JOURNAL OF FOREST SCIENCE, *55*, 2009 (3): 101–111

Photosynthetic response to different light intensities and water status of two main *Nothofagus* species of southern Patagonian forest, Argentina

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ABSTRACT: Net photosynthetic rate (*A*) and stomatal conductance (*gs*) of leaves of *Nothofagus pumilio* and *N. antarctica* seedlings were measured at different photosynthetic photon flux densities (PPFDs, 20, 150, 250, 500, 750, 1,000, 1,500 and 2,200 µmol/m^2/s) and after 60 minutes of shade to determine the rate of photosynthetic induction after seedling transition from low to high PPFD. *A* and *gs* were also measured to evaluate the responses to water stress and the tolerance to waterlogging. *N. pumilio* had higher *A* values than *N. antarctica* for all light intensities measured with the maximum value of 16.8 µmol CO$_2$/m^2/s saturated at 1,000 µmol/m^2/s PPFD. Values of *gs* for both *Nothofagus* species also declined as light intensity decreased. The times required for full induction of *A* were 18 and 27 minutes after the increase of PPFD (full sun) for *N. pumilio* and *N. antarctica*, respectively. There was a negative relationship between *A* and water stress, expressed as pre-dawn leaf water potential (ψ_{lp}), where the reduction in *gs* was the main factor that reduced *A* in both species. Tolerance to waterlogging was related with changes in *A* and *gs* where *N. antarctica* was more tolerant. These photosynthetic results of *Nothofagus* leaves to fluctuating light, water stress and waterlogging could be used to estimate the responses of regeneration to environmental conditions under the dynamics of natural stands, after silvicultural practices and for restoration plans.

Keywords: *Nothofagus*; shade; photosynthesis; water stress; waterlogging

The cool temperate forest of Southern Patagonia (Santa Cruz and Tierra del Fuego provinces, Argentina), dominated by the deciduous species *Nothofagus pumilio* and *N. antarctica*, occurs from 46° to 56°S and ranges in elevation from the sea level to more than 2,000 m a.s.l. Seedlings of these *Nothofagus* species often respond to the creation of canopy gaps from small treefalls but inadequate seedling establishment is associated with insufficient soil moisture (Veblen et al. 1996). Seedling establishment and growth can also be affected when primary forests are managed under silvicultural practices such as aggregated retention, shelterwood system or selective cuts by changing the availability of light intensity and soil water content. Furthermore, National and Provincial Governments of Argentina have been promoting restoration plans with native species in degraded areas of Patagonia. However, there is a lack of knowledge to evaluate the response of these plantations with *N. pumilio* and *N. antarctica* seedlings related to environmental factors such as water and light requirements.

Plants in natural environments can experience frequent fluctuations in irradiance from full sun to deep shade caused by overstorey shading and within canopy shading (Knapp, Smith 1987). When plants experience a change from high to low irradiance, a photosynthesis deactivation process occurs due to a reduction in stomatal conductance (*gs*) (Kirschbaum et al. 1988) and an increase in biochemical limitations (Tinoco-Ojanguren, Pearcy 1993). In plants transient from low to high irradiance there is an increase in photosynthesis rate (*A*) to the maximum *A*, defined as the 'induction phase' of photosynthesis (Sassenrath-Cole, Pearcy 1994), and it dependents on the previous activity status of photosynthetic enzymes and on *gs* (Pearcy et al. 1996). Several authors have linked regeneration growth to light availability in *Nothofagus* forest (Martínez Pastur et al. 1999; Caldentey et al. 2005). However, the physiological adaptability of leaves to a fluctuating light environment, related to the net photosynthesis of *Nothofagus* seedlings has received little attention.

In addition, woody plants strongly vary in their responses to water stress and waterlogging (Sun et al. 1995) by influencing their physiology and morphology. *N. antarctica* grows on poorly drained (wetlands) or on drier eastern sites of the Andes mountain near the Patagonian steppe compared with *N. pumilio*, which grows in better conditions with respect to soil water availability (Veblen et al. 1996). In this context, the soil water status may play an important role in the distribution of these species and seedling establishment after harvesting.

Therefore, an investigation of leaf photosynthetic responses to light and water availability will reveal the potential for abiotic regulation of *Nothofagus* seedlings regeneration and ecology.

In this study, the focus is on *Nothofagus* seedlings where plants experience sunlight fluctuations and different soil water conditions similar to those likely to be experienced in the native forest or in forest under silvicultural practices. The aim was to evaluate the effect of light intensity and the induction state, water stress and tolerance to waterlogging on net photosynthesis and stomatal conductance of individual leaves of the two major *Nothofagus* species in Southern Patagonia.

MATERIALS AND METHODS

Table 1. Characteristics of two-years-old seedlings of *Nothofagus pumilio* and *Nothofagus antarctica*. Standard error of the mean (s.e.m.) is presented in brackets

Species	Mean height (mm)	SLA ($m^2/g \times 10^3$)	Nitrogen (mg/g)
N. pumilio	100 (5)	128 (9)	27.8 (3.2)
N. antarctica	110 (4)	98 (12)	21.2 (2.8)

Two-years-old seedlings of *Nothofagus pumilio* from Cerro Catedral, Bariloche (41°11'S and 71°28'W) and *N. antarctica* from Chapelco, Neuquen (40°13'S and 71°17'W) were grown in the experimental nursery of INTA (National Institute of Agricultural Technology) located in San Carlos de Bariloche (41°08'S and 71°19'W). Seedlings were grown in 4-litre pots containing a mix of peat (50%), coarse sand (10%) and clay-loam soil (40%). The mean monthly temperature during the growing season (September–April) ranged from 6.1°C to 16.2°C with a mean maximum monthly temperature of 23.2°C. The N content of leaves was determined using the Kjeldahl technique after photosynthesis measurements (Table 1). Samples were dried in a forced draft oven at 65°C for 48 hr and ground in a mill containing a 1-mm stainless steel screen. The leaf area and dry weight values of individual leaves were used in the calculation of the specific leaf area (SLA) (Table 1).

