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Short communication

Annual nature of the growth rings of *Araucaria araucana* confirmed by radiocarbon analysis

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ABSTRACT

During the late 1950s and early 1960s, thermonuclear tests added considerable amounts of ^{14}C to the atmosphere. Since that time the amount has declined, which is owed to the exchange and dispersal of ^{14}C into the Earth's carbon reservoirs, creating an isotopic chronometer (the ^{14}C bomb peak) to all living organisms. In this study, we make use of this alternative radiometric method to compare dendrochronological ages with the ^{14}C signature of wood samples, as a way to independently confirm the annual nature of the growth rings of *Araucaria araucana* trees growing in a northern Patagonia (38°52′S, 70°34′W) site, represented by a forest of old living specimens of ca. 900 years. High precision ^{14}C-AMS of selected tree-rings from two trees confirms the annual frequency of the ring formation against an existing dataset of atmospheric ^{14}C values in the SH Zone 1–2, validating these tree specimens for further use on improving the temporal resolution of the ^{14}C age calibration curve for the Southern Hemisphere.

1. Introduction

The Earth's atmosphere produces radiocarbon (^{14}C) by cosmic ray secondary neutron collisions with nitrogen (e.g ^{14}N (n, p) ^{14}C). Once in the atmosphere the ^{14}C is oxidized to form $^{14}CO_2$, and later absorbed by trees as part of the photosynthesis process to finally be fixed in the wood cell walls during each growth season (Miyake et al., 2013). Therefore, a permanent record of the annual ^{14}C activity in the atmosphere has been tracked by the carbon assimilated in the growth rings of trees, resulting in an useful tool for reconstructing past environmental conditions. Moreover, and because ^{14}C ages are not true calendar ages, ^{14}C analysis of dendrochronologically or cross-dated tree rings have been successfully used to establish a ^{14}C calibration curve for the conversion of ^{14}C ages to calendar ages (Stuiver and Braziunas, 1993; Reimer et al., 2013; Hogg et al., 2013).

With the testing of nuclear weapons during the late 1950s and early 1960s high concentrations of ^{14}C were released into the atmosphere (Lerman et al., 1969; Levin and Hesshaimer, 2000). In the Northern Hemisphere (NH) the amount of artificial carbon in the atmosphere reached its maximum in 1963–1964 (Nydal and Lovseth, 1996; Hua et al., 2013) while in the Southern Hemisphere (SH) the maximum was associated with the calendar year of 1965 (Hua et al., 2000, 2003). The observed delay of 1–2 years on ^{14}C concentrations between the two Hemispheres is due to particular mechanisms in the atmospheric mixing (Hua et al., 1999). However, the relative rapid exchange of ^{14}C between the atmosphere, oceans and biosphere (Levin and Kromer, 2004; Levin et al., 2008) has enabled researchers to investigate the mechanics of carbon mixing and exchange processes, as well as modelers to analyze the pathway of ^{14}C, the dispersal of bomb carbon into the upper layers of the atmosphere, and its residence times (Stuiver and Braziunas, 1993). Moreover, the pronounced spike of the ^{14}C in the atmosphere produced by the nuclear bombs along with its subsequent decline after these nuclear tests (confirmed by continuous observations at several locations worldwide) allow researchers to use it today as a tracer. In this way, ^{14}C dating can be used to test the annual pattern of the growth ring formation of tree species, especially in tropical and sub-tropical regions (e.g. Worbes and Junk, 1989; Biondi and Fessenden, 1999; Biondi et al., 2007; Wils et al., 2009; Santos et al., 2015; Andreu-Hayle et al., 2015).

