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WOOD PRETREATMENT PROTOCOLS AND MEASUREMENT OF TREE-RING STANDARDS AT THE OXFORD RADIOCARBON ACCELERATOR UNIT (ORAU)

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ABSTRACT. This article presents the pretreatment protocols for wood samples processed at the Oxford Radiocarbon Accelerator Unit (ORAU), including recent implementation of a purification method to α-cellulose for non-routine samples. We examine the long-term reproducibility of measurement on wood samples at ORAU through the >1000 ^{14}C determinations made on known-age tree-ring standards processed in each AMS wheel since our present High Voltage Engineering Europa (HVEE) AMS system came on-line in September 2002. A discussion of background measurements is also provided.

INTRODUCTION

A new HVEE tandem accelerator mass spectrometry (AMS) system was accepted at ORAU in September 2002 (Bronk Ramsey et al. 2004). Since then, this system has been in routine use for AMS ^{14}C dating. Under regular operating conditions, ≥2 known-age wood samples are included in each AMS wheel (of ≤57 targets) as internal laboratory standards to verify process reliability (including chemical pretreatment).

The known-age tree-ring standards used at ORAU (either current standards or, shown in italics, standards no longer used but that have been used since September 2002) are

- Decadal (dendrochronologically dated) Irish bog oak samples (ORAU laboratory sample nr *P9029*, P9030, *P9031*, P9032, P9033, P9034, *P9035*, P14622 and *P14623*);
- Third/Fourth International Radiocarbon Intercomparison (TIRI/FIRI) wood (Scott 2003) (ORAU lab nr P5439, P10672, *P10673*, *P10675*, *P10677*, and P10679);
- Recent decadal (dendrochronologically dated) bristlecone pine (ORAU lab nr *P180* and *P181*);
- A background age Irish bog oak sample (Chelford log; ORAU lab nr P431).

Both the laboratory wood standards and unknown-age wood samples processed at ORAU are treated routinely with an acid-base-acid-bleach (ABA-bleach) chemical pretreatment (described below).

Recently, the processing of New Zealand kauri (*Agathis australis*), contributing to the extension of the pre-Holocene terrestrial calibration curve (Hogg et al. 2013), has required the application of a more stringent pretreatment protocol to refine wood to more pure α-cellulose. The development of this method is also discussed below. It should be noted that this α-cellulose purification pretreatment does not supersede the routine ABA methods; rather, it is an additional method for particular wood sample types. The high-precision measurements required for extending the terrestrial ^{14}C calibration curve (and identifying high-frequency signals within it) mean that it is particularly important to isolate the most reliable (chemically resistant) wood fraction for dating (i.e. α-cellulose; Hogg et al. 2006). The wood samples for α-cellulose purification must be preserved well enough to withstand the more rigorous chemical pretreatment and there must be sufficient starting weight (i.e. requiring ~75 mg of wood, compared to as little as 5–10 mg for the routine ABA-bleach protocol). In general (and as demonstrated herein), the routine ABA-bleach pretreatment procedure is sufficient to yield reliable, reproducible ^{14}C determinations for the overwhelming majority of wood samples dated at ORAU.

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METHODS

The regular wood pretreatment methodology applied at ORAU consists of an acid-base-acid-bleach (ABA-bleach) methodology (ORAU pretreatment code UW), as follows (Brock et al. 2010):

- Take shavings from wood with scalpel (typically, 20–100 mg);
- Solvent wash sample to remove additional contamination, if required (details discussed below).
  1. Remove sedimentary and other contaminant carbonates with 1M HCl for 20 min at 80°C.
  2. Remove any organic acid contaminants with 0.2M NaOH for 20 min at 80°C. If required, the NaOH may be replaced one or more times until the solution remains colorless.
  3. Remove any dissolved atmospheric CO_2 with 1M HCl for 1 hr at 80°C.
  4. Bleach (5% wt/vol $NaClO_2$, pH3) for ≤30 min (depending upon fragility) at 80°C to remove all but the carbohydrate portion of the sample (i.e. removing lignins and also mobile fractions, such as waxes and resins).
- After each stage, rinse ≥3× with ultrapure (Milli-Q™) water.
- Freeze-dry sample prior to combustion, graphitization, and AMS stages.

