associated with leaf ageing, while Chl$_{a+b}$ and N content per unit of mass decreased compared with control. Thus, low Ca in the nutrient solution may be producing an actual deterioration instead of a redistribution of resources. In consequence, the timing of leaf shedding may change depending on nutrient mobility and/or physiological effects on the leaf senescence processes.

Changes in $L_p$, $L_d$, and leaf longevity are determinants of the leaf age population dynamics and structure affecting the flux of leaves into and out of a standing population (Nilsen et al., 1987; Reich et al., 2004). How many leaves of each age are living in the plant and a diagram of leaf age structure allow it to be determined if the leaf population will grow, decline, or experience no noticeable change in numbers. In spite of the differences in the total number of leaves per plant between treatments, both control and low-Ca plants have a leaf age structure with a pyramidal shape, indicating an increase in leaf population over time. However, it is important to note that this supposition is based on the structure of leaf age in whole plants growing for a period of 70 d. It is possible that the leaf population in plants of either treatment changes in their age structure in the long term because the leaf survival probability curve as well as the number of leaves that it is possible to maintain in a plant are finite and, with the passage of time, the leaf production rate tends to decrease and the number of leaves stabilizes (Kikuzawa et al., 2008). Additionally, in low-Ca plants, the diagram shows the mean percentage of fully expanded young leaves to be slightly higher than that of newer leaves (Fig. 2D). This suggests that, over time, low-Ca plants may experiment a progressive reduction in the total number of leaves per plant.

Finally, the leaf survival probability curve of Ipomoea pes-caprae was modelled by a sigmoid pattern (Fig. 2B) very rarely reported in plant species. Reich et al. (2004) also found in some tropical trees that the mortality risk decelerated at older leaf ages compared with middle-aged leaves. A mortality curve with a sigmoid pattern has been observed in animal populations and is an intrinsic property of time-to-event traits that are affected by genetic and environmental factors (Fox and Moya-Laraño, 2003). By contrast, in plant species, survivorship curves are mostly of Type I, where the number of survivors declines exponentially throughout, or of Type II, with constant leaf mortality at all ages (Deevey, 1947; Bazzaz and Harper, 1977; Williams-Linera, 2000).

Gas exchange and nutrients. No relationship was found between Ca supply in the nutrient solution and maximum photosynthetic rate ($A_{\text{max}}$). Similarly, in B. vulgaris and Zea mays, Ca deficiency diminishes photosynthetic CO$_2$ uptake by only 10–15%, suggesting that, in general, the Ca requirement of higher plants for metabolic activities may be relatively small (Repka et al., 1971; Terry and Huston, 1975). Values of $A_{\text{max}}$ in this study are similar to those reported by Ripley (2001) in Ipomoea pes-caprae.

In this species, $A_{\text{max}}$ showed a typical pattern in relation to the development stage of the leaves, first increasing when leaves reach their physiological maxima and then decreasing with senescence. A similar pattern has been found in many species (Thomas and Stoddart, 1980; Šesták et al., 1985; Shirke, 2001). Low $A_{\text{max}}$ in newly unfolded leaves is associated with high dark respiration rate and low g$_{st}$, pigment content, and amount of photosynthetic enzyme (Shirke, 2001). In contrast, a decrease in $A_{\text{max}}$ in senescent leaves is associated with a decrease in stomatal and mesophyll conductance, chlorophyll content, and enzyme activity (Thomas and Stoddart, 1980; Šesták et al., 1985; Hidema et al., 1991; Loreto et al., 1994; Shirke, 2001).

