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Gas Exchange and Leaf Specific Mass of Different Foliar Cohorts of the Wintergreen Shrub *Aristotelia chilensis* (Mol.) Stuntz (Eleocarpaceae) Fifteen Days Before the Flowering and the Fall of the Old Cohort

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ABSTRACT

In the beginning of the spring Aristotelia chilensis *sheds the old cohort, forms a new one and flowers. Fifteen days before the flowering and the fall we measured net photosynthesis (A), transpiration (E), water use efficiency (WUE), specific leaf mass (SLM) and area and mass of leaves per branch in old and new cohorts. Under low irradiance the old cohort exhibited higher mean values of A and WUE on area bases ($P<0.05$). Under high irradiance A and WUE on mass bases were higher in new cohort due to its lower SLM (42.10 gm^{-2}) in relation to old cohort (79.53 gm^{-2}). Because old cohort showed 3.7 times more dry mass per branch it was able to counterbalance its lower performance on mass bases. It was concluded that old cohort was an important source of carbon during two powerful sink processes in the beginning of the spring: new leaves cohort construction and flowering.*

Key words: *Aristotelia chilensis*, foliar cohorts, photosynthesis, specific leaf mass

INTRODUCTION

The leaves of some temperate species persist through one winter until the emergence of the new leaves cohorts. These species exhibit a wintergreen phenology (*sensu* Bell & Blis, 1977). The conservancy of the old leaves during the unfavorable period was related to nutrient resorption before the leaves abscission and increase of the seasonal or the life-time carbon gain (Chabot & Hicks, 1982; Jonasson, 1995). However, at least in orchid *Tipularia discolor*, the wintergreen phenology is also related to light availability. In this species, the single leaf emerges in autumn when above canopy of the deciduous forest is open (October-November) and shows quickly its maximum seasonal net assimilation (8.5 µmol $CO_2.m^{-2}.s^{-1}$ in November) (Tissue *et al.*,1995). During March, the single leaf of *T. discolor* fixed only 3.3 µmol . $m^{-2}.s^{-1}$ and finally it senesces during late April immediately following the forest canopy closure.

Notwithstanding, the overwintering leaves of the wintergreen *Cornus canadensis* did not show any photosynthetic activity before the leaf-on in early spring (April), probably due to injuries during the winter. Nevertheless, this species was able to maintain low rates of net assimilation under low

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temperature long into the fall even after the first frost (Landhäusser *et al.*, 1997). Adaptation in this species was more likely related to their ability to function at low temperature. Beyond overcoming the first frost as in *C. canadensis*, the wintergreen fern *Polystichum acrostichoides* exhibited the capacity for positive net photosynthesis even during moderate winter days (Minoletti & Boener, 1993).

Nutrient resorption, expansion of life-time carbon assimilation (*e.g.* net photosynthesis during moderate winter days), leaf flushing and rapid development when above canopy is leaf-off are some features that enable wintergreen species in North hemisphere to acquire more resources for completing successfully its life cycle. However, nothing is known about the ecophysiology of the Andean wintergreen species *Aristotelia chilensis*.

Aristotelia chilensis (Mol.) Stuntz (Eleocarpaceae) is a temperate wintergreen shrub of the Andean-patagonic territory of Argentina that grows in open areas or under forest canopy (Damascos,1998) in regions with medium (1500 mm) or high (1800-3000 mm) precipitations (Dimitri,1972).

Wintergreen phenology in *A. chilensis* could be an event depending on carbon source from old cohort. This dependency probably occurs because the construction of new cohort and the flowering are strong sink processes and both occur in the beginning of the spring when overwintering cohort still persists. We tested this hypothesis comparing the gas exchange, the specific leaf mass and the leaf area per branch between the new and old cohorts of *A. chilensis* during the beginning of the spring under field conditions.

MATERIALS AND METHODS

The individuals of *A. chilensis* present under the forest canopy of the dominant evergreen species *Nothofagus dombeyi* (Fagaceae) were studied in a preserved area at the city of San Carlos de Bariloche, Argentina (41° 04' S-71° 08' W, 800 m a.s.l.). The annual mean precipitation in the study area is between 2000-2220 mm and annual mean temperature is 11°C (Barros *et al.*, 1983). The rainy period embraces autumn and winter and the growth season coincides with the dry period (spring and summer).