For a minority of the more fragile wood samples, the bleaching step is not applied, and the samples are given a WW ORAU pretreatment code. An asterisk (*) suffix is added to either the UW or WW pretreatment codes if the sample has required a solvent wash prior to the main ABA pretreatment stages. Such a solvent wash typically comprises sequential washes with acetone, methanol, and chloroform, and would be applied if samples were known to, or appeared to have been contaminated, often as a result of particular conservation treatments (including application of consolidants, preservatives, glues, varnish, etc.). (See Brock et al. 2010 for more detailed description of solvent extraction procedures applied.)

The α-cellulose pretreatment methodology (ORAU pretreatment code UA), proceeds as follows:

- ~75 mg of wood sampled with fine-bladed microplane, shaving across rings;
- Bleach (1.5% wt/vol. $NaClO_2$ + 0.06M HCl) at 70°C, repeated 4× over 24 hr.

More rigorous ABA methodology:

1. 4% (1.12M) HCl for 20 min at 70°C;
2. 17.5% wt/vol NaOH for 1 hr at room temperature, with ultrasonication and under a constant N_2 environment;
3. 5% (1.4M) HCl for 10 min at 70°C;
   - Rinse with ultrapure water after each stage (typically, 3×, 5×, 5–6×);
   - Freeze-dry sample prior to combustion, graphitization, and AMS.

This UA methodology is a revision upon the UA* methodology described by Hogg et al. (2013). Previously, an initial stage to remove mobile phases (principally resins) using a Dionex ASE® (accelerated solvent extraction) system was applied (performing 2 × 5 min static cycles, 150% flush volume of acetone, followed by 2 × 5 min static cycles, 150% flush volume of ultrapure water, both at 100°C and 116 atm. pressure). As described in the following section, this stage was found to be unnecessary and, indeed, led to inconsistent ^{14}C determinations. Accordingly, this solvent extraction stage has now been abandoned.

As noted by Brock et al. (2010), all glassware is thoroughly washed and then baked at 500°C for a minimum of 3 hr to ensure complete removal of any organic contaminants prior to use. Throughout the pretreatment process, individual samples are continually monitored and treatments adjusted accordingly. Samples are usually treated in 12- or 30-mL glass tubes. In the latter case, samples are separated after each chemical/water rinse through centrifugation, settling, and decanting; in the former case, 45–90 μm pore size polyethylene Ezee-filters™ (Elkay, UK) are employed for this purpose, having been precleaned by ultrasonication in ultrapure water for 20 min and subsequently passed through fresh ultrapure water prior to use (Brock et al. 2013).

The quoted ^{14}C measurements include uncertainties in pretreatment, combustion, graphitization, target pressing, and AMS background. As presented by Wood et al. (2010), a combustion background (derived from direct combustion of our internal laboratory nylon standard) of 0.0007 ± 0.0010 mg of modern C added to a sample is applied. There is no additional background correction for wood pretreatment (or pretreatment of all other plant material) over and above that for combustion and AMS.

RESULTS AND DISCUSSION

Over the last 10+ years that our HVEE AMS has been in operation (September 2002–June 2013), 1104 AMS measurements of known-age tree-ring standards have been performed at ORAU, using our routine pretreatment protocols (1077 pretreated as UW, 20 as UW*, and 7 as WW). Figure 1 illustrates the normalized deviations (given in terms of standard deviation, σ) between the mean ^{14}C determination of these tree-ring standards and their expected ^{14}C values. These are plotted against the theoretical spread of data about the mean that would be expected according to a standardized Gaussian distribution. The agreement between the empirical and expected values is good, demonstrating the long-term reproducibility of ^{14}C measurement on wood at ORAU.