The decline in $A_{\text{max}}$ with leaf senescence was rather sudden in control plants compared with plants growing with low Ca supply. The values of these negative slopes are similar to those reported in two tropical tree species with mean leaf longevity between 74 d and 94 d (−0.02 and −0.25 μmol m$^{-2}$ s$^{-1}$ d$^{-1}$, respectively; Kitajima et al., 2002). In control plants, a sudden reduction in $A_{\text{max}}$ from fully expanded young leaves to the last measured leaf age suggests that, even if the leaves continued to live longer, they would not contribute to the growth of the plant, supporting the hypothesis that the leaves will be shed at the time when the maximum lifetime carbon balance is attained (Field and Mooney, 1986; Kikuzawa, 1991; Mediavilla and Escudero, 2003). In addition, the linearity of the decline in $A_{\text{max}}$ with leaf age is more pronounced in species with
continuous leaf production and when it is caused primarily by resource redistribution to newer leaves (Kitajima et al., 1997; Ackerly, 1999). In fact, both $L_p$ and N resorption were higher in control plants compared with low-Ca plants. In low-Ca conditions, $A_{\text{max}}$ continued to be relatively high during the leaf lifespan even though leaf longevity was shorter compared with control plants. Contrarily, Kikuzawa (1991) predicted that the negative slopes of the regression of photosynthetic capacity against leaf age ($a_{\text{bl}}$) in the model would be steeper for species with shorter leaf longevities. However, the decline in photosynthetic rate with leaf age generally is a consequence of self-shading (Hidema et al., 1991; Ackerly, 1999; Hikosaka, 2005; Kikuzawa et al., 2008), but in *Ipomoea pes-caprae*, shading is small due to its extended growth pattern.

Following the model of Kikuzawa (1991), the estimated $x$ intercept in the linear regression analysis of the $A_{\text{max}}$–leaf age relationship is an extrapolation of the leaf age at which photosynthetic capacity would reach 0 and approximate the mean leaf longevity. In *Ipomoea pes-caprae*, the leaf longevities estimated using the $x$ intercepts were 7% and 71% higher than those obtained with the leaf survival curve. Similarly, in different tropical tree species, $x$ intercepts were greater than actual leaf longevities (Kikuzawa, 1991; Kitajima et al., 1997, 2002). Larger discrepancies between the $x$ intercepts and the actual leaf longevities in low-Ca plants compared with control plants may be caused by a non-linear decline in photosynthetic capacity with leaf age. In addition, after reaching $t_{50}$, it is only possible to measure from leaves that are still alive, while those that exhibit a faster physiological decline and die early are not represented; consequently, $A_{\text{max}}$ is overestimated as the leaf classes become older, concealing what takes place during the final senescence phase and the $x$ intercepts (Dungan et al., 2003).

Stomatal conductance ($g_s$) tends to decrease with leaf ageing and was always lower in controls than in low-Ca plants (Table 2). In fact, Ca plays a role in the control of leaf gas exchange due to its regulation of stomatal closure (McLaughlin and Wimmer, 1999; Song et al., 2008). Young et al. (2006) showed that CO$_2$-induced stomatal movements are attenuated when cytosolic Ca$^{2+}$ concentration is experimentally repressed. Then, a lower leaf Ca$^{2+}$ concentration per mass unit in low-Ca plants compared with control plants may affect $g_s$ responses. Values of $g_s$ in fully expanded leaves are similar to those reported for different species growing in coastal dunes and show the same tendency along leaf life (Pavlik, 1983; Ripley, 2001). In general, $E$ was higher throughout the leaf lifespan in plants under low-Ca conditions than in controls. The Ca flux to the xylem is influenced markedly by transpiration, which could produce changes in the amount of Ca supplied to the shoot and lead to plant development disorders (Stebbins and Dewey, 1972; McLaughlin and Wimmer, 1999; Song et al., 2008). Then, a higher $E$ during the leaf lifespan in low-Ca plants compared with control plants may be related to the need to increase leaf Ca concentration.

In both treatments, after leaf expansion, WUE decreases with leaf ageing (Fig. 3B). Lower WUE is often observed during leaf senescence and is a consequence of a more sudden decrease in $A_{\text{max}}$ with leaf ageing than that of $g_s$ (Sobrado, 1992; Escudero and Mediavilla, 2003). In leaves older than 35 d, WUE was always lower in low-Ca plants than in control plants, implying that short-term water efficiency was reduced. In spite of a high $A_{\text{max}}$ at shedding, in plants under low Ca, a low WUE during the leaf lifespan may increase the cost of maintaining old leaves in terms of water balance and, consequently, leaves should be discarded as soon as their WUE declines below the mean water efficiency of the canopy (Nelson et al., 2002; Munne-Bosch and Alegre, 2004).