The leaf bud of the shrub *A. chilensis* opens in late September and the leaves are almost totally expanded during the beginning of November. The fall of the old leaves occurs during late November. Flowers are produced between late October and the beginning of November and the fruits mature during early December (Damascos, unpublished data).

Net photosynthesis (A, μmol.m^{-2}.s^{-1}), transpiration (E, mmol.m^{-2}.s^{-1}) and the CO_2 intercelular concentration (Ci, ppm) were measured in the beginning of the growing season (November, 5 and 7, spring of 1998) using a portable infra red gas analyzer Li-cor 6200, connected to a leaf chamber that comprises ¼ lt. and 22.1 cm^2. The measurements of leaf gas exchange were carried out under field conditions in two days with different photosynthetic photon flux density (PPFD) which was measured directly by a sensor of quanta located on the leaf chamber. These measurements were performed in cloudy (low irradiance) and cloudless (high irradiance) days.

Gas exchange were determined on 4-5 old and 4-5 new leaves from old and new cohorts respectively on two plants at each day when the first direct solar irradiance achieved the leaves of *A. chilensis* passing through the canopy of *Nothofagus dombeyi* (between 10:30-12:30h). Only leaves without signs of predation or infection were investigated. For each record the leaf was kept for 20 seconds in the chamber for decay of CO_2 and increase of water vapor. Each adjusted slope that represented the decrease of CO_2 or the increase of the water vapor was used directly by the logger of Li-cor 6200 for calculating of A, E and Ci values of leaf gas exchange. The values expressed on mass bases were obtained by dividing the values of gas exchange results on area bases by the mean values of the specific leaf mass (SLM, g.m^{-2}).

Five plants were randomly selected during autumn (April/98) and in spring (November/98) for determining the SLM and the degree of senescence in different cohorts. Seventy autumn leaves were selected (senescing-achlorotic leaves per plant) by shaking the plant and collecting the fallen leaves along with 70 old leaves per plant (green leaves) that persisted on the branches after shaking. As spring leaves 70 old leaves per plant were selected that emerged in anterior spring in November 1997 and 70 new leaves per plant that emerged during November 1998. The SLM was obtained detaching one foliar disc per leaf (avoiding the major veins) performing five groups of 70 discs per cohort, each one from different plants prior selected. The discs were dried up under 60°C till constant weight. Five groups of 70 discs per cohort were weighted separately and the mean value per cohort was extracted.

During the middle of November 1998, the length of 150 old and new leaves from five different plants previously selected were measured for comparing the degree of expansion of the new cohort (emerged in November 1998) before the fall of the old cohort (produced in November 1997). All leaves from five branches with 60-65 cm of length were collected from five plants previously selected in order to determine the weight and estimate the area of the new and old cohorts per branch immediately before the fall of the old cohort. The leaves were dried up till constant weight and the value of the total area per branch was estimated utilizing the SLM previously determined.

The mean values of A, E and WUE from old and new cohorts of leaves were compared using a Student t test (Sokal & Rohlf, 1981). The same test was applied for the following three comparisons: between mean values of SLM obtained in autumn and in spring from old leaves, between mean values of SLM obtained in spring from old and new leaves and between mean values of SLM obtained in autumn from senescent and old leaves. The autumn SLM of the senescing and old leaves of each plant, the area of spring old and new leaves by branch and the weight of these foliar types were subjected to the two-population (paired) t-test (Sokal & Rohlf, 1981).

RESULTS

The mean values of irradiance in cloudy and in cloudless days were essentially the same for both cohorts studied (Table 1). Table 2 indicates the average values of intercellular concentration of CO_2 (Ci) from old and new cohorts. The Ci average values in both cohorts were in agreement with the available irradiance for photosynthetic process: high mean values under low irradiance (around 330 ppm) and low mean values under high irradiance (around 180 ppm). We found only one significant difference in Ci average values between new and old cohorts under low irradiance (higher values for new cohort).

Table 3 shows the mean values of the morning gas exchange measurements on area bases obtained in old and new cohorts in the beginning of the spring. Old cohort showed higher or at least equal mean values than new cohort in relation to A, E or WUE under low or high irradiance. Conversely, when A, E and WUE were expressed on mass bases (Table 4), the new cohort showed higher or at least equal mean values than old cohort.

