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RADIOCARBON DATING OF CHINESE ANCIENT TEA TREES

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ABSTRACT. The jungles of Linyun and Longlin Autonomous Prefecture, located in the heart of the southwestern Guangxi Zhuang Autonomous Region of China, are home to the oldest tea trees (*Camellia sinensis*) in the world. In the absence of regular annual rings, radiocarbon (^{14}C) dating is one of the most powerful tools that can assist in the determination of the ages and growth rates of these plants. In this work, cores were extracted from large ancient tea trees in a central Longlin rain forest; extraction of carbon was performed with an automated sample preparation system. The ^{14}C levels in the tree cores were measured using accelerator mass spectrometry (AMS) at the University of Tsukuba. These measurements indicated that contrary to conventional views, the ages of trees in these forests range up to ~700 years, and the growth rate of this species is notably slow, exhibiting a long-term radial growth rate of 0.039±0.006 cm/yr. It was demonstrated that ^{14}C analyses provide accurate determination of ages and growth rates for subtropical wild tea trees.

KEYWORDS: Age, AMS, Ancient tea trees, ^{14}C, *Camellia sinensis*.

INTRODUCTION

The tea tree, *Camellia sinensis*, is a species of small evergreen tree, the leaves and leaf buds of which are used to produce tea. This species belongs to the genus Camellia of flowering plants in the family theaceae. Camellia sinensis is mainly cultivated in tropical and subtropical climates in areas with at least 127 cm (50 inches) of annual rainfall. The Chinese tea tree, native to southwestern China, is a small-leafed bush with multiple stems that reaches a height of about 3 m. The utilization of tea plant to produce tea can be traced back to more than 3000 years ago. The jungles of Linyun and Longlin Autonomous Prefecture, deep in the southwestern Guangxi Zhuang Autonomous Region of China, are home to the oldest tea trees in the world. In these regions, the ancient tea trees are believed to have ages from several centuries to over a millennium (Chen and Chen 2007), and the ages of tea trees provide critical information for understanding the dynamics of tea tree populations, determining historical patterns of disturbance, and developing sustainable forestry practices. However, tea trees are not a species which reliably produce a clearly determinable annual growth ring, due to the very slow growth rates and similar seasonal climate at high elevations reaching 1500 m (Chen 1994). In the absence of annual rings, radiocarbon dating (Chambers et al. 1998; Vieira et al. 2005; Patrut et al. 2011; Pearson et al. 2011; Ehrlich et al. 2017) and stable isotope dating (Poussart et al. 2006; Loader et al. 2011) are of the most powerful tools that can potentially determine the ages and growth rates of these plants. In this work, cores were extracted from large ancient tea trees in a central Longlin rain forest. The ^{14}C levels in samples were then measured using accelerator mass spectrometry (AMS) jointly by Guangxi Normal University and the University of Tsukuba. It was demonstrated that ^{14}C analyses provides accurate determination of ages and growth rates for

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Figure 1 Location of tea tree discussed in text. Map image adapted from Google Earth.

wild tea trees, and contrary to conventional views (Chen and Chen 2007), the ages of trees in these forests range up to ~700 years. The growth rate of this species is shown to be very slow: a mean radial growth rate of 0.039 cm yr^{-1}, something rarely quantified in previous forest inventories and life-history studies.

MATERIALS AND METHODS

Sites and Sampling

The ancient tea tree is located on the border areas of three Provinces (Guangxi, Guizhou and Yunnan), 250 km from Nanning in the Guangxi Province, 250 km from Guiyang in the Guizhou Province, and 350 km from Kunming in the Yunnan Province, southwest of China (Figure 1). The GPS coordinates are 24°21'23.58"N, 106°27'43.62"E, and the altitude is 1700 m. Mean annual rainfall and temperature in the area is 1698 mm and 20.4°C, respectively (Wei et al. 2015).

