

High-throughput α -cellulose extraction method for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ stable isotope ratio analysis of tree water use efficiency in loblolly pine



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INTRODUCTION

Wood stable isotope ratio ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) sheds insight to water use efficiency (WUE) in loblolly pines in Tier II and III sites. Drought-induced reduction of stomatal conductance results in lower ^{13}C content in assimilated carbohydrates and in plant structures. The relationship between $\delta^{13}\text{C}$ in plant tissue and WUE has thus been established. But $\delta^{13}\text{C}$ is also influenced by photosynthetic rate. Because the effect of photosynthesis on the difference between $\delta^{18}\text{O}$ in plant tissue and in source water is small (Barbour 2007), the combination study of ^{13}C and ^{18}O of plant tissue would provide information to separate stomatal and photosynthetic effects on water use efficiency. Due to its local and temporal fixation and high relative abundance within the wood (McCarroll and Loader, 2004), α -cellulose has been the preferred sample material for this purpose. To obtain true annual resolution in the isotopic record, only latewood would be used for α -cellulose extraction because it is produced from current year assimilates (McCarroll and Loader, 2004). Various methods for α -cellulose extraction from wood are available. However, due to the large number and low weight of latewood samples we would obtain for PINEMAP, traditional methods are no longer suitable. Therefore, based on a method by Wieloch et al. (2011) from Potsdam Dendro Laboratory, we developed this high-throughput approach for α -cellulose extraction from loblolly pine wood samples.

PRINCIPLE OF α -CELLULOSE EXTRACTION

Grind or slice wood samples	Pretreatment (exclude resin) <ul style="list-style-type: none"> Use organic solvents for conifers only 	Bleaching for holocellulose <ul style="list-style-type: none"> Use acidified NaClO_2 solution 	NaOH treatment for α -cellulose <ul style="list-style-type: none"> Use 17.5% NaOH solution
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COMPARISON OF METHODS

Various methods for α -cellulose extraction are available. We chose the following methods for comparison based on literature review of the topic and recommendations from experts of this area.

Methods	Leavitt and Danzer (1993)	Loader et al (1997)	Yokoyama et al (2002)	A method from Dr. Nakastuka in Nagoya University, Japan	Wieloch et al (2011)
	Medium:	High:	Low:	Medium:	High:
Equipment requirement	Soxhlet extractor, special filter paper bags	Customized Soxhlet extraction thimbles, oversized ultrasonic bath	Buchner funnels	ultrasonic cleaner, customized polyethylene filters	customized multiple sample isolation system, Buchner funnels
Processing time	3 days	2 days	2 days	4 days	5 days
Working time /processing time	2/3	3/4	1/2	2/3	1/4
Bleaching and NaOH treatment					
No. of samples per batch	75 rings	50 rings	8 rings	60 rings	320 rings
Conduct multiple batches by one person	No	No	Two batches	No	NA
Popularity	Popular in dendroclimatology	Popular in dendroclimatology	Used in wood science, but not in dendroclimatology	Not published, but used at this laboratory for a long time	New method. Used in Potsdam Dendro Laboratory
Pretreatment					
Extra equipment required	No	Yes	No	No	NA
Processing time	2 days	1 day	8 days	1 day	

SELECTION CRITERION

Throughput: About 2700 samples will be analyzed for PINEMAP. The traditional methods of Loader et al. (1997) or Leavitt and Danzer (1993) would require 1-2 years of working time. The throughput of methods was the primary selection criterion.

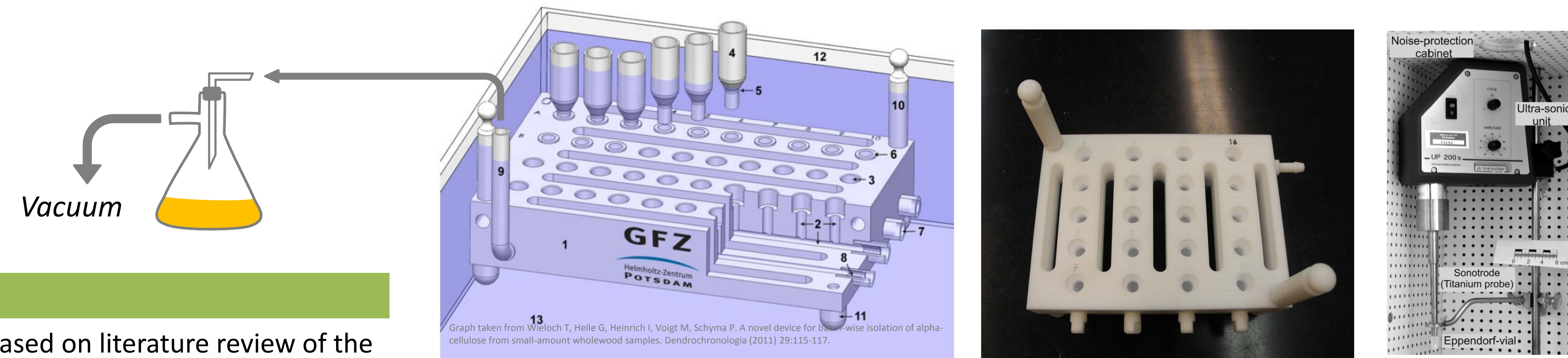
Individual sample size: the weight of latewood samples for PINEMAP is 1/5 of the wood samples commonly used for α -cellulose extraction. The limited sample size poses additional constraints on acceptable α -cellulose extraction methods.

PRETREATMENT

α -cellulose is typically extracted from resin-free wood samples. This step effectively doubles the extraction time. It has been suggested that in some species the resin exclusion pre-treatment did not alter the $\delta^{13}\text{C}$ signatures of the α -cellulose extract (Rinne et al. 2005). A preliminary analysis with loblolly pine samples has shown the α -cellulose yield to be more consistent with resin-excluded samples compared to un-treated samples ($28.3 \pm 1.6\%$ and $37.8 \pm 8.4\%$, respectively). For PINEMAP samples, instead of the commonly used approach of high-temperature processing in Soxhlet extractor (Loader et al., 1997), we will try to use acetone incubation as described by Yokoyama et al. (2002).

BLEACHING AND NaOH TREATMENT

We chose the method by Wieloch et al. (2011) for both steps. Wieloch et al. developed a multiple sample isolation system for solids (MSISS) for treating large numbers of small wood samples simultaneously. It consists of a drainage module, Buchner funnels, a water bath, and a hot plate. The funnels are equipped with sintered glass disks so that reaction would take place in funnels when no vacuum aspiration is applied to the drainage module (the left graph below). We made our drainage module at Precision Instrument Machine Shop of NCSU (the middle graph below).



SAMPLE HOMOGENIZATION

The obtained α -cellulose under the above procedure is a mixture of small chips and fibers. After testing several methods, we decided to follow Laumer et al. (2009), powderizing it with an ultrasonic homogenizer (the right graph above, taken from Laumer et al. 2009)

FUTURE WORK

The whole system for cellulose extraction was established and a test run have been conducted. Samples from a Tier2 site at Plymouth, NC, have been extracted and will be analyzed for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$.

The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ information would be used to for model parameterization (Aim 2), characterize variation in tree WUE by genotype, tree age, and region, and provide a physiological understanding of underlying mechanisms for the relationship between regions and genotype under different climate change scenarios.

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The photos for the process for α -cellulose extraction