Table 1 - Mean and range of the Photosynthetic Photon Flux Density values (PPFD, low and high irradiances) on new and old cohorts during the gas exchange measurements in the beginning of the spring. Means followed by the same letters indicate absence of significant statistical difference in one way ANOVA ($P<0.05$, n=9)

PPFD (μmol m^{-2} s^{-1})	**Old leaves**	**New leaves**
Low irradiance	79 a	63 a
	32 – 127	44 – 77
High irradiance	581 a	603 a
	989 – 2063	669 – 2045

Table 2 - CO_2 intercellular concentration (Ci) and leaf temperature (T) of the old and new cohorts under low (LI) and high (HI) irradiances during the morning in the beginning of spring. Data are presented as mean ± standard deviation. Means followed by same letters indicate absence of significant statistical difference in one way ANOVA ($P<0.05$, n=9)

	PPFD*	**Old leaves**	**New leaves**
Ci	LI	328±13 a	343±14 b
(ppm)	HI	190±32 a	178±19 a
T	LI	12.4±0.18 a	12.6±0.51 a
(°C)	HI	21.2 ± 3.92 a	21.8±1.95 a

[*] PPFD: Photosynthetic Photon Flux Density (see Table 1)

Table 3 - Mean values of leaf gas exchange expressed on area bases (m^2) for the new and old cohorts of *Aristotelia chilensis* measured under low (LI) and high (HI) irradiance during the morning in the beginning of the spring. Net photosynthesis (A, μmol.m^{-2}.s^{-1}); transpiration (E, mmol m^{-2} s^{-1}) and water use efficiency (WUE, μmol CO_2/mmol H_2O). Data are presented as mean ± standard deviation . Means followed by same letters indicate absence of significant statistical difference in one way ANOVA ($P<0.05$, n=9)

	PPFD*	Old Cohort	New Cohort
A	LI	2.99±0.83a	2.00±0.50b
	HI	8.42±1.90a	9.53±1.90a
E	LI	1.34±0.04a	1.37±0.19a
	HI	1.95±0.28a	2.11±0.31a
WUE	LI	2.23±0.64a	1.50±0.47b
	HI	4.84±1.50a	4.55±0.31^{a}

[*] PPFD: Photosynthetic Photon Flux Density (see Table 1)

Table 4 - Mean values of leaf gas exchange expressed on mass bases (kg) for the new and old cohorts of *Aristotelia chilensis* measured under low (LI) and high (HI) irradiance during the morning in the beginning of the spring. Net photosynthesis (A, μmol $kg^{-1}s^{-1}$), transpiration (E, mmol $kg^{-1}s^{-1}$) and water use efficiency (WUE, μmol CO_2/mmol H_2O). Data are presented as mean ± standard deviation. Means followed by same letters indicate absence of significant statistical difference in one way ANOVA (P<0.05, n=9)

	PPFD*	Old leaves	New leaves
A	LI	37.10± 0.36 a	43.58±10.92a
	HI	104.54±15.87a	207.93±26.7b
E	LI	16.69±1.66 a	29.79±4.10 b
	HI	23.46±10.09 a	46.03±6.76 b
WUE	LI	27.75±7.96 a	32.79±10.25a
	HI	60.11±18.65 a	99.25±10.6b

[*] PPFD: Photosynthetic Photon Flux Density (see Table 1)

Table 5 shows the foliar dry weight and the total relative area of the old and the new cohorts per branch of *A. chilensis* in the beginning of the spring at fifty days before the total fall of the old cohort. The area per branch was equivalent between the new and old cohort but the last one performed 2.7 times more mass per branch.

Table 5 - Foliar dry weight and the total relative area of the old and the new cohorts per branch of *Aristotelia chilensis* in the beginning of the spring at fifty days before the total fall of the old cohort. Data are presented as mean ± standard deviation and the range of the values (minimum-maximum). Means followed by same letters indicate absence of significant statistical difference in one way ANOVA (P<0.05, n=10)

	Leaves	
	Old	New
Dry Weight (g)	6.82 ± 2.39 a 3.60 - 10.20	1.82 ± 0.99 b 0.90 - 3.10
Area by branch (cm^2)	809.94 ± 236.57a 427.54- 1211.35	432.29 ± 236.58a 213.77 - 285.02

Punctual values of A, E and WUE from Tables 3 and 4 as a function of photosynthetic photon flux density values (PPFD) from Table 1 are shown in Figure 1.