Having a large trunk, multiple stems distant from each other, and large leaves, ancient tea trees are easily recognized (Figure 2). All giant ancient tea trees greater than 50 cm in circumference at breast height (cbh) were marked with a red paint number mark at approximately 1.3 m above the ground. The cbh of each tree, none of which forms buttresses, was measured with a synthetic fabric diameter tape at a point approximately 5 cm below the label. Measurements indicated that the cbh of the ancient tea trees of interest are in the range of 80 to 189 cm. Wood cores were extracted using an increment borer (inner diameter: 0.5 cm, length: 45 cm) from ancient tea trees located in a central Longlin rainforest (LL-1, LY-1, and LY-2) in Aug 2016, as shown in Figure 1. In all cases, the sampling height varied between 1.20 and 1.30 m. Some of the trunks contained a heart-rot hole such that the center points of the trunks could not be located, and were excluded from this study. Finally, 10 wood cores of approximately 45 cm in length and 0.5 cm in diameter were collected.

Sample Preparation

Each core was checked carefully under the microscope to find the starting point of growth (pith), and then the wood was dissected and dried under vacuum in a freeze dryer. The wood sample was transferred to a glass tube and washed with MQ water in an ultrasonic bath followed by decanting the supernatant. This step was repeated until no further fine dust was present.

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Figure 2 A view of a Chinese ancient tea tree in Guangxi province.

The resulting samples were first soaked in a 1.2 N HCl (60°C) solution for 2 hr. The first acid treatment was repeated 3 times. Subsequently, the samples were soaked in a 1.2 N NaOH (60°C) solution for 2 hr. This treatment was repeated 3 times until impurities were removed. After soaking in 1.2 N HCl (room temperature) all night, samples were repeatedly rinsed with ultrasonic water to attain complete neutrality. Second, samples were bleached with hot $NaCl_2O_5$ / HCl (1.2 N) solution to remove the lignin. The mass of $NaCl_2O$ added into the HCl solution was 1.5 times larger than the original wood mass (10 mg) and the solution temperature was set at 90°C. After the color of solutions became colorless, we stopped the $NaCl_2O$ treatment. Finally, the samples were washed in hot MQ water (60°C) for 30 min followed by decanting the supernatant. This process was repeated 3 times to ensure complete neutralization of the solution, and then the samples were dried in a vacuum freeze dryer before further processing. Undergoing these processes, we obtained hollocellulose which appeared as white fibers.

The hollocellulose samples corresponding to 1 mg C were then combusted to CO_2 and purified in vacuum lines using the automatic sample preparation system coupled with an elemental analyzer at the University of Tsukuba, Japan (Matsunaka et al. 2018); the analyzer is a modified version of the instrument used by Kato et al. (2014). The resulting CO_2 was graphitized by hydrogen reduction under the catalytic action of ion powder and then finally pressed into the cathode cones for AMS.

^{14}C-AMS Measurement at UTTAC

Radiocarbon measurements were performed at the 6MV AMS facility at the University of Tsukuba (UTTAC) (Sasa et al. 2015; Shen et al. 2019a, 2019b). The $^{14}C/C$ values were determined by simultaneous measurements of $^{13}C^{4+}$ and $^{12}C^{4+}$ current by the offset Faraday cup on the high energy side and the count rate of ^{14}C on the multianode detector. The mean value of $^{14}C/C$ ratio in each sample was determined by normalizing the measured value against the values of six standard samples (three from NIST-SRM4990C; one each from IAEA-C1, IAEA-C6, and IAEA-C8). Nine 5-min runs were performed for each sample. The abundance values and uncertainties are based on the averages and the relative standard deviations of the nine runs, respectively. The measurement error of the

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system was 2.0‰ for the graphite of NIST-SRM4990C (HOX-II), and the background levels including the pretreatment were below 0.08 pMC (percent modern carbon) (57,400 BP) for the graphite of IAEA-C1. The concentration of ^{14}C expressed as ^{14}C Fm (fraction modern), which is the isotopic-fractionation-corrected value, was calculated according to the method of Mook and van der Plicht (1999).

$$Fm = \frac{\left(\left({}^{14}C/{}^{12}C_{sample} - {}^{14}C/{}^{12}C_{BKG}\right) \quad \left[1 - 2 \times \left(25 + \delta^{13}C_{sample}\right)/1000\right]\right)}{\left(0.7459 \text{ x } \left({}^{14}C/{}^{12}C_{HOX-II} - {}^{14}C/{}^{12}C_{BKG}\right)\left[1 - 2 \ \times \left(25 + \delta^{13}C_{HOX-II}\right)/1000\right.\right)} \tag{1}$$

where, $\delta^{13}C_{sample}$ and $\delta^{13}C_{HOX-II}$ are isotopic fractionations for samples and HOX-II standard.

