

# A Detailed Investigation Of Pretreatment Protocols For High Precision Radiocarbon Measurements Of Annual Tree-Rings

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## Abstract:

Much discussion has revolved around proper sample types and pretreatment methods to generate a calibration dataset that would allow for the most accurate representation of an unknown sample's calendar age. Since the development of radiocarbon dating, tree-rings have played a vital role in the creation of global calibration datasets, with these samples being subjected to varying degrees of both physical and chemical pretreatments. With an international move away from multi-ring composite measurements and toward higher resolution annual and/or sub-annual measurement of radiocarbon content, and with the on-set of a major new research project using, primarily the highly resinous North American Bristlecone Pine, we set out to review sample preparation procedures. The question became; which fraction, and therefore how much pretreatment is really necessary in order to extract the most representative and replicable C<sup>14</sup> determination in a tree-ring for any given growth year, and could it be both specimen as well as species dependent? For this study, a total of four samples, were chosen. These samples consisted of two rings from two different years from Bristlecone Pine and two rings from these same years from Irish Oak. These rings were each treated with a series of progressively more aggressive chemical treatment allowing for the extraction of different fractions from each ring. An analysis of the different pretreatment methods, as well as interspecies variance is discussed.

## Introduction:

The recent move to increase the precision and resolution of the calibration curve used to determine the calendar age of radiocarbon dated samples has led to a new round of discussions centered on determining the best sample type, as well as which fraction of such samples, is best suited for inclusion in the calibration curve. Tree-rings from several different species have already been included in past calibration curves, with several studies looking at different wood fractions and pretreatment protocols [1], [2], [3]. It is generally agreed that alpha cellulose is both the most robust and stable of the different fractions of wood and therefore thought by many to be the most appropriate for C<sup>14</sup> analysis. However, each subsequent wood fraction extraction increases both the time and cost for each sample. This can become prohibitive as the number of samples increases. One must also keep in mind the added issue of loss of sample mass during each subsequent extraction which can become problematic for raw samples of small mass. We chose to look at these different fractions in order to determine the statistical necessity of extraction down to the alpha cellulose fraction. The different pretreatment methods used were: no pretreatment (whole raw wood), ABA (Acid/Base/Acid) pretreatment (holocellulose + Lignin fraction) [4], ABA plus bleaching (holocellulose fraction) [5] and ABA plus bleaching plus base (alpha cellulose fraction) [5]. A similar study was carried out by J. Southon on old Kauri and Spruce in 2010 for samples at or near the limit of C<sup>14</sup> dating [1]. In order to determine if there was a potential species dependence, and/or ring age dependence, two different species with two single year tree-rings from each were chosen for this study. These two species were: Bristlecone Pine (*Pinus longaeva*) from the White Mountains of California, and Irish Oak (*Quercus petraea*) from Timahoe, Co. Laois, Ireland. The two years chosen were the 1507 BC and the 1617 BC rings, samples that have been securely dated in calendar years by dendrochronology.