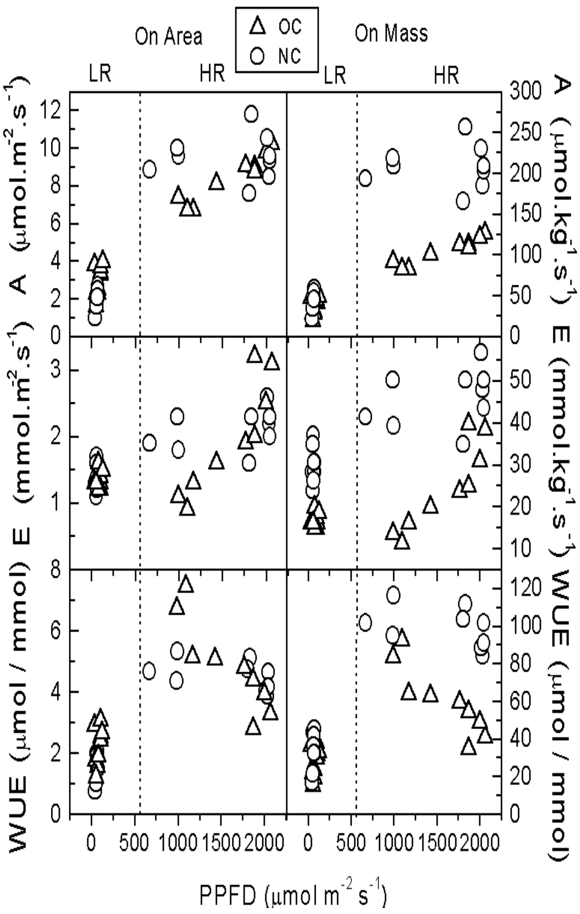

Figure 1 - Values of leaf gas exchange as a function of photosynthetic photon flux density (PPFD) in new (NC, O) and old cohorts (OC, Δ) of *Aristotelia chilensis* in the beginning of the spring and measured under low (LR) and high (HR) irradiance. The values are expressed on area (m^2, left) and on mass bases (kg, right). From the top to the bottom: net photosynthesis (A); transpiration (E) and water use efficiency (WUE, A/E).

All values of A under low irradiance were over the light compensation point and under high irradiance they showed the saturation tendency. Values from new cohort and old cohorts appeared more clearly separated on mass bases. Fig. 2 shows that the SLM accompanied the leaf development of *A.chilensis*. It increased toward leaf maturity and decreased toward leaf senescence. There was not significant difference (t, P <0.05) in relation to the length of the old and the new cohorts in the beginning of the spring (Fig. 2).

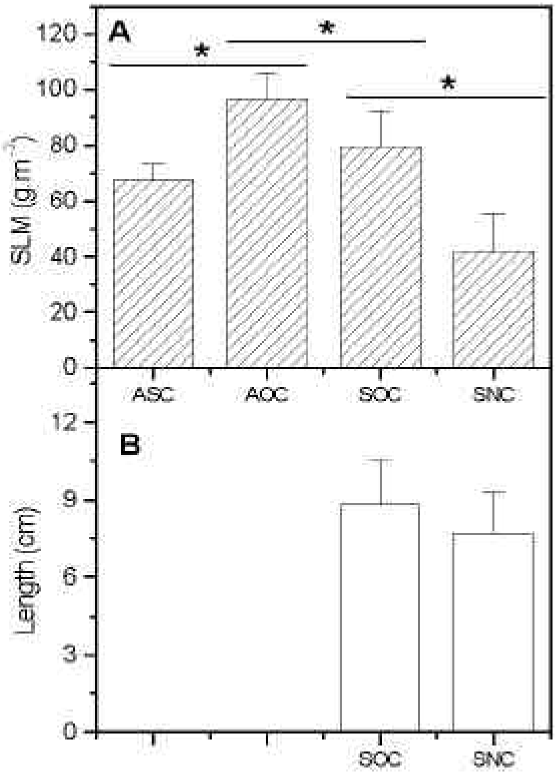

Figure 2 - Mean values (bars) and standard deviation (vertical lines on the top of the bars) of specific leaf mass (SLM, A) and the length of the leaves (Length, B) from old and new cohorts in autumn and in spring: autumn senescent cohort (ASC); autumn old cohort (AOC); spring old cohort (SOC) and spring new cohort (SNC). Asterisks (*) indicate statistical differences in one way ANOVA between two foliar cohorts at $P<0.05$, n= 5.