The ^{14}C ages were finally calculated as follows:

$$T({}^{14}C\,age) = -\frac{1}{\lambda} ln\ (Fm) \tag{2}$$

$$\text{where } \lambda = \ln 2/T_{1/2} = 0.693/5568 = 1/8033a^{-1} \tag{3}$$

RESULTS AND DISCUSSION

Tree Age

Fraction modern values and radiocarbon dates of the 10 samples which collected from Longlin and Lingyun are listed in Table 1. Radiocarbon dates and errors were rounded to the nearest year. The resulting radiocarbon values were calibrated with the OXCAL 4.3 program (Ramsey 2009) to determine the age of wood grown before 2016 by using the calibration curve INTCAL13 (Reimer et al. 2013) for the northern hemisphere. Only samples from trees with diameter at breast height (dbh) greater than 20 cm were used for ^{14}C analysis because trees smaller than this were unlikely to be old enough to obtain accurate age estimates (i.e., greater than 250 yr). The 2σ probability distribution for the radiocarbon dates, with a relative area corresponding to the 95.4% confidence interval, was chosen to calculate the calendar ages. Each 2σ probability distribution corresponds to one or several ranges of calendar years. From these values, the 2σ range with the highest probability was selected and was used to calculate the calibrated cal BP ages and the corresponding errors. Tree ages were derived from calibrated cal BP ages extrapolated (from AD 1950) to AD 2016.

Chinese ancient tea trees appear to commonly reach ages that are rarely attained by any other shrub tree. For the 10 ancient trees sampled, the calibrated age ranged from 300 years to 700 years, which put them in the era from the Chinese Qing Dynasty to the Yuan dynasty. All trees with a dbh >25.0 cm were older than 350 years, and the oldest tree dated by ^{14}C had a dbh of 57.32 cm with the age of 682 years. It is well-known that radiocarbon dating can be problematic when the sample age is less than 250 yr old because of fluctuations in atmospheric ^{14}C content during this period (Stuiver et al. 1998). To obtain the younger Chinese tea tree size-age information, the dbh of 12 plantation tea (the same genus as ancient tee tree, under the identical environment) with known ages (20–50 yr age) in the nearby area were measured; the age versus tree size (diameter at dbh) relationship was plotted as displayed in Figure 3, and the tree size was significantly correlated with age (P<0.001). The mean error of ages for ancient tree samples was approximately 40 yr with a range of 30–50 yr. As calibrated age decreases, particularly after AD 1700, relative error becomes larger. Thus, estimates after AD 1700 are of limited utility. However, the errors obtained before AD 1700 contained small enough relative error (4–12%) to accurately estimate age. With the life

Table 1 Data sheet for Chinese ancient tea tree dating at UTTAC.