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Studies of past SH atmospheric ^{14}C are limited and mostly concentrated to the eastern sector of the Hemisphere (e.g. Fig. 1 in Hua et al., 2012). Therefore, the need of newer long living tree-ring archives is evident. Early attempts of using South American woods for expending the ^{14}C calibration curve have encountered some problems. Several ^{14}C analyses using *Araucaria angustifolia* (Paraná Pine) at 27′S, 50′W (1960–1969) of Kikata et al. (1992a, 1993) cited in Hua et al. (1999; Fig. 8) were considered erroneous, due to either the presence of carbohydrate reserves from the previous year into the current analyzed tree-ring and/or lack of a proper chemical pretreatment before ^{14}C measurements. When, we compared the *A. angustifolia* dataset in all the articles in which it was reported, e.g. Kikata et al. (1992a,b) and Kikata et al. (1993), we identify a discrepancy that appears to be originally generated in Table 2 in Kikata et al. (1993), and later carried over unnoticed into Hua et al. (1999) compilation. The ^{14}C value associated with 1961 in Table 2 Kikata et al. (1993) is incorrect because the ^{14}C measurement was either not performed or failed to produce a result, and the value reported actually belongs to the year 1958, as per Fig. 6 in Kikata et al. (1992a). Assuming the values shown in figures are correct, the *A. angustifolia* dataset of Kikata et al. (1992a,b; 1993) is in good agreement with the rest of the SH curve. Lisi et al. (2001) reproduced the overall layout of the bomb pulse from the *A. angustifolia* wood at 22°50′S, 46°04′W based on 13 single tree-ring ^{14}C measurements obtained by liquid scintillation counters, but no further measurements were performed. Subsequently, the concept of investigating the annual rhythms preserved in southern American tree-rings for the purpose of improving the ^{14}C calibration curve was stagnated for about 10 years or so. Recently, Santos et al. (2015) revisited the *A. angustifolia* measurements at 22°50′S, 46°04′W from AD 1927–1997 by using high-precision (0.2–0.3%) ^{14}C-AMS. The validation of the annual formation of the *A. angustifolia* tree rings was carried out by 83 ^{14}C dates of holocellulose extracts from pre-dated dendrochronological wood layers, and their agreement with the existing SH ^{14}C compilation datasets (e.g. McCormac et al., 1998, 2002; Hogg et al., 2002; Hua et al., 2013), led to a prompt investigation of other *Araucariaceae* species from South America with promising ancient wood archives that could refine the current SH ^{14}C calibration curve (Hogg et al., 2013).

Araucaria araucana (Molina) K. Koch shows distinct growth rings that have been used for inferences of present and past climatic variability (Holmes et al., 1979; Villalba et al., 1989; Mundo et al., 2012a; Villalba et al., 2012; Hadad et al., 2014) and different disturbing events, e.g. fires (González et al., 2005; Mundo et al., 2013) or frosts (Hadad et al., 2012). Here, we took advantage of the ^{14}C excess injected into the atmosphere by nuclear bomb tests during the late 1950s and early 1960s to validate the *A. araucana* annual growth rings from a site vegetated with veteran standing trees of multi centennial ages at northern Patagonia of Argentina (LaMarche et al., 1979; Hadad et al., 2014). Throughout our results we indicate that this species can be successfully used for complementing the existing SH atmospheric ^{14}C calibration curve.

2. Materials and methods

2.1. Tree and site description

In the Andes temperate areas in northern Patagonia of Argentina and Chile the *A. araucana* forests occur. The tree is an endemic conifer mostly distributed between 900 and 1800 m altitude and between 37° 20′ and 40° 20′ S (Roig and Villalba, 2008). Its wood's anatomy reveals a yellow ochre uniform color, with no difference between sapwood and heartwood. Growth rings are clearly visible under hand lens and obviously under microscope (Fig. 1A) (in web version). The density is approximately 0.5 g/cm^3 (Díaz-Vaz, 1984), therefore it's an easy wood material to be used in both sampling and laboratory stages of manipulation. The growing activity of *A. araucana* is related to the austral summer temperature conditions (particularly between October and March) occurring in the northern Patagonia (Roig and Villalba, 2008). This species reaches ages near the millennium (LaMarche et al., 1979; Hadad et al., 2014) and their ring-width patterns reflect the yearly climate variability (Villalba et al., 1989; Mundo et al., 2012b; Muñoz et al., 2013; Hadad et al., 2014). Because of this background, dendrochronological analysis of *A. araucana* has allowed the development of many ecological and paleoclimatic inferences (Roig and Villalba, 2008).