*Ipomoea pes-caprae* photosynthetic capacity and leaf N content are highest at full expansion and thereafter decline linearly with leaf age. Many studies have found that the decrease in photosynthetic capacity with leaf ageing is paralleled by decreases in leaf N content (Thomas and Stoddart, 1980; Lajtha and Whitford, 1989; Sobrado, 1992; Kitajima et al., 1997; Escudero and Mediavilla, 2003). Parallel changes in photosynthetic capacity and N content with leaf ageing take place because photosynthetic proteins are degraded progressively through senescence and the nitrogen compounds are translocated to other organs, leading to a reduction in the leaf N content and, consequently, in $A_{\text{max}}$ (Makino et al., 1984; Hidema et al., 1991; Hikosaka, 1996). PNUE in leaves 7–21 d old was 25% lower than in completely expanded mature leaves. This may indicate a different N partition within photosynthetic proteins (thylakoid versus carboxylase enzymes) and/or mesophyll conductance changes during leaf lifespan (Makino et al., 1984; Sobrado, 1992; Loreto et al., 1994; Hikosaka, 1996; Marchi et al., 2008).

**Maximum carbon assimilation in whole plants.** A decrease in total leaf area negatively affects the carbon gain at the whole-plant level if the photosynthetic rate is not enough to compensate for the reduced leaf area per plant. In this study, plants growing under low Ca conditions had a lower number of leaves per plant and smaller leaf size, but the maximal initial $A_{\text{max}}$ of fully expanded young leaves was not significantly affected by the low Ca supply compared with control plants. Consequently, it is possible to assume that the maximum carbon assimilation in whole plants is significantly reduced in low-Ca conditions compared with control plants. However, it is necessary to consider the effect of leaf age on photosynthetic capacity and age structure of the population of leaves in order to estimate the actual total carbon gain of the whole foliage at a particular moment (Kitajima et al., 1997, 2002; Koyoma and Kikuzawa, 2009). In *Ipomoea pes-caprae* growing under low Ca conditions, $A_{\text{max}}$ at shedding represents 72% of that of young leaves, but leaf lifespan was shorter than in control plants. Additionally, there were no significant changes in the age structure of the leaf population between treatments. Consequently, the amount of carbon gain in the whole plant...
is still low compared with control plants and was positively correlated with total leaf area (Fig. 5B, inset). Thus, it is established that leaf area in the canopy increases with \( L_p \), which is proportional to the rate of photosynthesis in the whole plant (Ackerly, 1999; Koyoma and Kikuzawa, 2009).

Conclusions

Leaf turnover rate and longevity are affected by the growth period, indicating that changes in the development state and/or source-sink relations may affect leaf demographic parameters. A low Ca supply in the nutrient solution has only a small negative effect on the maximal initial \( A_{\text{max}} \) of young leaves but affects negatively the leaf production rate and longevity. It is not clear if Ca deficiency may shorten leaf lifespan by accelerating leaf senescence physiological processes or because the advantage of an increased leaf longevity disappears due to the limited redistribution of Ca. In spite of the changes in leaf demography as a consequence of a low Ca supply, the age structure of the leaf population reaches a similar pattern to that of control plants in a way that the number of leaves per plant continues to increase and plant survival is likely. In I. pes-caprae, the lower number of leaves in all leaf age classes was the most important characteristic that determined the reduction in the maximum \( \text{CO}_2 \) assimilated per plant in low-Ca conditions compared with control plants. Finally, this study highlights the significance of simultaneously using the total leaf demographic census and the assimilation rate along with leaf lifespan data in order to understand the performance of whole plants under constrained conditions. However, more studies are required to evaluate the possible changes in \( A_{\text{max}} \) with leaf age and leaf demography associated with non-mobile nutrient limitation and with development phases.

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