	Fraction Moderm		^{14}C ages (yr BP)					Calibrited cal age(cal yr BP)		Tree ages(yr)[b]	
Wood sample ID	Best est.	1 σ	Best est.	1σ	Tree diameter at dbh (cm)	Mean growth rate(mm yr^{-1})	Cal AD range 2σ (95.4%)[a]	Best est.	2σ	Best est.	2σ
1#Longlin	0.931	0.28%	575	23	51.6	0.38	**1308–1362 (61.4%)** 1386–1416 (34.0%)	615	27	681	27
2#Longlin	0.966	0.22%	274	18	28.0	0.38	1520–1592 (42.3%) **1622–1665 (51.4%)** 1784–1794 (1.7%)	306	22	372	22
3#Longlin	0.961	0.26%	323	22	39.5	0.42	**1488–1604 (75.2%)** 1610–1643 (20.2%)	404	58	470	58
4#Longlin	0.964	0.28%	298	23	32.8	0.36	1499–1502 (0.5%) **1513–1600 (67.9%)** 1616–1652 (27.0%)	393	44	459	44
5#Longlin	0.927	0.26%	605	22	57.3	0.42	**1298–1370 (74.3%)** 1379–1404 (21.1%)	616	36	682	36
9#Llingyun	0.961	0.28%	321	23	27.4	0.29	**1490–1603 (75.3%)** 1612–1644 (20.1%)	403	57	469	57
10#Llingyun	0.983	0.24%	138	20	23.9	0.41	**1670–1779 (41.6%)** 1798–1891 (38.2%) 1908–1943 (15.6%)	225	55	291	55
11#Llingyun	0.967	0.30%	271	25	39.2	0.53	1520–1578 (37.6%) 1582–1591 (1.6%) **1623–1667 (52.4%)** 1783–1796 (3.8%)	305	22	371	22
13#Llingyun	0.981	0.27%	156	22	20.8	0.39	1666–1706 (16.4%) **1720–1784 (37.6%)** 1832–1882 (12.1%) 1914– (18.7%)	198	32	264	32
15#Llingyun	0.970	0.28%	247	23	26.1	0.36	1528–1550 (4.2%) **1633–1670 (66.1%)** 1780–1800 (22.9%) 1943– (2.3%)	298	19	364	19

Abbreviations: AD is Anno Domini; BP is before present, i.e., before AD 1950; and ^{14}C BP is the radiocarbon-dated years before AD 1950.

[a]The highest probability 2σ range for each sample is in bold type. The relative areas of 2σ ranges for a radiocarbon date correspond to the 95.4% confidence interval.

[b]The tree ages were calculated from the calibrated cal BP ages extrapolated (from AD 1950) to AD 2016.

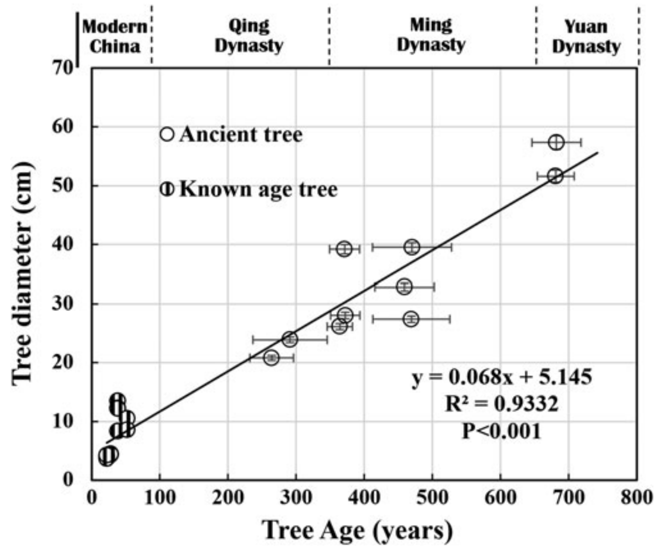

Figure 3 Tree age versus tree size relationship for Chinese ancient tea trees.

span vs. diameter relationship (Figure 3) and diameters from a the larger population of trees, the better understanding of the life span distribution of the Chinese ancient tee trees in this region would be obtained.

Growth Rate

We also study the relationship between the mean growth rate of an individual tree with the life span of the tree. The grow rate calculated from the age of the tree center and the tree diameter is a mean growth rate over the life span of the tree. The plot is shown in Figure 4. The mean growth rate was also highly correlated with age ($P<0.001$), but the relationship was nonlinear. With the increase of the age, the long-term growth rate decreases slowly before finally reaching a balanced value of approximately 0.039 cm yr^{-1}, which is less than the average growth rate of all trees studied in Figure 3, including the younger known age trees, and is a considerably better value for predicting the age of ancient trees with ages > 250 yr. Growth rates for tea trees also varied as a function of dbh. For all data combined, mean growth rate decreased slightly with size up to approximately 20 cm dbh (>250 yr age).