Wood core samples were collected from Primeros Pinos (PP) (Fig. 1B) site, at the western side of Neuquén province in northern Patagonia, Argentina (38° 52′S, 70° 34′W) and at an elevation of 1628 m (Fig. 2). Dominant forests are open stands at the forest-steppe ecotone, growing in a rocky ground with a sandy matrix that provides a well-drained substratum. The regional climate is characterized by a mean annual air temperature of 12.4 °C (the mean of the warmest and coldest months are 19.8 °C and 5.1 °C, respectively), with the annual precipitation around 500 mm, although it is mainly concentrated in the winter months (De Fina, 1972).

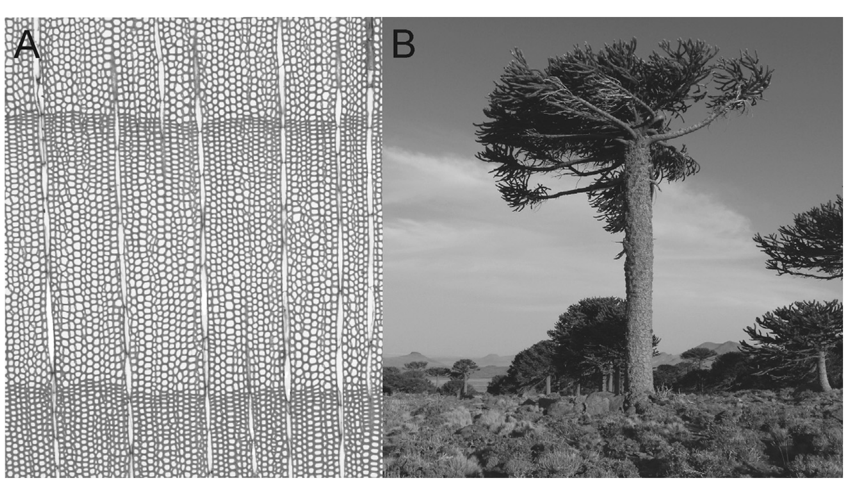

Fig. 1. A) Transverse section view of the *A. araucana* tree ring's anatomy and, B) the *A. araucana* forest stand at the Primeros Pinos study site.

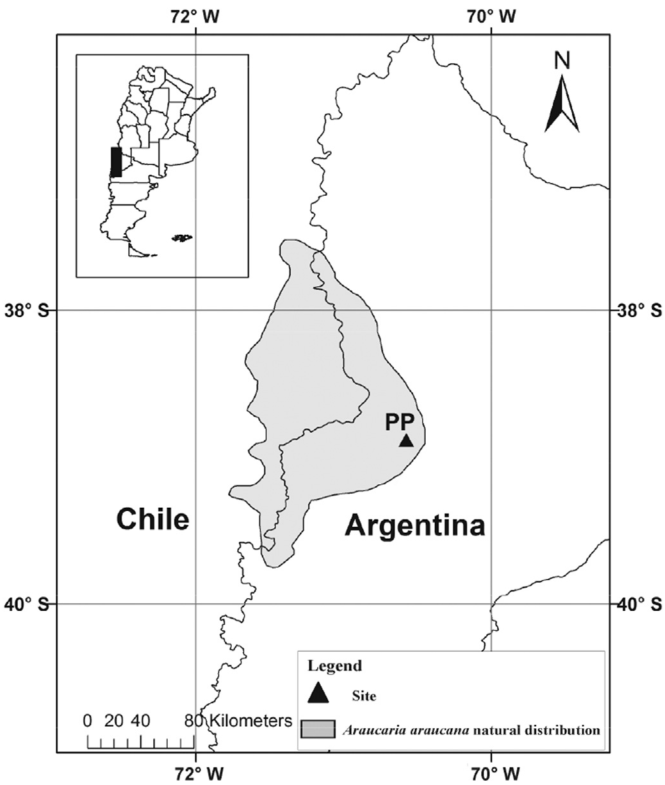

Fig. 2. Study area in the northern Patagonia, Argentina where wood cores from *A. araucana* were taken. PP: Primeros Pinos. Shaded gray correspond to the natural distribution of *A. araucana* forests.

