A conifer-friendly high-throughput α-cellulose extraction method for ^{13}C and ^{18}O stable isotope ratio analysis

Wen Lin[1], Asko Noormets[1], JC Domec[1,3], John King[1], Ge Sun[2], Steve McNulty[2]
[1]North Carolina State University, Raleigh, NC, USA; [2]USDA Forest Service, Raleigh, NC, USA;
[3]Bordeaux Science Agro UMR TCEM 1220, FRANCE

DEPARTMENT of FORESTRY and ENVIRONMENTAL RESOURCES

INTRODUCTION

Wood stable isotope ratios ($\delta^{13}C$ and $\delta^{18}O$) offer insight to water source and plant water use efficiency (WUE), which in turn provide a glimpse to potential plant responses to changing climate. To use the effects of recent severe droughts on the WUE of loblolly pine throughout Southeastern USA as a harbinger of future changes, an effort has been undertaken to sample the entire range of the species for the isotopic composition in a consistent manner.

Collect tree cores → Chisel latewood from the desired ring → Extract α-cellulose from the latewood → Obtain δ13C and δ18O data → Convert to WUE

- Field
- *NCSU Tree physiology lab*
- *NCSU Tree physiology lab*
- Isotopic analysis lab
- *Computer*

Although α-cellulose is broadly seen as the best indicator of plant water status (Gaudinski et al., 2005; McCarroll and Loader, 2004), the extraction of α-cellulose from wood becomes a rate-limiting step in such an endeavor due to its requirements on intensive time and labor input.

Pretreatment to remove extractives	Bleaching for holocellulose	Purification for α-cellulose
• Use organic solvents	• Use acified $NaClO_2$ solution	• Use 17% NaOH solution

The recent development of a multiport extraction system (MSISS, Multiple Sample Isolation System for Solids) in the Potsdam Dendro Laboratory (Wieloch et al., 2011) allows up to at least 4-fold higher throughput compared to the most commonly used techniques. However, this method doesn't include a pretreatment step for removing the extractives, which is often required for softwood species. Traditional pretreatments (Green, 1963; Loader et al., 1997) could not be adapted for the multiport extraction system. On the other hand, the α-cellulose extraction method developed by Yokoyama et al. (2002) used a simple substitute (acetone).

Therefore, **the objective of the current study** was to test if the acetone pretreatment is suitable for application in MSISS by comparing the results to those with traditional extraction techniques.

TWO PRETREATMENTS

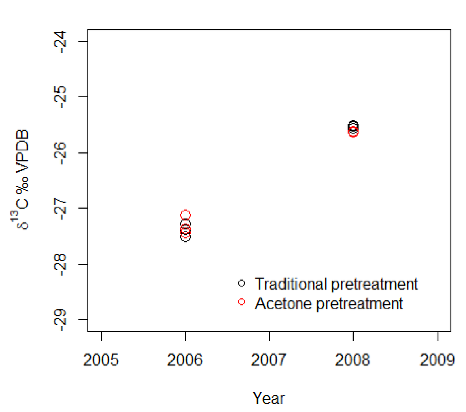
Traditional pretreatment by Loader et al. (1997): reflux wood samples in Soxhlet extractors using 2:1 toluene and denatured alcohol for at least 6 hours.

Acetone pretreatment by Yokoyama et al. (2002) (slightly revised): soak dried wood slivers in water overnight, then in acetone for 8 days , and change acetone every 2 days.

PREILMINARY RESULTS

Using latewood samples of a loblolly pine from Washington County, NC, we tested the effects of pretreatments and contrasting climate (wet vs. dry years) on both δ13C and δ18O of α-cellulose.

ANOVA indicated there was no significant difference between traditional and acetone methods for both δ13C and δ18O (p-values 0.99 and 0.12, respectively). The effect of climate was significant ($p<0.01$). No interaction between climate and pretreatment was significant.