Net photosynthesis (*A*, μmol $CO_2/m^2/s$) and stomatal conductance to water vapour (*gs*, mol H_2O per m^2/s) were measured simultaneously using a Li-6400 Portable Photosynthesis System (LiCor, Lincoln, Nebraska, USA). *A* and *gs* were measured on a random sample of the youngest fully expanded intact leaves (leaf age: 20 ± 5 days) located on top of the main stem of each seedling. Measurements were taken only on the sunlit leaves because there are differences in leaf structure, pigment composition and thickness between sun and shade leaves (Lichtenthaler et al. 2007). All measurements were taken at midday ± 1 hour on sunny days during several days in December 2002 and January 2003.

Pre-dawn leaf water potential (ψ_{lp}) was measured on the same day as photosynthesis measurements. Values for ψ_{lp} were obtained from a random sample of the youngest fully expanded leaves with a pressure chamber (Model 1002, PMS Instrument Co., Corvallis, Oregon, USA). Daily courses of air temperature were recorded using a digital thermometer (logging interval 5 minutes, resolution ± 0.2°C) to calculate daily mean air temperature.

Instantaneous photosynthetic and stomatal conductance responses to light intensity

Forty well irrigated whole seedlings of both species were exposed for 60 min to each of eight light intensities: 20, 150, 250, 500, 750, 1,000, 1,500 and 2,200 (full sunlight) $\mu mol/m^2/s$ PPFD before measurements of instantaneous rates of *A* and *gs*. The experiment was arranged in a completely randomized single-seedling plot design, and each treatment (light intensity) contained five plants of each species. Different black shade cloth which covered a 2.3 × 1.8 m area supported horizontally on a vertically adjustable metal frame was used to provide shading. Light intensity was monitored with quantum sensors installed above and below the shade source and recorded every 5 min. All measurements were taken when water (pre-dawn leaf water potential –0.01 to –0.12 MPa) and temperature (18–23°C) were not limiting (Read 1985).

Photosynthesis and *gs* were also measured at 1, 2 and 10 min after the return to full sunlight for seedlings previously exposed to 60 min of shade (95 to 100 μmol/m^2/s PPFD) to calculate the induction state at these times.

Instantaneous photosynthetic and stomatal conductance responses to water status

Twenty plants of each species were selected for measurements of instantaneous *A* and *gs* responses to water stress. The experiment was set up with two water stress treatments in a completely randomized single-seedling plot design, and each treatment had ten plants of each species. The treatments were: (*i*) well watered seedlings where the soil water content was maintained near field capacity throughout the experiment (pre-dawn leaf water potential –0.01 to –0.12 MPa), and (*ii*) seedlings which were left unwatered until negative net photosynthesis values occurred.

Another group of twenty seedlings were totally flooded to evaluate the tolerance to waterlogging in the two species. For this, the pots were placed in fresh tap water containers. The water was replaced daily. An extra group of twenty plants was used as a control by maintaining the soil water content in pots near field capacity. *A* and *gs* measurements were taken at 1, 10 and 20 days since the start of the flooding treatment.

All measurements were taken when the temperature (18–23°C) (Read 1985) was not limiting and in full sun conditions (1,700–2,000 μmol/m^2/s PPFD).

Data analysis

The measured values of *A* in μmol CO$_2$/m^2/s and *gs* in mol H$_2$O/m^2/s were transformed by dividing the observed values by values obtained in non-limiting conditions (before shade and irrigated plants) to give a standardized index value that ranged from 0 to 1. A value of 1 ($A_s = 1$ or $gs_s = 1$) corresponds to the maximum value found for *A* or *gs* in non-limiting conditions.

The induction state of the leaf (*IS*) at any time (IS_t) was calculated (Equation 1) from measurements collected 1, 2 and 10 min after the return to full sunlight for plants previously exposed to 60 min of shade (95–100 μmol/m^2/s PPFD). Data were analyzed as one-way ANOVA with three replicates

$$IS_t = (A_t/A_{ss}) \times 100 = A_s \times 100 \qquad (1)$$

where:

A_t – the assimilation rate at time *t*, measured in minutes from the light increase,

A_{ss} – the steady-state, light saturated assimilation rate after induction is complete ($A_s = 1$ in the present work).

Thus ($A_s \times 100$) can be used to calculate the induction state, which is equivalent to IS_t calculated in Equation (1). IS_1, IS_2, IS_{10} serve as indicators of the stomatal and RuBisCO limitations imposed by the induction requirement (Pearcy et al. 1996) at $t = 1$,