## 50 **Methods:**

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52 Tree-ring samples were obtained from a research project involving Bristlecone Pine tree-ring chronologies  
53 developed by Matthew Salzer [7] and Irish Oak tree-ring chronologies developed by David Brown [8]. With  
54 the aim being to dissect sufficient material from each species/year for multiple replicate measurements, two  
55 growth years (1617 BC and 1507 BC) were selected in which environmental conditions in both California  
56 (for Bristlecone Pine) and Ireland (for Irish Oak) were favorable, with respect to their species, for “wide”  
57 tree-ring formation. From each ring, four, 2mg fractions were weighed out and put directly into 9mm quartz  
58 combustion tubes for CO<sub>2</sub> extraction (*No Pretreatment*). The remaining samples were weighed and then  
59 treated with our standard ABA pretreatment [4]. This method consists of an overnight soak at 70° C in 3N  
60 HCl. The samples were then rinsed to a neutral pH with Type-1 (ultra-pure) water and immersed in a 1.2N  
61 NaOH at 70° C overnight. The following morning the level of humic contaminants was noted and the  
62 samples were rinsed with Type-1 water. The samples were put back into NaOH for 2 hours at 70° C and  
63 monitored. This step was repeated until all humic contaminants were removed. The samples were again  
64 rinsed to a neutral pH with Type-1 water and then immersed in a 3N HCl at 70° C overnight to neutralize  
65 any trace amounts of NaOH and to drive off any absorbed CO<sub>2</sub>. The samples were dried in a vacuum oven  
66 at room temperature and weighed to determine mass loss during pretreatment (*ABA Pretreatment*) [4]. Four  
67 2mg fractions were separated and placed directly into a 9mm quartz combustion tube for CO<sub>2</sub> extraction.  
68 The remainder was weighed and then placed into a bleaching solution overnight at 75° C. The bleaching  
69 solution is mixed in-house in 1 liter batches using the following ingredients: 810ml Type-1 water, 20ml 3N  
70 HCl, 27g Sodium Chlorite. Fresh batches are made weekly as the bleaching efficiency of the solution  
71 decreases as the chlorine evolves over time. Spot checks are also conducted on the pH of the solution in  
72 order to ensure that the pH stays within the appropriate range (between 2 and 4) to ensure bleaching  
73 efficiency is not compromised. The following morning the samples were checked and re-bleached when  
74 necessary. Once fully bleached, the samples were rinsed with Type-1 water, dried in a vacuum oven at room  
75 temperature and weighed to determine mass loss during the bleaching (*holocellulose Extraction*) [5]. When  
76 possible, four, 2mg fractions were separated and placed directly into a 9mm quartz combustion tube for CO<sub>2</sub>  
77 extraction. Unfortunately, one of the samples (1507 BC Bristlecone Pine) only yielded enough holocellulose  
78 to allow for three, 2mg fractions. The remainder was reweighed and then placed into 17.5% NaOH at room  
79 temperature for 2 hours, rinsed to a neutral pH with Type-1 water, acidified with 3N HCl for 1 hour, rinsed  
80 to a neutral pH with Type-1 water, dried in a vacuum oven at room temperature, and weighed to determine  
81 mass loss during the extraction (*Alpha Cellulose Extraction*) [5]. This fraction was separated into 2mg  
82 portions and placed into 9mm quartz combustion tubes for CO<sub>2</sub> extraction.

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84 Copper(II) oxide was added to the packed combustion tubes in a ratio of 100mg CuO per 1mg of expected  
85 carbon. The combustion tube was placed on a vacuum system and evacuated to approximately 10<sup>-5</sup> torr. The  
86 CuO/sample was isolated from vacuum and combusted on line at approximately 1000° C. The liberated  
87 gases were passed through two water (dry ice/isopropyl) traps and a copper/silver furnace at 450° C in order  
88 to purify the CO<sub>2</sub>. The purified CO<sub>2</sub> was manometrically quantified and frozen into a vessel. Iron powder  
89 was weighed out at a ratio of 2mg of iron to 1mg of carbon, and put into a 6mm quartz tube. Approximately  
90 100mg of zinc granules were also put into a separate 6mm quartz tube. These tubes, along with the vessel  
91 containing the purified CO<sub>2</sub>, were attached to a graphite reduction vacuum apparatus. After evacuating the  
92 iron and zinc tubes, the CO<sub>2</sub> was introduced, the zinc and iron were heated to 450° C and 550° C,  
93 respectively, and the gas was allowed to reduce to iron carbide powder using standard iron/zinc reduction  
94 technique [6]. Once the reduction was complete (>98%), the resulting “graphite” was taken for AMS  
95 analysis. All measurements were performed on our 3 MV NEC Pelletron particle accelerator. Each sample  
96 was separately measured at least 3 times, with the resulting data being combined using standard statistical  
97 techniques (weighted mean). The data from identical samples were then combined, again using standard  
98 statistical techniques. These steps were taken to limit dependence on both processing as well as potential  
99 equipment instability.

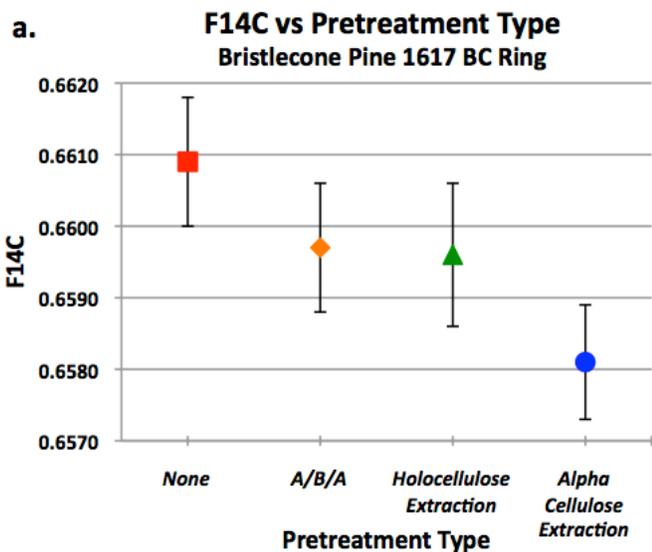
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## 101 Discussion and Results:

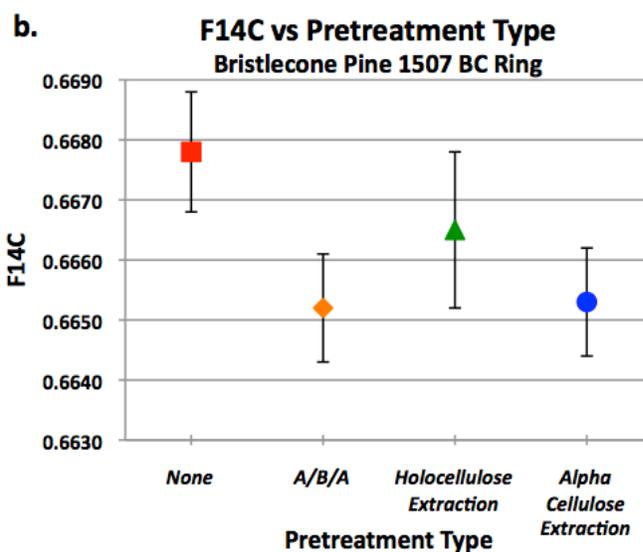
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103 It is agreed that alpha cellulose is the most stable fraction of a piece of wood and that for wood from certain  
104 preservation environments the step to alpha cellulose can be essential to remove younger contaminants  
105 (Southon and Magana, 2010 [1]). However, there is discussion as to whether or not one needs to go to the  
106 length of extracting this fraction in all cases in order to ensure accurate, concise and reproducible results for  
107 high-resolution tree-ring dating. For example, in the study by Southon and Magana [1], four out of the five  
108 wood samples tested indicated that wood pretreatment via ABA methods yielded results that were not  
109 significantly different to  $C^{14}$  values for holo or alpha cellulose extractions. In the hopes of bringing some  
110 clarity to this discussion two sets of known-age tree-rings, (1617 BC and 1507 BC) from two different  
111 species from differing geographic and ecological regions, were chosen. Four different fractions of these  
112 rings (each subsequent fraction extracted from the previous pretreatment), were measured for radiocarbon  
113 and stable carbon isotope content.

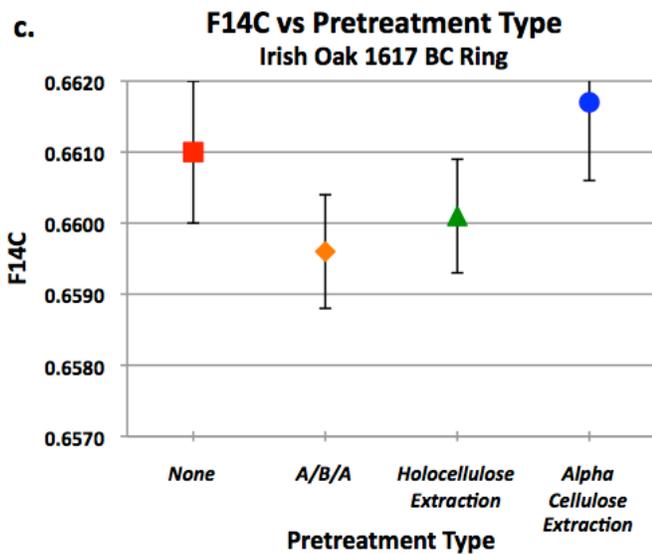
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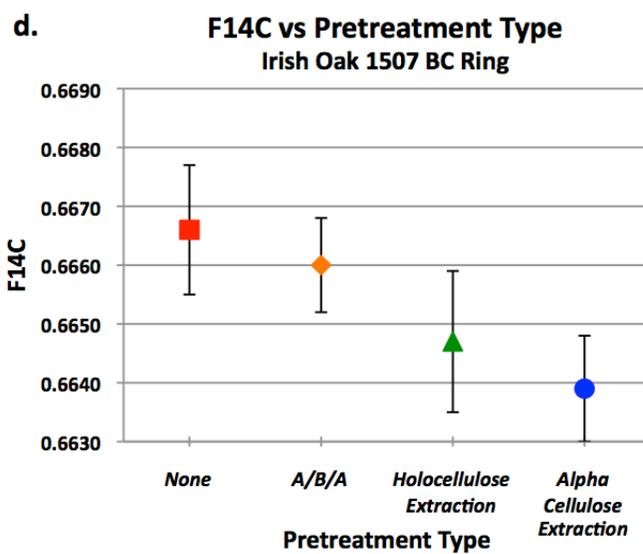
117 Fig. 1a