Based on this study, we believe that the ^{14}C dating method is effective for determining the life span of long-lived trees, such as tea trees, when the age of a given tree is more than 250 yr old. The error of age (30–50 yr) was small enough to determine the estimated lifespan of this species. This study is the first attempt to estimate the mean radial growth rate of Chinese ancient tea trees by applying the ^{14}C dating. Generally, the growth rates at younger stages were relatively rapid. With the trees becoming mature, the long-term growth rate will decrease slowly and finally reach a balanced value (balance between gross production and respiration), and reproduction gradually begins to limit their growth as an individual becomes larger (Bazzaz et al. 1987; Enquist et al. 1999). The maximum-sized

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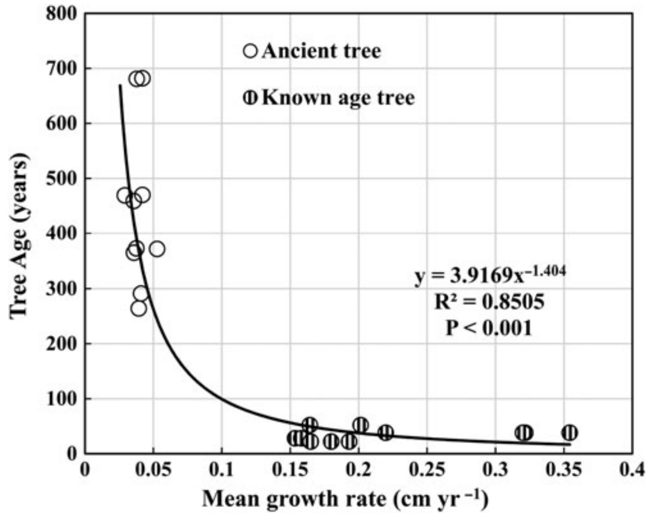

Figure 4 Tree age versus mean growth rate relationship for Chinese ancient tea trees.

Chinese ancient tea tree named "Jingxiu" was found to have a diameter of 184 cm in the Yunnan province. Only a rough minimum age of more than 3000 yr was estimated with the girth measurement, by Wang et al. in 1982 (Xu 2008). This age estimating method based on girth measurements may include large errors, but it is considered to be consistent with our estimated age (~ 4700 year) derived from the long-term growth rate in this study.

CONCLUSIONS

This study's findings provide a first look at the ages and growth rates of wild ancient tea trees in southwestern China. We performed carbon dating of ancient tea trees from Guangxi, Southwestern China by using AMS radiocarbon analysis. The IntCal13 curve was used to calibrate the measured radiocarbon dates. Based on the dating results, we suggest that the oldest age of the collected ancient tea tree sample from southwestern China was 682 ± 36 years old, which located it in the era of the Yuan dynasty. Ancient tree size and mean growth rate are both significantly correlated with age. The long-term mean growth rate was estimated to be 0.039 ± 0.006 cm/yr in this study. Theoretically, under identical environmental conditions (e.g. mean annual rainfall, altitude, mean annual temperature, etc.), the biggest/largest Tea trees would likely be the oldest. This is consistent with the size-age relationships demonstrated in this study so we suggest that the ages of tea trees in SW China could be reliably estimated by the size of the tree.

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REFERENCES

Bazzaz FA, Chiarlello NR, Coley PD, Pitelka LF. 1987. Allocating resource to reproduction and defense. BioScience 37:58–67.

Chambers JQ, Higuchi N, Schimel JP. 1998. Ancient trees in Amazonia. Nature 391:135–136.

Chen G. 1994. Tea tree age determination method. Taiwan Agricultural Research 2(1):40. In Chinese.

Chen Z, Chen P. 2007. The rich ancient tea tree resources are the best proof for the origin of the world tea tree. Agricultural Archaeology 5:257–267.

Ehrlich Y, Regev L, Kerem Z, Boaretto E. 2017. Radiocarbon dating of an olive tree cross-section: New insights on growth patterns and implications for age estimation of olive trees. Frontiers in Plant Science 8:1918.