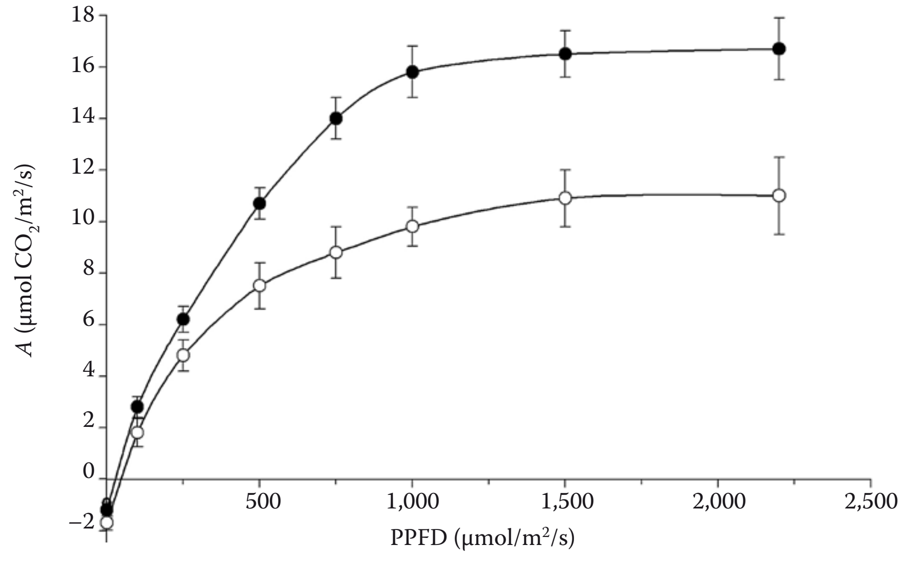

Fig. 1. Net photosynthesis rate (*A*) against different light intensities (photosynthetic photon flux density, PPFD) for *Nothofagus pumilio* (●) and *Nothofagus antarctica* (○) leaves. Bars indicate standard error of the mean (s.e.m.)

2 and 10 min, respectively. Also, the time required to reach 90% of full induction (T_{90}) after 60 min of shade (95–100 µmol/m^2/s PPFD) was calculated for each species as described by Urban et al. (2007).

Overall, 272 photosynthesis and *gs* measurements were taken. Of these: 80 were used to quantify the responses to light intensity, 72 for the responses to water stress, and 120 for the tolerance to waterlogging.

Statistical analyses were carried out using the Genstat statistical package (GENSTAT 5 1997). Standard errors of means (s.e.m.) were used to evaluate least significant differences (lsd) at the 0.05 probability level for mean separation of the studied variables.

Table 2. Induction state (IS) (%) at 1, 2 and 10 min from the light increase (full sun at 2,000–2,200 µmol/m^2/s PPFD) for *Nothofagus pumilio* and *Nothofaus antarctica* seedlings exposed for 60 min to 95–100 µmol/m^2/s PPFD (severe shade)

	IS_1	IS_2	IS_{10}
N. pumilio	55	67	75
N. antarctica	44	52	62
LDS	4.3	1.3	3.5
Significance	***	***	***

*** $p < 0.001$

RESULTS

Net photosynthetic rate (*A*) and stomatal conductance (*gs*) responses to light intensities

Light intensity caused a pronounced effect on the rate of net photosynthesis as a function of PPFD (Fig. 1). *N. pumilio* had higher *A* values than *N. antarctica* for all light intensities measured. The maximum *A* values were 16.8 and 11.1 µmol CO$_2$/m^2/s saturated at 1,000 µmol/m^2/s PPFD for *N. pumilio* and *N. antarctica*, respectively, but as shade increased net photosynthesis decreased. From 1,000 to 20 µmol/m^2/s PPFD, *A* decreased from its maximum value to –0.042 and –0.6 µmol CO$_2$/m^2/s for *N. pumilio* and *N. antarctica*, respectively. The light compensation point for *N. pumilio* was lower (25 µmol/m^2/s PPFD) compared with *N. antarctica* with a mean value of 48 µmol/m^2/s PPFD. The apparent photosynthetic quantum efficiency also showed differences between species being 0.038 and 0.025 µmol CO$_2$/µmol PPFD for *N. pumilio* and *N. antarctica*, respectively.

Values of *gs* for both *Nothofagus* species also declined as light intensity decreased (Fig. 2). The maximum *gs* values were 0.51 and 0.42 mol H$_2$O/m^2/s at high light intensities (1,500–2,200 µmol/m^2/s PPFD) for *N. pumilio* and *N. antarctica*, respectively. From this, the rate of decline was similar between species (0.0002 mol H$_2$O/m^2/s per µmol PPFD/m^2/s).

Fig. 3 shows the relationship between standardized values of net photosynthetic rate (A_s) and stomatal conductance (gs_s) in response to different light intensities. A standardized value of $A_s = 1$ corresponds to A = 16.8 µmol CO$_2$/m^2/s for *N. pumilio* and A = 11.1 µmol CO$_2$/m^2/s for *N. antarctica*, or *A* in non-limiting conditions. A value of $gs_s = 1$ corresponded to the maximum *gs* value of 0.51 mol H$_2$O/m^2/s for

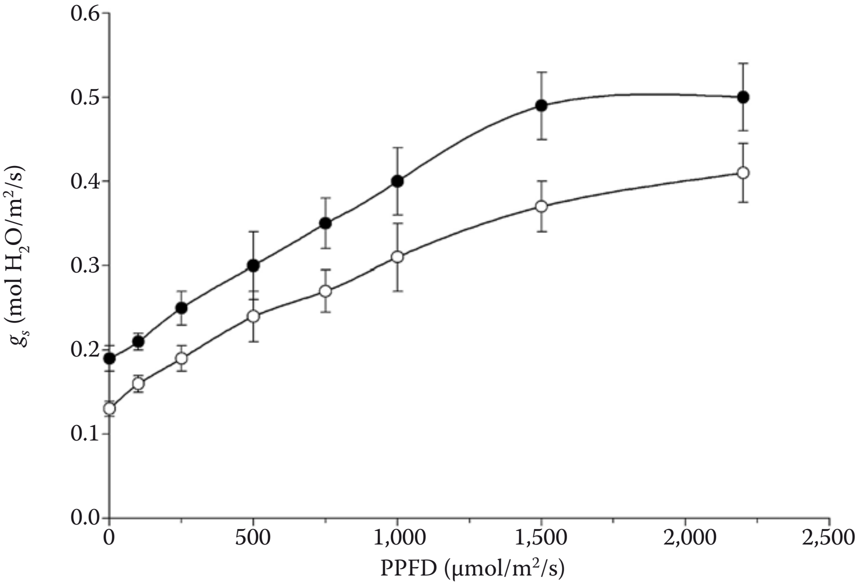

Fig. 2. Stomatal conductance (*gs*) against light intensities (photosynthetic photon flux density, PPFD) for *Nothofagus pumilio* (●) and *Nothofagus antarctica* (○) leaves. Bars indicate standard error of the mean (s.e.m.)