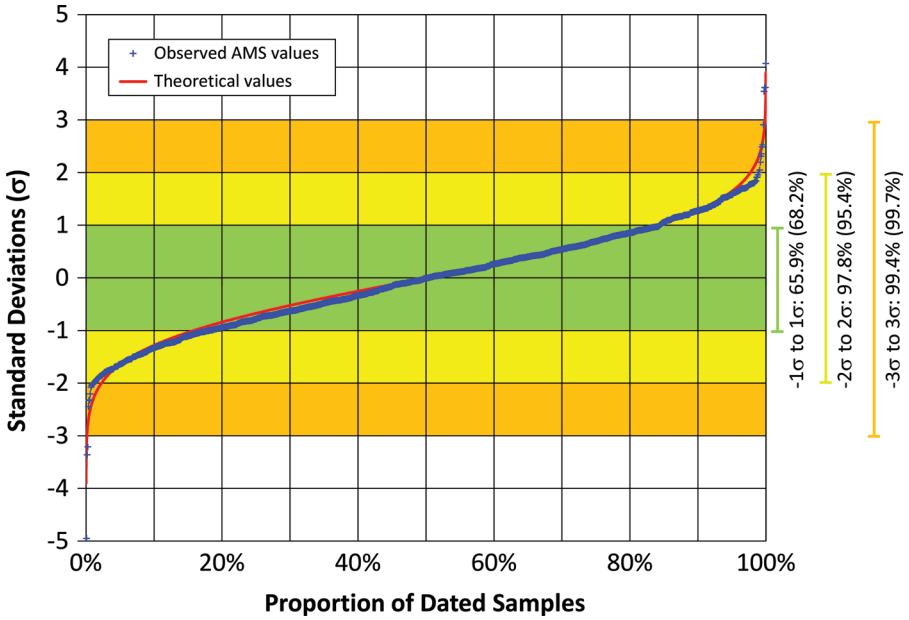

Figure 1 The observed normalized deviation of ^{14}C determinations from their expected values for known-age wood standards at ORAU (i.e. P180, P181, P9029, P9030, P9031, P9032, P9033, P9034, P9035, P10675, P10677, P10679, P14622, and P14623) between September 2002 and June 2013 (n = 1104), as compared to that expected from a standardized Gaussian distribution. The 1σ, 2σ, and 3σ ranges of the observed deviations from their "true" values are indicated on the right-hand side of the figure; theoretical (expected) values are shown in parentheses.

Between September 2002 and June 2013, 87 ^{14}C measurements have been obtained on background-age wood standards at ORAU with our routine UW pretreatment (i.e. P431, P4539, P10672, and P10673), plus a single UW pretreatment of background age kauri (P29851). These measurements are plotted against the combustion yield of each sample (given in mg C) in Figure 2. These demonstrate generally "good" background F^{14}C values, but with occasional younger outliers, most notably for the Chelford log (P431). The Chelford log is actually a very fragile sample, and we attribute these outlying measurements to variable preservation, and perhaps inhomogeneous contamination from unknown sources. The sturdier wood samples, including the sole kauri measurement, do not show this scatter.