2.2. Wood cores

Wood samples were obtained from two trees of *A. araucana*, and two increment cores per tree (4 increment cores) were taken at breast height (1.30 m). In the laboratory, wood samples were air dried and sanded with progressively fine sandpaper to highlight the visual of the tree-ring annual boundary structure. The calendar age of the growth rings was determined by applying the Schulman's method (Schulman, 1956), which states that the annual ring'date correspond to the year's growth begins. Subsequently, the ring widths were measured with a Velmex measuring system with a precision of 0.001 mm and the quality control of the derived tree-ring series was made according to standard visual cross-comparison methods proposed by Stokes and Smiley (1968) and by statistical cross-dating procedures carried out with the COFECHA programme (Holmes, 1983). The statistical data resulted from the cross-dating procedures applied to the *A. araucana* wood material used in this study should be referred to Hadad et al. (2014).

2.3. Sample preparation and AMS ^{14}C analysis

Wood cores comprising of ~10 years were sent to the Keck Carbon Cycle Accelerator Mass Spectrometer (KCCAMS) facility for ^{14}C sample processing and analyses. Tentative dates were obtained from individual growth rings by assuming that the outermost ring (next to the end of each individual core) was formed by the assigned year provided by the Dendrochronology Laboratory at Mendoza and that each ring was annual. We manually chose 2 rings per core by cutting them along their visible annual tree ring markings on each end, under the microscope. The markings were indicated by distinct differences in color from lighter (earlywood) or less dense (~3 mm in length) to darker (latewood) or more dense (~1 mm in length) wood segments. Besides the known time frame of each core provided by the dendrochronological analysis, the orientation of the core and consequently the actual calendar year associated with each edge were unknown. Therefore, each separated wood sample taken from both ends of each core, was initially designated by its time frame, followed by the letters A or B until measurements were performed. Then the actual calendar year could be properly identified by the alternative ^{14}C bomb pulse radiometric method.

In order to remove unwanted compounds of each sample, 10–20 mg of wood was subjected to a holocellulose extraction procedure. First they were placed in a 13 mm test tube, of which an acid-base-acid (ABA) treatment was then applied according to an established protocol (Santos and Ormsby, 2013). Following ABA, the wood material was then treated using 1 N HCl with 1 M $NaClO_2$ at 70 °C, in a fume hood for 4 h to isolate holocellulose. The holocellulose was then rinsed with warm water until a reading of pH > 6 (Southon and Magana, 2010). All samples were then placed on a 50 °C heat block until they were dry.

To prepare the samples for ^{14}C-AMS measurements, 1.5–2 mg of holocellulose were added to pre-baked quartz tubes, along with 60–70 mg samples of CuO and a 2 mm Ag wire. Each tube was evacuated, sealed, and then baked at 900 °C for 3 h. The CO_2 produced was cryogenically purified and converted to graphite by the hydrogen reduction method (Santos et al., 2004). The graphite samples were then pressed for placement into the wheel, and then measured by AMS. The samples were measured together with wood blanks and standards for background corrections (e.g. subfossil woods Queets-A and FIRI-H, and aliquots of IAEA-C3 cellulose – Santos and Ormsby, 2013; Santos et al., 2015). For isotopic fractionation corrections, we use the online $\delta^{13}C$-AMS values (Santos et al., 2007). The ^{14}C results reported here are in terms of "Fraction modern carbon" or just $F^{14}C$ following the recommendation of Reimer et al. (2004). The $F^{14}C$ notation is equivalent to the notation of "percent Modern Carbon (pMC)" of Stuiver and Polach (1977), since 1.0 $F^{14}C$ = 100.0 pMC, and cannot be affected by the year of the measurement.