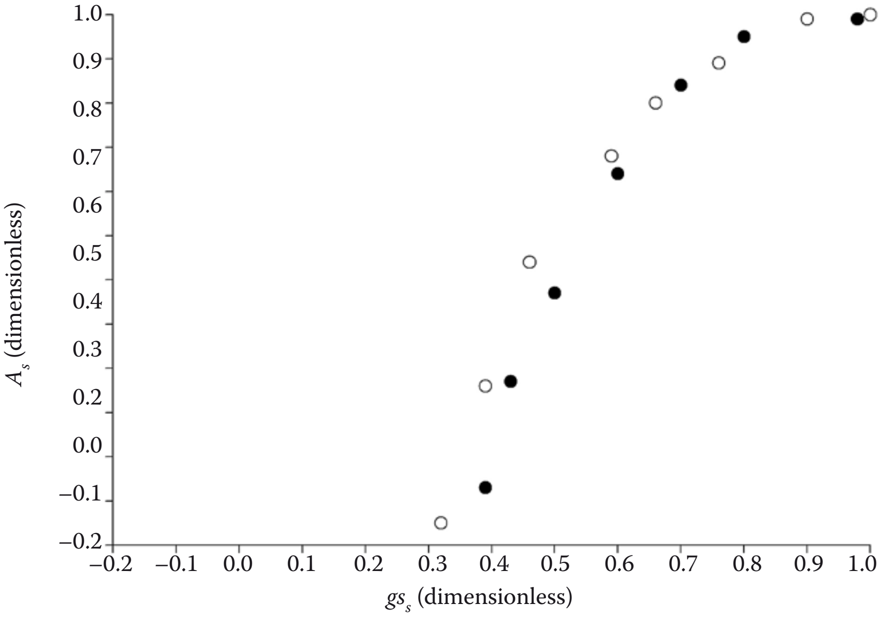

Fig. 3. Relationship between standardized rate of net photosynthesis (A_s) and standardized rate of stomatal conductance (gs_s) for *Nothofagus pumilio* (●) and *Nothofagus antarctica* (○) leaves in response to different light intensities where temperature and water were not limiting

N. pumilio and 0.42 mol $H_2O/m^2/s$ for *N. antarctica* found in non-limiting conditions. The relationship showed a positive relationship between gs_s and A_s against changes in light intensities, but values of gs_s close to 0.3–0.4 were related to negative values of A_s.

Induction of *A* after 60 minutes under shade

There were distinct aspects to the induction process of *A* after 60 min adaptation to low PPFDs between species (Table 2). The IS_1 for *N. pumilio* plants was 20% higher than for *N. antarctica* plants. However, after 10 min the relative differences in IS_{10} decreased between the species studied. The time required to reach full induction ($A_s = 1$) was dependent on the species and the *IS* previously experienced by seedlings. The times required to reach 90% of full induction of *A* (T_{90}) after 60 min of shade (95 to 100 µmol/m²/s PPFD) were 19 and 28 minutes for *N. pumilio* and *N. antarctica*, respectively.

Net photosynthetic rate (*A*) and stomatal conductance (*gs*) responses to water stress

The effect of water stress, expressed as pre-dawn leaf water potential (ψ_{lp}), on *A* was analyzed in the optimum temperature range. The range of ψ_{lp} in

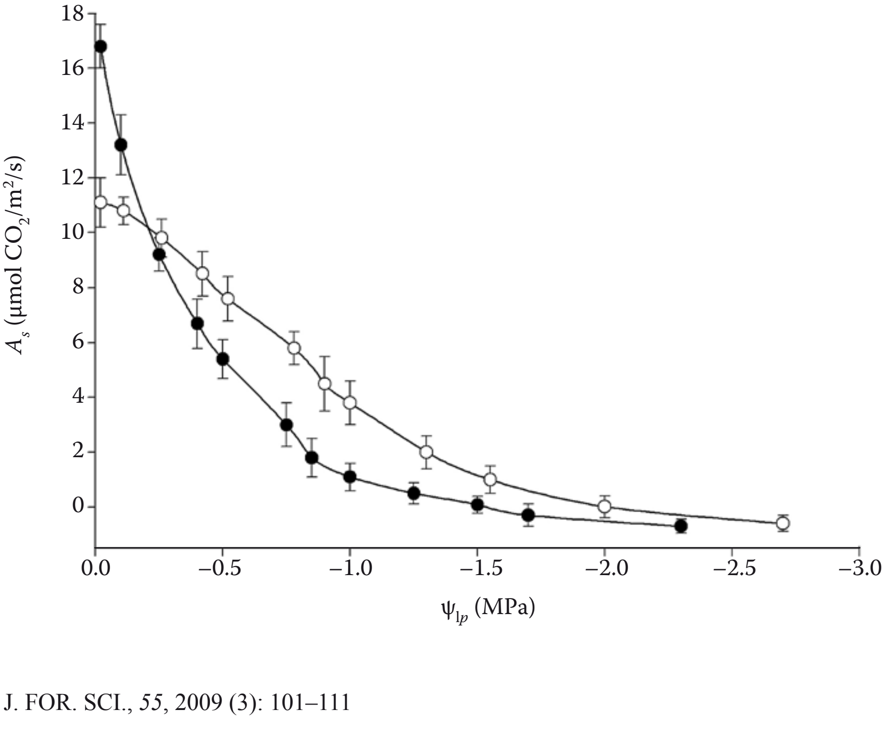

Fig. 4. Response of net photosynthesis rate (*A*) to water stress expressed as pre-dawn leaf water potential (ψ_{lp}) for *Nothofagus pumilio* (●) and *Nothofagus antarctica* (○) leaves where temperature and light were not limiting. Bars indicate standard error of the mean (s.e.m.)