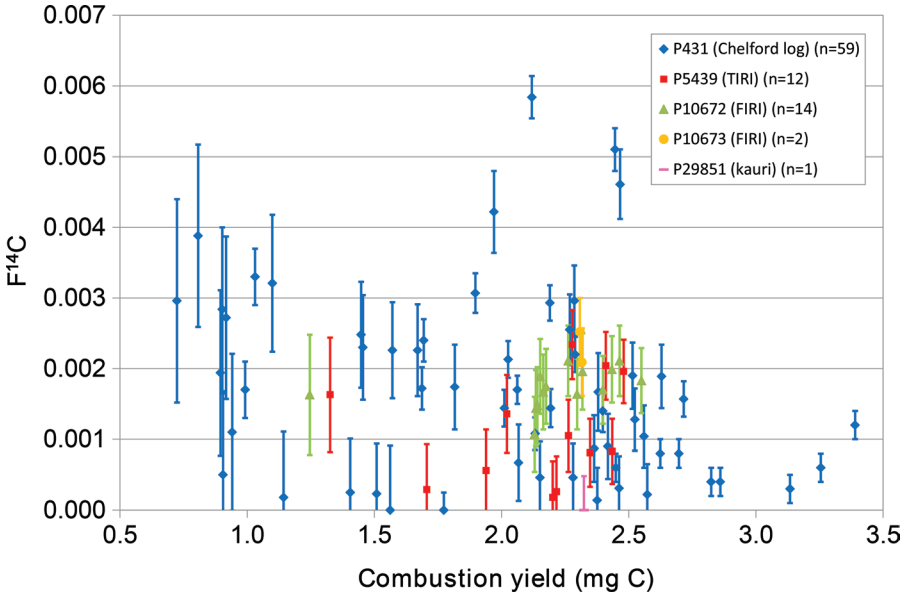

Figure 2 F^{14}C values (±1σ) for the background-age wood standards at ORAU (with routine UW pretreatment applied), plotted against combustion yield (mg C).

^{14}C dates issued by all ^{14}C laboratories are issued with unique sample identifiers; the ORAU lab issues dates with an OxA- prefix, while non-standard/experimental research measurements are differentiated by use of an OxA-X- prefix. Over the time period September 2002–June 2013, 1001 wood samples have been processed at ORAU and given the OxA- identifiers, comprising 726 samples processed with the UW pretreatment protocol, 183 with UW*, 71 with WW, 11 with WW*, and 10 processed with other "non-routine" chemistries for sample-specific reasons. A further 190 wood samples have been treated more "experimentally" (including kauri, see below), and given our OxA-X- sample identifiers accordingly.

In deriving the UA (α-cellulose) methodology described above, a variety of methods and variations were compared; specifically, these centered on the solvent extraction prior to the main α-cellulose purification. These included (i) the previous UA* methodology (as published by Hogg et al. 2013, and described above); (ii) a solvent extraction using the Dionex ASE system, applying the same settings as with the UA* method, but only flushing through with ultrapure water, rather than with acetone followed by ultrapure water; and (iii) a Sohxlet extraction, using acetone for ~8 hr, allowing the sample to dry overnight, and a second Soxhlet extraction with ultrapure water for a further ~8 hr. The routine ORAU UW method was additionally performed for comparison.

Figure 3A shows the results of applying these 5 methodological variations on kauri of background age (Oxygen Isotope Stage 7). Figure 3B shows the equivalent data for a modern (AD 2006/2007) kauri sample. Additionally, these 5 methodological variations were performed on six decadal blocks of kauri for which initial data (generated with the UA* pretreatment protocol) appeared anomalous compared with data obtained at the Waikato and University of California, Irvine (UCI) laboratories. The ORAU data are plotted in Figure 3C, along with the consensus values from multiple replicate measurements performed at UCI.