Herein, we chose to produce several replicates from the pre-to post-bomb period, for two main reasons: a) to assure that the chemical pretreatment chosen (holocellulose extraction rather than alpha-cellulose) can produce samples for meaningful ^{14}C investigations. Thus, concerns regarding wood pretreatments failing on removing recently produced photosynthates into older growth rings (as sapwood is converted to heartwood) (Worbes and Junk, 1989; Hoper et al., 1998) could be properly addressed; and b) to show that we can achieve a high degree of reproducibility when using *A. araucana*, and therefore that this tree species should be considered as a potential candidate to further complement the datasets in the SH ^{14}C calibration curve. Twenty-two samples from 4 increment cores obtained from two trees were successfully measured, corresponding to the calendar years of 1930 (n = 5), 1950 (n = 5), 1955 (n = 1), 1965 (n = 3), 1970 (n = 1), 1990 (n = 2), 2000 (n = 3) and 2010 (n = 2).

3. Results and discussion

Statistics of the 4 increment cores involved in this study are in Table 1. The mean of tree ring widths was between 1.02 mm and 1.29 mm, while the minimum was 0.27 mm and the maximum was 3.26 mm, respectively. The average correlation of the 4 series against the reference master chronology derived from COFECHA was $r = 0.63$, considering a segment length of 142 years. No false or missing rings in the 4 analyzed series were detected.

In Table 2 and Fig. 3 we show the $F^{14}C$ values of the 22 tree rings analyzed for the period between 1930 and 2010. Several replicates from initially unprocessed wood was processed and measured by

Table 1
Statistics of ring-width 4 increment cores of *A. araucana*.

Code	Time span	Mean of ring width (mm) ± SD	Minimum ring width (mm)	Maximum ring width (mm)
PP3A	1871 2011	1.02 ± 0.38	0.32	2.04
PP3B	1900 2011	1.02 ± 0.41	0.30	2.02
PP4A	1910 2012	1.46 ± 0.69	0.27	3.26
PP4B	1890 2012	1.29 ± 0.46	0.40	2.27

Table 2
Measured ^{14}C, showed as a Fraction Modern Carbon, ($F^{14}C$; Reimer et al., 2004) from the tree rings of *A. araucana* at Primeros Pinos, Argentina.

Ring formation (year AD)	Samples	UCIAMS#	$F^{14}C$[a]	±1σ	$F^{14}C$ average	stdev
1930	PP3A	141324	0.9788	0.0014	0.9788	0.0027
1930	PP3B	141325	0.9765	0.0013		
1930	PP4A	141328	0.9809	0.0013		
1930	PP4B	141330	0.9759	0.0015		
1930	PP4B	141331	0.9821	0.0015		
1950	PP3A	141323	0.9689	0.0015	0.9735	0.0035
1950	PP3B	141326	0.9755	0.0013		
1950	PP4A	141327	0.9751	0.0013		
1950	PP4B	141329	0.9709	0.0013		
1950	PP4B	141332	0.9772	0.0013		
1955	PP3A	141335	0.9913	0.0013	N/A	N/A
1965	PP3A	141333	1.5914	0.0022	1.5982	0.0060
1965	PP3B	141336	1.6024	0.0022		
1965	PP4A	141338	1.6009	0.0022		
1970	PP4A	141339	1.5165	0.0021	N/A	N/A
1990	PP3A	141342	1.1522	0.0015	1.1166	0.0012
1990	PP4A	141344	1.1539	0.0016		
2000	PP3A	141346	1.0907	0.0016	1.0758	0.0027
2000	PP3B	141347	1.0908	0.0014		
2000	PP4A	141349	1.0955	0.0014		
2010	PP3B	141345	1.0523	0.0014	1.0510	0.0019
2010	PP4A	141348	1.0496	0.0014		

N/A – not applicable.
[a] Represents the ^{14}C content of the sample. ±1σ: standard error of $F^{14}C$.

^{14}C-AMS methodology. Those replicates were paired in six groups according to the calendar year they belong as in Table 2 and, their $F^{14}C$ mean values and standard deviations (e.g. $F^{14}C$ average and stdev respectively). Although those datasets are relatively small for an in depth statistical analyses, the following can be drawn. Four among six sets show high precision, with the standard deviation of less than 0.3%, and individual values within ±2σ of the mean. The 1950, 1965 sets, however, show slightly larger standard deviations, which are clearly dominated by 1–2 measurements in each set (e.g. UCIAMS #141323 and 141332 for 1950, and #141333 for 1965). Those are clearly not a case of misidentified rings (Hua et al., 2003). Since no suspicious errors could be attributed to those ^{14}C results, they cannot possibly be eliminated from the averaged $F^{14}C$ values, and standard deviations calculated.