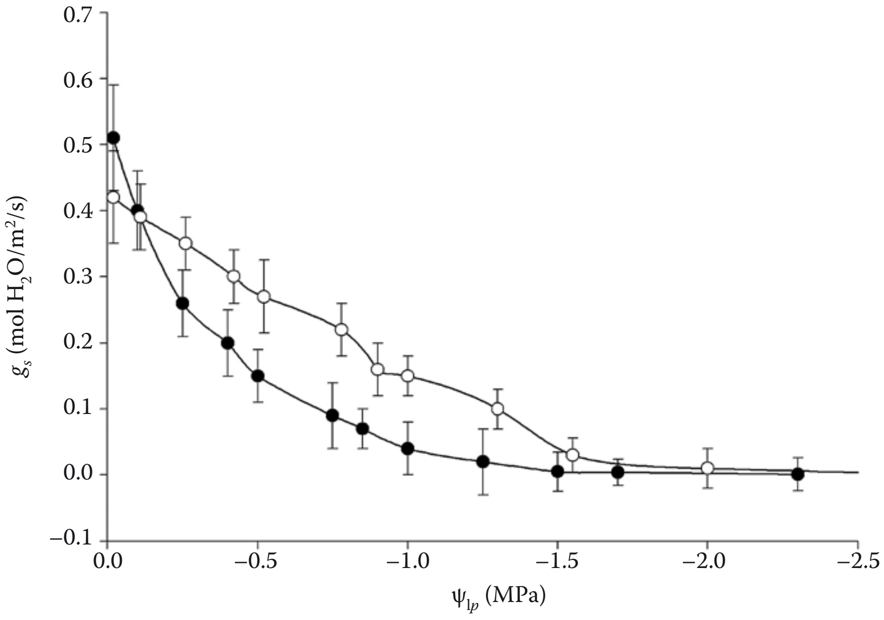

Fig. 5. Response of stomatal conductance (*gs*) to water stress expressed as pre-dawn leaf water potential (ψ_{lp}) for *Nothofagus pumilio* (●) and *Nothofagus antarctica* (○) leaves where temperature and light were not limiting. Bars indicate standard error of the mean (s.e.m.)

the present work was from −0.02 MPa to −2.3 and −2.7 MPa for *N. pumilio* and *N. antarctica*, respectively. There was a negative relationship between *A* and ψ_{lp} (Fig. 4) and between *gs* and ψ_{lp} (Fig. 5). The maximum values of *A* of 16.8 µmol $CO_2/m^2/s$ ($A_s = 1$) and 0.51 mol $H_2O/m^2/s$ ($gs_s = 1$) of *gs* for *N. pumilio* were measured at −0.02 MPa. From this point, *A* decreased at the rate of 1.13 µmol CO_2 per m^2/s per 0.1 MPa of ψ_{lp} (Fig. 4) and *gs* at the rate of 0.034 mol $H_2O/m^2/s$ per 0.1 MPa (Fig. 5) as water stress increased down to −1.5 MPa. *N. antarctica* showed lower *A* and *gs* values under control conditions (irrigated plants) compared with *N. pumilio*, but declined smoothly as water stress increased. Thus, the maximum value of *A* for *N. antarctica* of 11.1 µmol $CO_2/m^2/s$ ($A_s = 1$) at −0.02 MPa decreased at the rate of 0.56 µmol $CO_2/m^2/s$ per 0.1 MPa of ψ_{lp} down to −2.0 MPa (Fig. 4). Similarly, the maximum value of *gs* of 0.42 mol $H_2O/m^2/s$ ($gs_s = 1$) declined at the rate of 0.020 mol $H_2O/m^2/s$ per 0.1 MPa (Fig. 5). Then, *A* reached a negative value from −1.7 MPa for *N. pumilio* and from −2.7 MPa for *N. antarctica*.

Least squares regression analysis showed a positive linear relationship between gs_s and A_s for changes in ψ_{lp} with a coefficient for the slope of 1.03 for *N. pumilio* and 1.07 for *N. antarctica* (Fig. 6). The negative ordinate axis intercept values showed that values of gs_s close to zero were related to negative values of A_s.