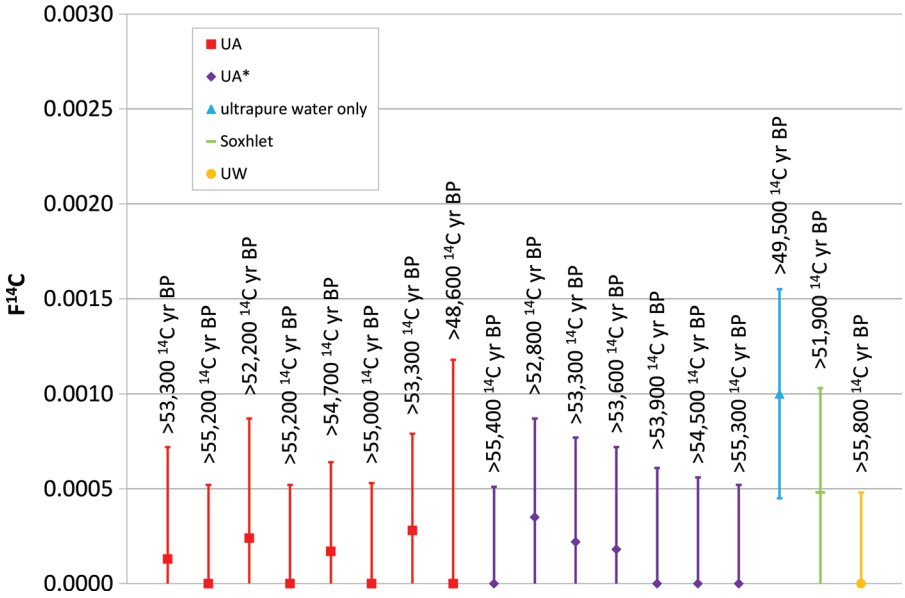

Figure 3A $F^{14}C$ determinations (±1σ) of background age (Oxygen Isotope Stage 7) kauri pretreated with 5 methodological variations.

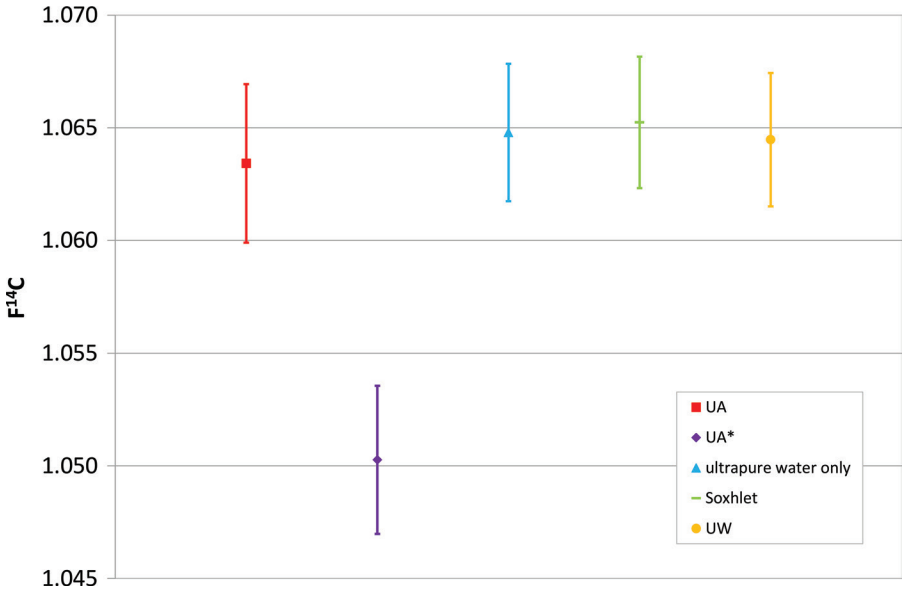

Figure 3B $F^{14}C$ determinations (±1σ) of modern (AD 2006/2007) kauri pretreated with 5 methodological variations.

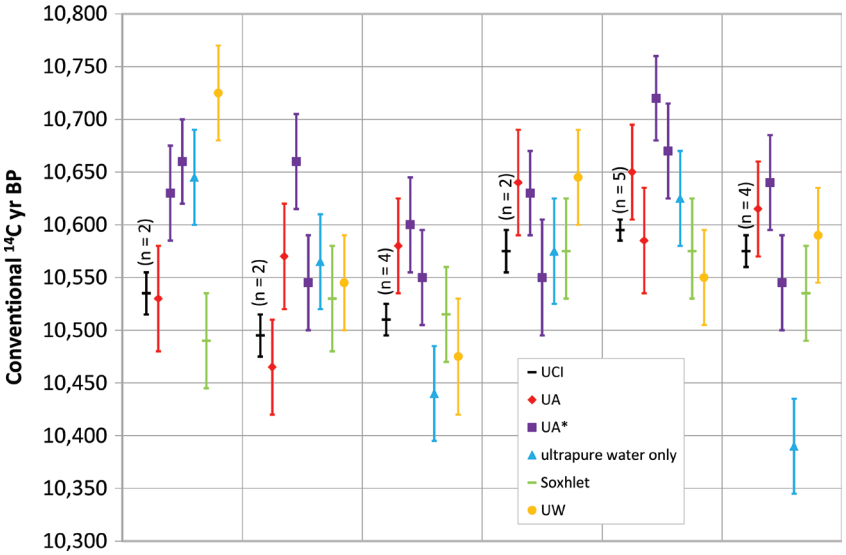

Figure 3C Conventional ^{14}C ages (±1σ) of six decadal kauri blocks pretreated with 5 methodological variations, as compared to consensus values from replicate measurements performed at UCI.