120 Fig. 1b



123 Fig. 1c



126 Fig. 1d

127 Figures 1a-d: Changes in fraction modern for each ring tested based on the pretreatment it received.

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As can be seen from the results, the data (Fig. 1a-d) for both growth years of the Bristlecone Pine as well as the Irish Oak show that for ABA and holocellulose pretreated samples, the radiocarbon content of the samples decrease from the un-treated samples. This would be consistent with the removal of a younger contaminate. However, there is little to no statistically relevant difference between ABA and holocellulose pretreatments. For all but the 1617 BC Irish Oak ring, as expected, we see a further decrease in radiocarbon content compared to the ABA and holocellulose pretreated samples. The increase in radiocarbon for the 1617 BC Irish Oak ring is consistent with either the addition of younger carbon, or the removal of an older contaminate. However, from a statistical standpoint, there is little to no difference in the fraction modern of samples that underwent an ABA or stronger pretreatment.

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As an additional check, a “common mean” Fraction Modern value was calculated for each ring. The “common mean” was calculated by taking the weighted mean for all fractions of a ring. The deviation from this “common mean” was then calculated for each pretreatment type. The deviation from this “mean” for each pretreatment type was then statistically combined (weighted mean) to see if any further information could be teased out of the data (Fig. 2).

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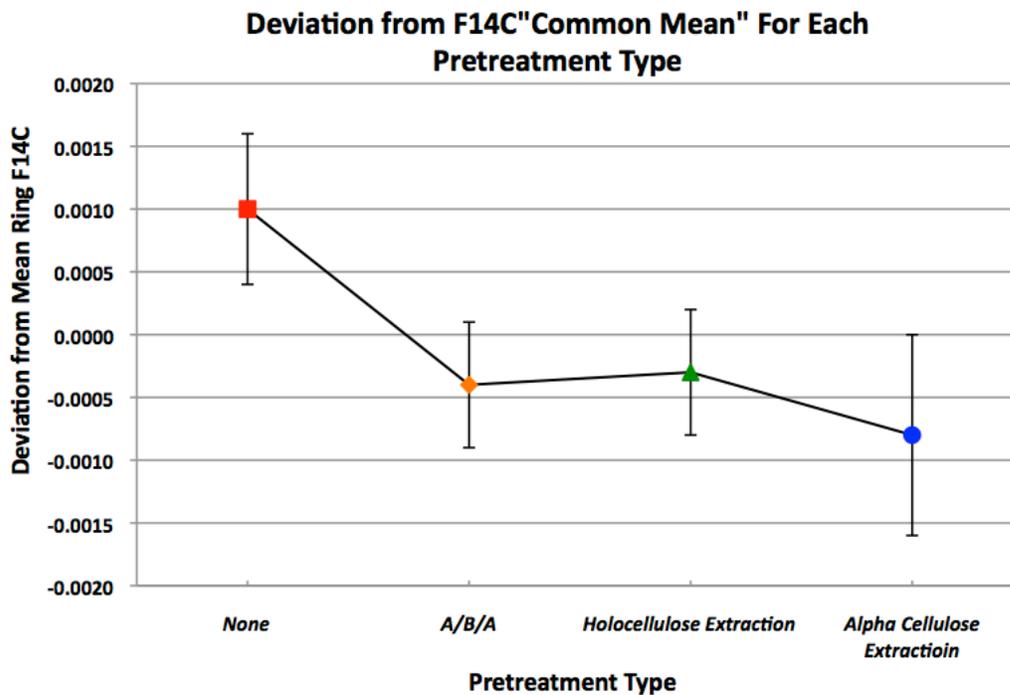


Fig. 2

Figure 2: Deviation from the “common mean” fraction modern for each pretreatment type

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As can be seen from the above figure, there is no statistically relevant difference between any of the pretreated fraction, although there is a significant difference between treated and untreated wood. Based on this data, no conclusive statement can be made as to what the “true” or most representative age for these samples are. The only conclusion we can really draw is that some pretreatment is necessary.