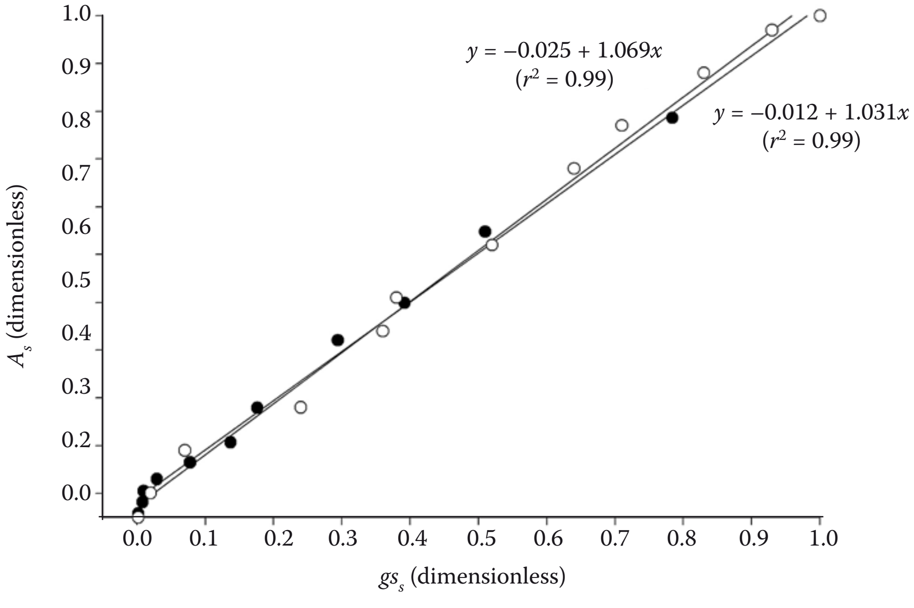

Fig. 6. Relationship between standardized rate of net photosynthesis (A_s) and standardized rate of stomatal conductance (gs_s) for *Nothofagus pumilio* (●) and *Nothofagus antarctica* (○) leaves in response to water stress where temperature and light were not limiting

Net photosynthetic rate (*A*) and stomatal conductance (*gs*) responses to waterlogging

In seedlings of both species there was a decline of approximately 35% in *A* and *gs* after one day of waterlogging compared with the control (Table 3). However, after 10 days of waterlogging the average decrease in *A* and *gs* was 83% for *N. pumilio* and 65% for *N. antarctica*. While *A* of *N. pumilio* seedlings reached negative values after 20 days of waterlogging, plants of *N. antarctica* had positive photosynthetic activity with higher *gs* values.

DISCUSSION

Effect of light intensity and induction of photosynthesis

The photosynthetic rate of individual *Nothofagus* leaves decreased with decreasing PPFD (Fig. 1). However, *N. pumilio* showed higher *A* values than *N. antarctica* over the whole range of PPFD (Fig. 1). In addition, the observed maximum *A* values of *N. pumilio* were slightly higher as compared to broad-leaved deciduous tree species (from 3.8 to 14.8 μmol $CO_2/m^2/s$) reported by Reich et al. (1995). These maximum values of *A* for *N. pumilio* can result from a higher RuBisCO amount and therefore a higher activity (Reich et al. 1995) which is consistent with higher leaf N concentration (Table 1). The minimum value of A_s for plants of both species grown at 100 μmol/m²/s PPFD was around 85% lower than for those grown in full sunlight conditions (Fig. 1). This was consistent with data found in *Nothofagus cunninghamii* by Read and Hill (1985), who reported a double increase of *A* after transition from 50 to 800 μmol/m²/s PPFD. A reduced *gs* under low light (Fig. 2) can subsequently explain the decrease of *A* (Pearcy 1988; Tinoco-Ojanguren, Pearcy 1993). Sharkey and Ogawa (1987) reported that stomata can respond to light absorbed by pigments within the guard cell and by responding to an agent from the mesophyll cells to the guard cells. However, the rate of *gs* limitation occurred more slowly than the *A* reduction rate and A_s reached negative values when leaf stomata still appeared partially opened (values of gs_s close to 0.3–0.4) (Fig. 3). This indicates that factors other than stomatal closure, such as biochemical limitations, caused the reduction of *A* under low PPFDs. A description of the non-stomatal limitations that affected photosynthesis was provided by Sassenrath-Cole and Pearcy (1994), who investigated the time course of deactivation of RuBisCO and FBPase (fructose-1,6-bisphosphatase) activities at low PPFD (35 μmol/m²/s). A similar mechanism may explain these results for *Nothofagus* seedlings.

A during induction was dependent on species responses to the previous low light intensity (95 to 100 μmol/m²/s PPFD) that the seedlings were exposed (Table 2). For example, IS_2 was 15% lower in *N. antarctica* plants exposed to 60 minutes of shade than in *N. pumilio* seedlings. Chazdon and Pearcy (1986) reported a decrease by 60% in IS_2 after 60 min at 7–10 μmol/m²/s for the shade-grown *Alocasia macrorrhiza*. According to Sassenrath-Cole and

Table 3. Response of net photosynthesis (*A*) and stomatal conductance (*gs*) to different days after waterlogging compared with the control (plants irrigated near to field capacity) for *Nothofagus pumilio* and *Nothofaus antarctica* seedlings. Measurements were taken when temperature was not limiting and in full sun conditions (1,700–2,000 μmol/m²/s PPFD)

A (μmol $CO_2/m^2/s$)	Days of waterlogging			
	control	1	10	20
N. pumilio	16.5	10.1	2.9	–0.3
N. antarctica	11.1	7.5	3.9	2.1
Significance	***	**	**	***
gs (mol $H_2O/m^2/s$)				
N. pumilio	0.50	0.33	0.09	0.001
N. antarctica	0.43	0.29	0.12	0.06
Significance	***	**	**	***

** $p < 0.05$, *** $p < 0.001$

Pearcy (1994) increases in stomatal conductance are the sole cause of increases in assimilation rate after 10 min of saturating PPFD when the enzymes are already fully activated. However, other factors than an increase in *gs* caused the increment in *A* during the first minutes of induction (Pearcy, Seemann 1990; Tausz et al. 2005). In contrast, stomatal opening may cause a continuing further increase in photosynthesis rate for up to 60 min (Pearcy et al. 1996).