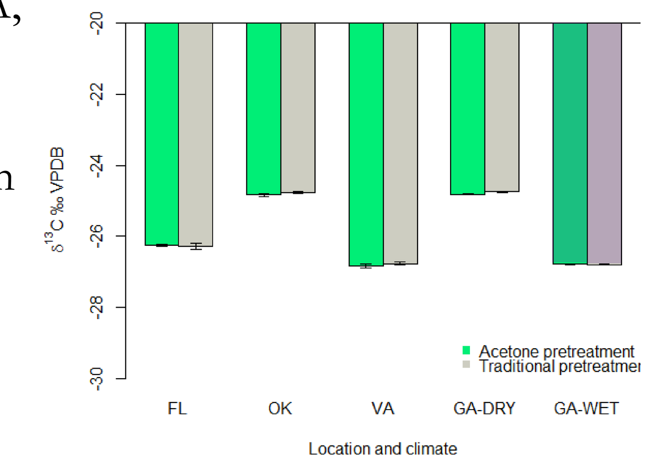


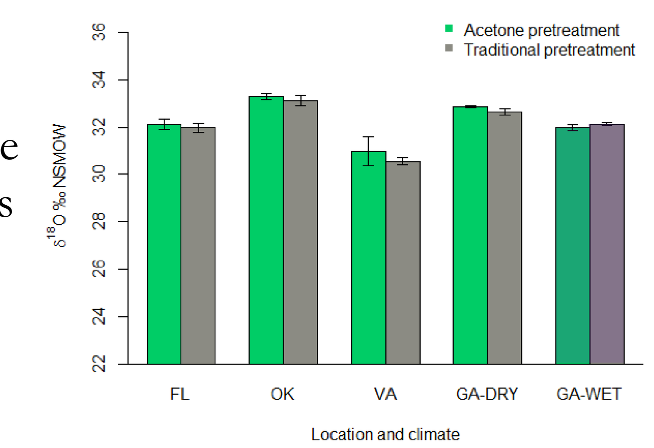
VERIFICATION STUDY AT A LARGER SCALE

Using latewood samples of loblolly pines from Clark County, GA, Buckingham County, VA, McCurtain County, OK, Alachua County, FL, α-cellulose was extracted and δ13C and δ18O measured.

Combined with preliminary data, the p-values from ANOVA with unbalanced designs for the main effect of pretreatment for δ13C and δ18O are 0.58 and 0.19, respectively. The effects of location were significant (p-values <0.01), while the interactions between location and pretreatment were not. Therefore, we conclude that acetone is effective in removing extractives from coniferous wood.

We also tested the effects of climate on δ13C and δ18O of α-cellulose extracted from latewood samples from GA, where experienced more exceptional droughts. The effects from climate were significant for both δ13C and δ18O (p-values <0.01). The interactions between climate and pretreatment were not significant as well.





FUTURE WORK

The precision of the isotope ratio mass spectrometry (IRMS) method is ±0.2‰ for carbon and ±0.3‰ for oxygen (Boettger et al., 2007). A few data points deviated from the mean values may be attributed to the incomplete bleaching step during the extraction process. The experimental protocol obtained from Potsdam Dendro Lab would be revised. Thus the hypothesis that the differences of the stable isotope results produced by two pretreatments fall within the precision of IRMS method would be tested using a revised protocol.

Sample purity of α-cellulose from different extraction techniques will be evaluated with infrared spectroscopy similar to Rinne et al. (2005).

The α-cellulose extraction method combined with acetone pretreatment for coniferous wood would be tested using wood samples of Norway spruce (*Picea abies*), Fraser fir (*Abies fraseri*), Ponderosa pine (*Pinus ponderosa*), Douglas-fir (*Pseudotsuga menziesii*), and black spruce (*Picea mariana*).

ACKNOWLEDGEMENTS

We acknowledge the generous help, insightful discussions and sharing of unpublished results by **Thomas Wieloch**, **Wei Liang**, and **Takeshi Nakatsuka** that formed the basis for the method reported here. **Jameel Hasan**, **Hou-min Chang**, **Ilona Peszlen**, and **Reza A. Ghiladi** generously allowed access to sample preparation and extraction equipment and were a valuable resource about wood chemistry and formation. **Andrew Laviner** , **Anu Sõber**, **Barbara Lachenbruch**, **Ben Bond-Lamberty**, **Brian Amiro**, **Geoff Lokuta**, **Madison Akers** and **Rodney Will** kindly provided wood samples.