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Stable isotope measurements were also conducted on each fraction of each ring. A similar “common mean” analysis was done on this data (Fig. 3).

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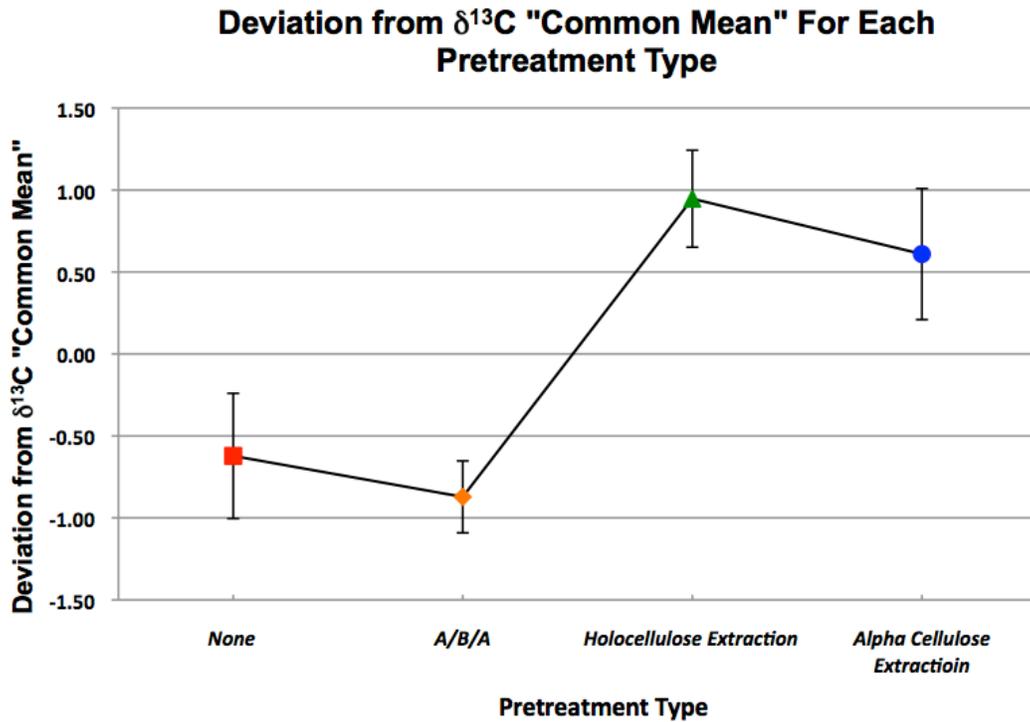


Fig. 3

Figure 3: Deviation from  $\delta^{13}\text{C}$  "Common Mean" for each pretreatment type.

From the above data, it is much easier to see that with each subsequent pretreatment, we are removing some fraction of the sample and changing the sample's isotopic composition. Here, no statistically relevant difference can be seen between untreated and ABA pretreated samples. However, a substantial difference can be seen between the untreated/ABA pretreated and the cellulose extracted sample, although no statistically relevant difference can be seen between holocellulose and alpha cellulose.

Also of interest, there seems to be a trend away from the accepted calibration curve in the direction of decreasing radiocarbon for wood samples that underwent, at a minimum, an ABA pretreatment (Fig. 4a,b). Although the current calibration curve is composed of multi-ring samples, it can still be used as a basis of comparison for this study as it should represent an averaged and smoothed measurement of radiocarbon across our two calendar dated years. The fact that there is a discrepancy may reflect this smoothing process, or perhaps indicate differences in pre-treatment methodology for samples making up the curve. When compared with the spread of the raw IntCal13 [9] data however, all points are within error of contemporary blocked measurement equivalents. So, the differences we are observing are minor in relation to the accepted spread of error on samples from the same calendar dated years between labs.