The rate of decrease in *A* under low PPFDs and the responses during induction could be used as physiological indicators to define shade-tolerant species from a physiological perspective. Thus, the species with a slow decrease in *A* when exposed to shade, or fast responses to induction (higher values of IS_1 and IS_{10} or lower T_{90}), would be classified as more shade-tolerant because they would increase the carbon photosynthetic gain. It could be expected that species with faster recovery during induction would allow greater sun utilization of fluctuating sunlight in native stands. We observed more rapid photosynthetic induction kinetics (T_{90}) in *N. pumilio* than in *N. antarctica* seedlings, and the values found in the present work were lower than those reported by Urban et al. (2007) for sun foliage of intermediate shade-tolerant *Fagus sylvatica* trees. In comparison Chazdon and Pearcy (1986) reported an IS_1 value of approximately 45 for leaves of the shade-tolerant *Alocasia macrorrhiza* after 60 min under shade (7–10 μmol/m^2/s PPFD). Kursar and Coley (1993) reported that the induction of *A* to 50% of eight shade-tolerant species of Panama's rainforest occurred very quickly ranging from 1 to 3 min after 15 min at < 10 μmol/m^2/s PPFD. Thus, *N. pumilio* was more 'shade tolerant' from a physiological perspective compared to *N. antarctica*.

Effect of water stress and waterlogging

There was a negative relationship between *A* and the water status of the plants expressed as pre-dawn leaf water potential (ψ_{lp}). Effects of water stress on *A* can be caused by stomatal and non-stomatal factors. In the present study, the reduction in stomatal conductance to water vapour was the main factor that reduced *A* in both species (Fig. 5). Moderate water-deficit stress reduces photosynthesis primarily by inducing stomatal closure (Chaves 1991). This stomatal behaviour confirms that both species showed a drought avoidance response to water stress (Fig. 6) rather than a stress tolerance response through osmotic adjustments. This is consistent with Alberdi (1987), who reported for *N. pumilio* and *N. antarctica* a lack of daily variation in the leaf osmotic pressures.

However, it is now recognized that the stomatal closure does not respond to changes in leaf water potential until a critical threshold level is reached. In the present study, this was confirmed when *A* reached a negative value from –1.7 MPa for *N. pumilio* and from –2.7 MPa for *N. antarctica* with positive values of gs_s (Fig. 6). This indicated that total respiration was higher than photosynthesis under severe water stress and that non-stomatal processes limited *A*. In contrast, Sun et al. (1995) found that seedlings of *Nothofagus solandri* and *Nothofagus menziesii* reached values of *A* and *gs* close to zero at –4.0 MPa of ψ_{lp} demonstrating a higher water stress tolerance than the species studied in the present work. Long-term severe water stress can decrease *A* values by increasing mesophyll resistance and by reducing the RuBisCO activity in water-stressed leaves (O'toole et al. 1976) due to the decreased CO_2 concentration in chloroplasts. The better response of *N. antarctica* to water stress compared with *N. pumilio* can be attributed to the more responsive stomatal closure (Fig. 5) and also to the lower value of SLA (Table 1) which is related to a greater concentration of structural constituents (abundant sclerenchyma and bigger vascular bundles) and thicker cuticle (Landsberg, Gower 1997).

Tolerance to waterlogging was related with changes in *A* and *gs*. In this context *N. antarctica* was more tolerant with positive photosynthetic activity and more opened stomata after 20 days of waterlogging compared with *N. pumilio* (Table 3). Sun et al. (1995) reported a more tolerant response to severe waterlogging for *Nothofagus solandri* and *Nothofagus menziesii* seedlings with a reduction of 50% and 65%, respectively, after 8 days of waterlogging. In our study, after 10 days of waterlogging the average decrease in *A* was 83% for *N. pumilio* and 65% for *N. antarctica*. Similarly, they found that the decrease in *A* was associated with a decline in *gs*, but also with a decrease in non-stomatal or residual conductance to diffusion CO_2 (related to mesophyll conductance and carboxylation efficiency), which resulted in severe damage to the photosynthetic apparatus of leaves.

Ecological and management implications

The consequence of the light responses found in the present work is likely to modify the efficiency of sunlight utilization in mature *Nothofagus* stands because the incoming irradiance (sun gaps versus shade area) is generally low due to the tree crown cover or occurs in intense sunflecks (lasting from seconds to 15 min). Since, the full photosynthetic induction occurred 18 and 27 min after the increase of PPFD in *N. pumilio* and *N. antarctica*, respectively, it is likely that *Nothofagus* leaves will be rarely fully induced under natural light regime. In contrast, Tausz et al. (2005) reported a faster response to simulated sunflecks of *Nothofagus cunninghamii* leaves with a 90% photosynthetic rate completed within 3 min. Furthermore, because *N. pumilio* showed faster photosynthetic induction (higher values of IS_1 and IS_{10}, and lower values of T_{90}), its leaves can use more efficiently sudden increases in irradiance (via sunflecks) compared with *N. antarctica*. More effective utilization of fluctuating light by *N. pumilio* leaves may significantly increase carbon gain and consequently may be important for regeneration growth underneath shaded canopies. The rate of decrease in A under different shade intensities could also be used as physiological indicators to define shade-tolerant species from a physiological perspective. Thus, species with a slow decrease or deactivation in A when exposed to shade would be classified as more shade-tolerant because they would increase the carbon photosynthetic gain. In this context, *N. pumilio* leaves showed a lower deactivation process of A under different shade intensities than *N. antarctica*. Further study to quantify the dynamics of the induction response in fluctuating light environments will be required to assess the quantitative role in photosynthetic activity.

Furthermore, *N. pumilio* had a higher net photosynthesis than *N. antarctica* over the range of PPFD when water is not limiting (Fig. 1). This indicates that *N. pumilio* seedlings can regenerate better than *N. antarctica* under denser canopies but also in big gaps created by silvicultural practices. Different regeneration harvesting systems have been used in *N. pumilio* forests to assist regeneration from selective cuts that create small light gaps to clear-cuts (Gea et al. 2004). According to the results of our experiment, *N. pumilio* seedlings can be adapted to new environmental conditions by increasing the photosynthetic rates when water is not limiting. However, when soil moisture becomes a limiting environmental factor (ψ_{lp} less than –1.7 MPa) the application of silviculture systems may fail because the photosynthetic response of *N. pumilio* seedlings will be reduced instead of the high light availability. However, predictions of natural regeneration trends based on our results are limited because there are interactions between light and other environmental factors such as temperature and water stress.