Enquist BJ, West GB, Charnov EL, Brown JH. 1999. Allometric scaling of production and life-history variation on vascular plants. Nature 401:907–911.

Kato K, Tokanai F, Anshita M, Sakurai H, Ohashi MS. 2014. Automated sample combustion and CO_2 collection system with IRMS for ^{14}C AMS in Yamagata University, Japan. Radiocarbon 56:327–331.

Loader NJ, Walsh RPD, Robertson I, Bidin K, Ong RC, Reynolds G, McCarroll D, Gagen M, Young GHF. 2011. Recent trends in the intrinsic water-use efficiency of ringless rainforest trees in Borneo. Philosophical Transactions of the Royal Society B: Biological Sciences 366(1582): 3330–3339.

Matsunaka T, Sasa K, Hosoya S, Shen H, Takahashi T, Matsumura M, Sueki K. 2019. Radiocarbon measurement using a gas/solid hybrid ion source and an automated sample preparation system at the University of Tsukuba. Nuclear Instruments and Methods in Physics Research B 204–208.

Mook WG, van der Plicht J. 1999. Reporting ^{14}C activities and concentrations. Radiocarbon 41:227–239.

Patrut A, Karl F, Van Pelt R, Mayne DH, Lowy DA, Margineanu D. 2011. Age determination of large live trees with inner cavities: radiocarbon dating of Platland tree, a giant African baobab. Annals of Forest Science 68(5):993–1003.

Pearson S, Hua Q, Allen K, Bowman DM. 2011. Validating putatively cross-dated Callitris tree-ring chronologies using bomb-pulse radiocarbon analysis. Australian Journal of Botany 59(1): 7–17.

Poussart PM, Myneni SCB, Lanzirotti A. 2006. Tropical dendrochemistry: A novel approach to estimate age and growth from ringless trees. Geophysical Research Letters 33(17).

Ramsey BC. 2009. Bayesian analysis of radiocarbon dates. Radiocarbon 51(1):337–360.

Reimer PJ, Bard E, Bayliss A, Beck JW, Blackwell PG, Ramsey BC, Grootes PM, Guilderson TP, Haflidason H, Hajdas I, et al. (2013). IntCal13 and Marine13 radiocarbon age calibration curves 0–50,000 years cal BP. Radiocarbon 55(4):1869–1887.

Sasa K, Takahashi T, Matsumura M, Matsunaka T, Satou Y, Izumi D, Sueki K. 2015. The new 6 MV multi-nuclide AMS facility at the University of Tsukuba. Nuclear Instruments and Methods in Physics Research B 361:124–128.

Shen H, Sasa K, Meng Q, Matsumura M, Masunaka T, Hosoya S, Takahashi T, Honda M, Sueki K, He M, et al. 2019a. Exposure age dating of Chinese tiankengs by ^{36}Cl-AMS. Nuclear Instruments and Methods in Physics Research B 459: 29–35.

Shen H, Sasa K, Matsumura M, Meng Q, Masunaka T, Hosoya S, Takahashi T, Honda M, Sueki K, He M, et al. 2019b. ^{36}Cl preparation method for Chinese Karst samples (Tiankeng). Nuclear Instruments and Methods in Physics Research B 458:126–129.

Stuiver M, Reimer PJ, Bard E, Beck JW, Burr GS, Hughen KA, Kromer B, McCormac G, van der Plicht J, Spurk M. 1998. IntCal98 radiocarbon age calibration, 24,000–0 cal BP. Radiocarbon 40:1041–1084.

Vieira S, Trumbore S, Camargo PB, Selhorst D, Chambers JQ, Higuchi N, Martinelli LA. 2005. Slow growth rates of Amazonian trees: consequences for carbon cycling. Proceedings of the National Academy of Sciences, USA 102:18502–18507.

Wei H, Liu S, Wang L, Huang M. 2015. Analysis on distribution characteristics of precipitation in Lingyun County in 50 years. Journal of Meteorological Research and Application 21:72–73. In Chinese

Xu WZ. 2008. The "Jingxiu" ancient tea tree. Private Technology 8(1):207. In Chinese