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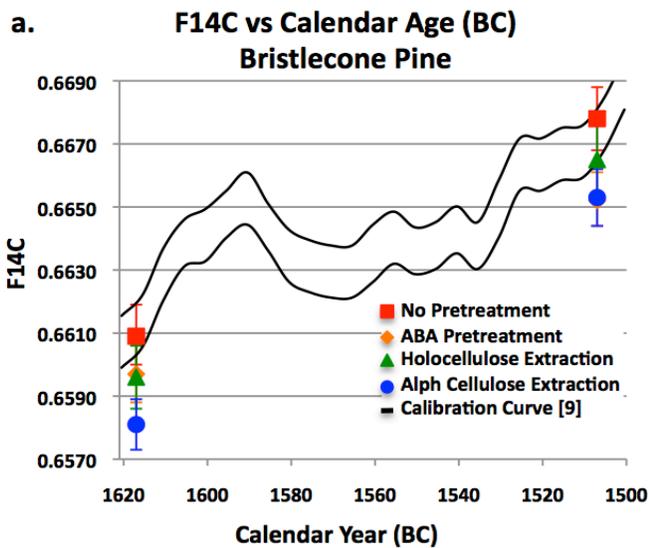


Fig. 4a

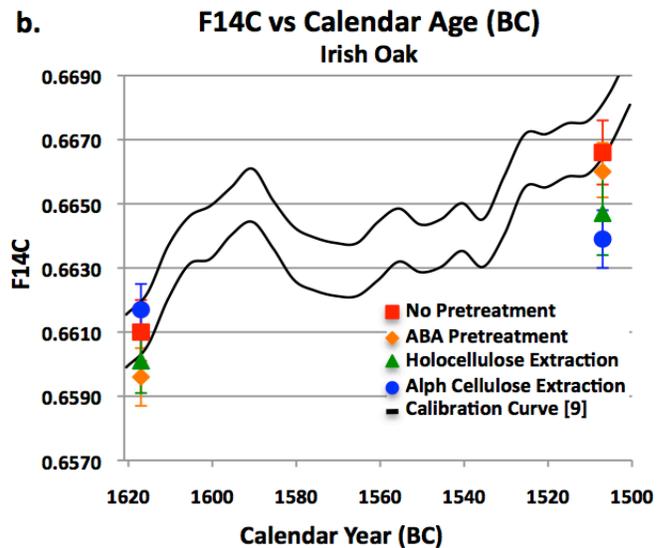


Fig. 4b

Figure 4a-b: Results for different pretreatments on Bristlecone Pine and Irish Oak compared to Intcal 13 [9].

### Conclusion:

For three of the four samples measured, there is very little evidence to suggest that any pretreatment beyond holocellulose extraction is necessary for annual high-resolution radiocarbon dating of either Bristlecone Pine or Irish Oak during this time period. The same conclusion can be drawn from the stable isotope measurements in which no statistically relevant difference can be seen between alpha and holocellulose. So, we concluded that if, as expected, the “true” age of the sample is associated with the most stable fraction (alpha cellulose) of the wood, for these species, during this time period, stopping the extraction at the holocellulose fraction yields statistically the same radiocarbon age. Unexpectedly, the result for the 1617 BC Irish Oak ring did not show this same behavior and is believed to be due to a technical error that occurred during the alpha cellulose extraction. Unfortunately, there was no sample remaining from this particular ring, so a repeat of the experiment to test this theory is unlikely. We hope to try on an adjacent ring to clarify this discrepancy. If, as expected, the adjacent ring falls in line with the other results, the aggressiveness of the pretreatment necessary for concise high-resolution dates appear to have no temporal or species dependence. We also note that comparison of the  $C^{14}$  measurements on different pretreated fractions of our two different wood species for the same years is very consistent, indicating little or no regional  $C^{14}$  variability between California and Ireland for the years tested. Of potentially greater import, the majority of the data (83%) falls below the accepted calibration curve [9] for this time period for both species. Samples with less or no pretreatment conform more closely to the curve. Other studies, including Miyake [10], have also noted offsets from the calibration curve when looking at annual resolution  $C^{14}$  data. The major annual data series now published for the same time period (Pearson [11]) shows that the off-set observed in the data presented here is sustained by both species of trees for a substantial time period (from around 1650 BC to 1540 BC). However, from 1700 BC to 1650 BC the same data sequence directly overlies IntCal13 [9]. Pretreatments for all samples in this time series were standardized holocellulose treatments, so this implies either that the off-set is more likely due to differences in pretreatments for different batches of raw data included in the current IntCal13 [9], or is related to the smoothing associated with the construction of the IntCal13 [9] curve. If the off-set observed is pretreatment related, it may be due to differences in the lab pretreatment protocols for samples currently included in IntCal13 [9].

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