Implementation of both Soxhlet extraction and the UA method (i.e. no solvent extraction) show much improved agreement with the consensus UCI data (as compared with the previous UA* method). There is a suggestion from these data that some "old" contamination is resulting in these sporadic anomalous values at ORAU. This is supported by the reduced F^{14}C value for the modern kauri sample with the UA* methodology applied. Although further analysis would be required to fully understand the cause of this aging effect, it is suspected that old carbon from the acetone solvent wash is reacting chemically with the cellulose. Any physical (i.e. non-chemical) effect would be presumed to be removed by the subsequent ultrapure water rinse and strong chemical pretreatment. It might therefore be surmised that any such chemical reaction might be favored by the conditions within the ASE system (i.e. 100°C temperature and 116 atm. pressure). The effect is not consistent, with the majority of UA* data (across tens of other decadal blocks, not shown here) giving good agreement with the Waikato and UCI values. But for these 6 samples (which were initially selected because they were the only samples from a batch of 20 decadal blocks that gave these erroneous data compared to the other two labs), replication of the UA* method could again yield anomalously old data, though the effect was not consistent. We therefore suspect that particular inhomogeneous characteristics within the wood additionally favor the aging effect.

All measurements of the background-age kauri yielded ^{14}C determinations of infinite age. There is therefore no evidence for any incorporation of younger carbon contamination. This also serves to confirm the lack of a need for any pretreatment background over and above the combustion background already included within the F^{14}C/^{14}C date calculation.

While both Soxhlet extraction and the UA method (i.e. no solvent extraction) give reliable data (for modern and background kauri, and as compared to the intermediate aged kauri dated independently at Waikato and UCI), we decided to adopt the UA method as our new α-cellulose pretreatment protocol at ORAU, primarily due to the reduced processing time required. As described by Hogg et al. (2013; see also Southon and Magana 2010), this is in line with the pretreatment procedure for α-cellulose purification of the kauri at UCI (i.e. no solvent extraction applied), in contrast to the Waikato pretreatment, which does implement an initial Soxhlet extraction stage. The choice not

to perform the Soxhlet extraction at ORAU is also in keeping with the tenet that when processing samples for ^{14}C dating, it is preferential to adopt the least convoluted methodology where possible. Any additional stages might lead to an increased possibility of introducing contamination to samples through additional sample handling, though this must be balanced against optimal purification of and optimal removal of contaminant from the sample.

Since the problem of the incorporation of old carbon did not come to light immediately, a significant proportion of the ^{14}C measurements contributing to the New Zealand kauri (*Agathis australis*) Research Project were performed with the original UA* protocol (as described by Hogg et al. 2013). However, since the effect seems to be sporadic, rather than systematic, we have no reason to disregard measurements performed with the previous method, except for where data were in statistical disagreement with the measurements performed at Waikato and UCI. In this limited number of cases, samples have been repeated with the adoption of the revised UA protocol.

CONCLUSIONS

Since our present HVEE AMS system came on-line at ORAU in September 2002, >1000 ^{14}C determinations have been obtained on in-house tree-ring standards of known age. These data have been shown to be within the expected range, showing good reproducibility, and giving confidence in the measurement of "unknown" samples dated at the lab (>1000 samples through the same period). The routine ABA(-bleach) pretreatment protocols for regular wood sample chemical pretreatment have been revisited (ORAU pretreatment codes UW and WW, with and without a final bleaching stage, respectively), and a revised UA pretreatment protocol for α-cellulose has been presented, following evidence suggesting that some old carbon contamination was affecting the reliability of the previously adopted UA* methodology.

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