REFERENCES

- D. McCarroll, N. J. Loader, Stable isotopes in tree rings. *Quaternary Science Reviews* **23**, 771 (2004).
- J. B. Gaudinski *et al.*, Comparative analysis of cellulose preparation techniques for use with 13C, 14C, and 18O isotopic measurements. *Analytical Chemistry* **77**, 7212 (2005).
- T. Wieloch, G. Helle, I. Heinrich, M. Voigt, P. Schyma, A novel device for batch-wise isolation of α-cellulose from small-amount wholewood samples. *Dendrochronologia* **29**, 115 (2011).
- J. W. Green, in *Methods in carbohydrate chemistry,* R. L. Whistler, Ed. (Academic Press, New York and London, 1963), vol. III, pp. 9-21.
- N. Loader, I. Robertson, A. Barker, V. Switsur, J. Waterhouse, An improved technique for the batch processing of small wholewood samples to α-cellulose. *Chemical Geology* **136**, 313 (1997).
- T. Yokoyama, J. Kadla, H. Chang, Microanalytical method for the characterization of fiber components and morphology of woody plants. *Journal of agricultural and food chemistry* **50**, 1040 (2002).
- W. Laumer *et al.*, A novel approach for the homogenization of cellulose to use micro-amounts for stable isotope analyses. *Rapid Communications in Mass Spectrometry* **23**, 1934 (2009).
- T. Boettger *et al.*, Wood cellulose preparation methods and mass spectrometric analyses of δ13C, δ18O, and nonexchangeable δ2H values in cellulose, sugar, and starch: An interlaboratory comparison. *Analytical Chemistry* **79**, 4603 (Jun, 2007).
- K. T. Rinne *et al.*, On the purification of alpha-cellulose from resinous wood for stable isotope (H, C and O) analysis. *Chemical Geology* **222**, 75 (Oct 20, 2005).

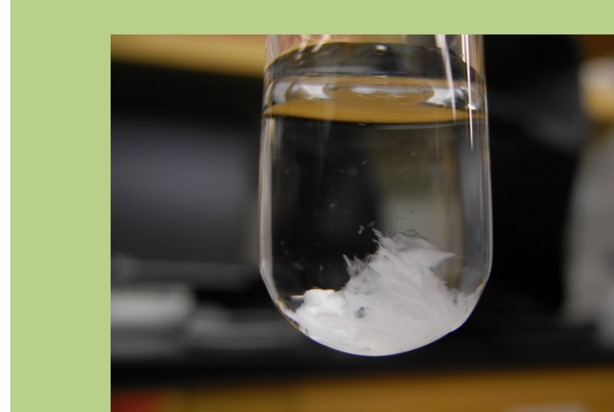
The process for α-cellulose extraction

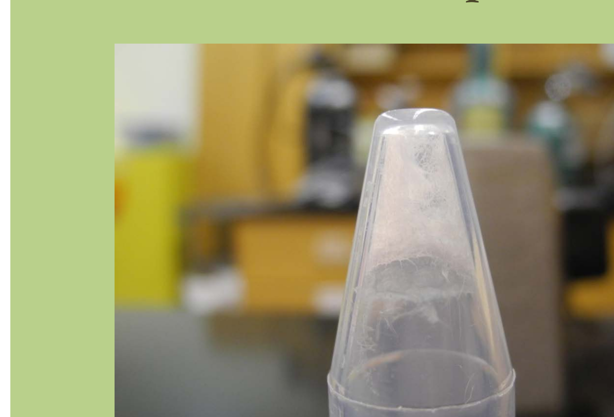

Taking cores


Making wood slivers


Acetone pretreatment


Bleaching and purification


α-cellulose in chips/fibers


Homogenized α-cellulose