DISCUSSION

The results of leaf gas exchange under high and low irradiance confirmed an important photosynthetic activity and water use in old cohort produced during the previous spring and preserved during winter till the flowering and the complete expansion of the new cohort. It was clear why the average values of A and WUE were greater in new cohort only on mass bases and at high irradiance (Table 4). The WUE values indicated that old cohort did not represent one higher demand of water resource to photosynthetic process during spring, which was not only the beginning of the growth season but also was the beginning of the dry period. The higher mean values of A and WUE obtained under low irradiance (Table 3) in old cohort revealed its adaptation in relation to microclimate conditions, considering that the data was taken in a population of *A. chilensis* growing under a forest canopy and that old cohort was overlapped by new cohort as it occurs in most plants (Harper 1989). Equal or smaller average values of intercellular CO_2 concentration (Ci, Table 2) in old cohort indicated the good performance of its enzymatic photosynthetic apparatus, even near the beginning of its senescence period (November 15-30). The mean values of A in old cohort on mass and area bases under high irradiance (8.4 μmol m^{-2} s^{-1} and 104 μmol.kg^{-1}. s^{-1}) were similar to the average values of photosynthetic capacity of the deciduous woody species (8.9 μmol.m^{-2}.s^{-1} and 78 μmol.kg^{-1}.s^{-1}, Prado & Moraes, 1997). Besides the values of gas exchange, the importance of the old cohort as significant source of carbon was evident from values of its area per branch (equivalent to new cohort) or from its mass per branch (3.7 times greater than new cohort, Table 5). Thus, if the old cohort could not achieve the mean value of A of the new cohort on mass bases under high irradiance, it was counterbalanced by its absolute value of mass per branch.

Leaf senescence has been determined by endogenous and exogenous events (Thomas & Stoddart 1980). However, if leaf aging and fall of the old cohort occurs just in the beginning of the growing season (spring) and simultaneously with the expansion of the new cohort as in *A. chilensis*, this means that senescence in old cohort is conducted strongly by internal events. These leaf life span physiologically determined should be able to initiate and organize the mass transport from the old cohort to the new one in order to change the old cohort from a carbon source at maturity to a mineral source during senescence (Reader, 1978; Thomas & Stoddart, 1980 and Harper, 1989) . This kind of changing is very rapid in *A. chilensis* as the old cohort changes its color from green to yellow in two weeks only. The same processes can get two moths in deciduous species.

Though the new cohort development was not totally completed during the beginning of spring (Fig. 2, lower SLM) it presented high performance of gas exchange mainly under high irradiance, where it showed the average photosynthesis values on area and mass bases (9.53 μmol m^{-2} s^{-1} and 208 μmol kg^{-1} s^{-1}) greater than the mean values from deciduous woody species cited above. The higher average values of A and WUE of the new cohort under high irradiance were conditioned by their lower SLM (42.1 g.m^{-2}) in relation to old cohort (79.5 g.m^{-2}, Figure 2).

From the results it was possible to show that the leaves conservation strategy during one winter (wintergreen phenology) was dependent on the photosynthetic activity of the cohort emerged at the previous spring in *A. chilensis* growing under the evergreen wet forest. This might be placed in terms of a significant carbon source from old cohort to the construction of the new cohort and the flowering in the beginning of the spring. Defoliated plants during autumn and spring before the emergence of the new leaves showed significant smaller percentage of flowers and lower initial growth of the branch in relation to intact plants (Damascos, unpublished data).

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RESUMO

No início da primavera *Aristotelia chilensis* perde as folhas da cohort mais velha, forma uma nova cohort e floresce. Quinze dias antes da floração e perda da cohort velha medimos: fotossíntese líquida (A), transpiração (E), eficiência do uso da água (EUA), massa específica da folha (MEF) e área e massa das folhas por ramo nas cohorts nova e velha. Sob baixa radiação a cohort velha apresentou valores médios maiores de A e EUA expressos em área ($P<0.05$). Sob alta radiação A e EUA expressos em massa foram maiores na cohort mais nova devido à sua baixa MEF (42.10 gm^{-2}) comparada à cohort mais velha (79.53 gm^{-2}). A cohort mais velha exibiu 3.7 vezes mais massa seca por ramo, compensando sua baixa performance expressa em massa. Concluímos que a cohort mais velha é uma importante fonte de carbono durante processos que representam fortes drenos na primavera: a construção de novas folhas

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