On the other hand, the relationships between A and ψ_{lp} (Fig. 4), between gs and ψ_{lp} (Fig. 5) and the response to waterlogging conditions (Table 3) are physiological variables that can help to understand the ecological and geographical distribution of native forest. The results highlighted that *N. antarctica* has competitive advantages to grow in harsh conditions (dry sites or wetlands) compared with *N. pumilio*. However, *N. antarctica* showed lower values of A and gs under non-limiting conditions (irrigated plants) being a species with poor competitive ability to grow on more moist soils or sheltered sites compared with *N. pumilio*. These features are consistent with the natural spatial distribution of these species in Patagonian native forests across a rainfall gradient where *N. antarctica* is a dominant species in forests of dry sites (xeric ecotone zones, exposed or rocky sites) or on poorly drained soils in wetlands or in bogs (Veblen et al. 1996).

Finally, the results from the present study can assist active restoration plans with native species in large degraded areas of Patagonia to restore environmental services (CO_2 fixation, clear water, biodiversity, landscape, and recreation) and to sustain local labour from timber industries. Native forest areas of *N. pumilio* and *N. antarctica* are irreversibly degraded by the combination of fire with animal grazing (particularly in drier eastern regions) with new vegetative cover of shrubs or grassland often dominated by exotic species that inhibit the germination and establishment of *Nothofagus* seedlings. Therefore, to accelerate the recovery of these *Nothofagus* forests, active rehabilitation is required. The response of A to water status expressed as pre-dawn leaf water potential (ψ_{lp}) from the present work (Fig. 4) can be used to estimate the response of *N. pumilio* and *N. antarctica* seedlings related to the degraded sites suitable to be restored. However, ψ_{lp} is a state variable which is difficult to obtain from field measurements. A solution to this practical limitation would be the use of a water basis model which predicts the plant water potential based on the masses of water in soil, roots and shoots, and processes of rainfall, drainage and evapotranspiration. The driving environmental variable of radiation is a readily available field measurement and can be used to estimate the responses of A of *N. pumilio* and *N. antarctica* seedlings in a particular degraded site for restoration.

CONCLUSIONS

Under non-limiting water conditions, *N. pumilio* had a higher net photosynthesis than *N. antarctica* over the whole range of PPFDs indicating that *N. pu-*

milio seedlings have a higher potential to regenerate better under denser canopies but also in big gaps created by silvicultural practices. The relationship between *A* and ψ_{lp}, between *gs* and ψ_{lp} and the response to waterlogging conditions are physiological variables that can help to understand the ecological and geographical distribution of *Nothofagus* native forest. The photosynthetic characteristics of *Nothofagus* leaves found in this manuscript related to fluctuating light, water stress and waterlogging could be used to estimate the responses of regeneration to environmental conditions under the dynamics of natural stands, after silvicultural practices and for restoration plans.

Acknowledgment

We thank Mario Pastorino for his help in plant collection and for organizing research facilities. We also thank Javier Gyenge and María Elena Fernández for technical assistance and instrument facilities.

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Received for publication July 25, 2008
Accepted after corrections October 22, 2008

Vliv intenzity světla a dostupnosti vody na fotosyntézu dvou druhů *Nothofagus* – dominantní dřeviny lesů jižní Patagonie (Argentina)

ABSTRAKT: Cílem experimentů bylo, na základě studia rychlosti čisté fotosyntézy (*A*) a stomatální vodivosti (*gs*), ohodnotit citlivost sazenic *Nothofagus pumilio* a *N. antarctica* k vodnímu stresu a jejich toleranci k podmáčení. Hodnoty *A* a *gs* byly stanoveny při různých intenzitách fotosynteticky aktivní radiace (PPFD = 20, 150, 250, 500, 750, 1 000, 1 500 a 2 200 µmol/m^2/s). Dále byla u sazenic přenesených z nízké (adaptace 60 minut) na vysokou (saturační) ozářenost studována rychlost indukce fotosyntézy. Sazenice *N. pumilio* vykazovaly při všech sledovaných intenzitách světla vyšší hodnoty *A* v porovnání s *N. antarctica*, přičemž maximální hodnoty *A* (16,8 µmol CO$_2$/m^2/s) bylo dosaženo při intenzitě světla 1 000 µmol/m^2/s. Při snižování intenzity světla docházelo u obou druhů *Nothofagus* ke snižování hodnot *gs*. Čas potřebný pro dosažení plné indukce fotosyntézy činil u *N. pumilio* a *N. antarctica* 18, resp. 27 minut. Byla pozorována nepřímá úměra mezi rychlostí *A* a mírou vodního stresu, který byl hodnocen na základě vodního potenciálu listů před svítáním (ψ_{lp}). Výsledky prokázaly, že hlavní příčinou redukce rychlosti *A* u obou studovaných druhů bylo snížení hodnoty *gs*. Tolerance k podmáčení rovněž souvisela se změnami hodnot *A* a *gs*, přičemž tolerance *N. antarctica* byla v porovnání s tolerancí *N. pumilio* vyšší. Získané poznatky o vztahu mezi fotosyntézou listů sazenic *Nothofagus* a vnějšími podmínkami (proměnlivá ozářenost, vodní stres, podmáčení) mohou být využity při odhadu dynamiky vývoje původních lesních porostů či přirozené obnovy lesních ploch po hospodářských zásazích.

Klíčová slova: *Nothofagus*; zastínění; fotosyntéza; vodní stres; podmáčení

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