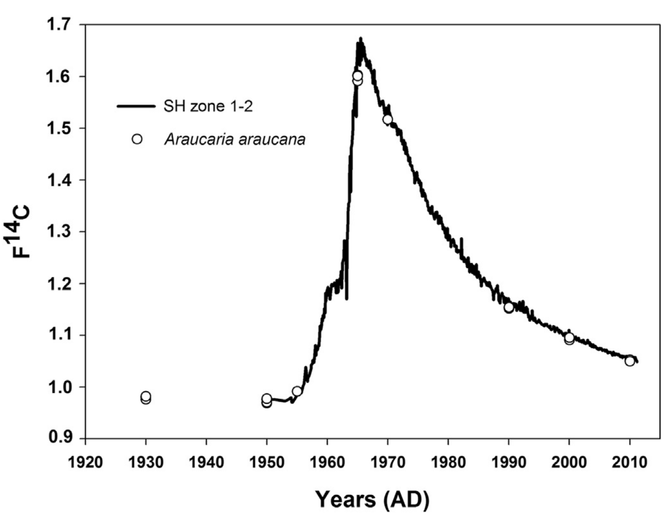

Fig. 3. $F^{14}C$ of *A. araucana* tree-ring samples (yellow circles) compared to the ^{14}C values of atmosphere in the SH Zone 1–2 (line blue, from Hua et al., 2013). Here we plotted all ^{14}C values listed in Table 2. Note that besides the good agreement of our *A. araucana* $F^{14}C$ data with SH Zone 1–2, most of the $F^{14}C$ replicate values overlaped, which can make it difficult to differentiate between individual values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The $F^{14}C$ signatures of the tree rings and the calendar year obtained by dendrochronological analysis was plotted against the SH calibration curve (Fig. 3). We found a good agreement between the annual $F^{14}C$ content of *A. araucana* tree rings at PP and the compiled monthly record for SH zone 1–2 (e.g. Hua et al., 2013). This implied that: 1) the growth rings are formed annually; 2) that both laboratories, the tree-ring and ^{14}C-AMS, are suitable for long-term single tree ring wood analysis; and 3) the *A. araucana* trees can also be used to derive long-term records of high-resolution ^{14}C signatures. In addition, studying a long-term species such as the case of the *A. araucana* from northern Patagonia of Argentina is of particularly importance, because long-term climate proxy data in this region are very scarce and a paleorecord ^{14}C time scale is essentially non-existent.

4. Conclusion and future directions

Although it appears that similar species of *Araucariaceae* in

South America could have equal growth responses (Kikata et al., 1992a,b; Kikata et al., 1993; Lisi et al., 2001; Santos et al., 2015), it is important to determine if different climatic regions can induce different patterns and frequencies of rings in these species.

The presence of annual rings has been previously reported in *A. araucana* trees, based on dendrochronological principles (LaMarche et al., 1979). However, in this study we confirm the annual nature of the growth ring formation in *A. araucana* through directly comparing selected calendar years assigned by dendrochronology cross-dating of individual wood layers and their ^{14}C signatures obtained by ^{14}C-AMS with the available SH compilation of the atmospheric bomb ^{14}C data (e.g. Hua et al., 2013).

These trees are presently available, and can be used to enhance the limited amount of true data in the SH ^{14}C calibration curve (Hogg et al., 2013). In this sense, the growth rings of *A. araucana* can be viewed not only as a powerful proxy indicator of climate variability, but its dendrochronologically-dated tree-ring samples can be also used to derive information on atmospheric ^{14}C values for the SH, at least for the past millennium (Hadad et al., 2014). Therefore, significant progress is expected in the near future once a network of dendrochronologies are developed with the *A. araucana* of Primeros Pinos, and similar species of the *Araucariaceae* family in South America, to fill in atmospheric ^{14}C data gaps.

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