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Pliocene Wood from the Gray Fossil Site

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By

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Abstract

The Gray Fossil Site in northeastern Tennessee preserves materials from a 5-million-year-old ecosystem, including wood from nearby trees. This study consists of three parts: conservation of wood remains, identification of taxonomic groups represented by the fossil wood, and measuring the organic content of fossil wood from the Gray Fossil Site. When excavated, wood specimens from the site are saturated due to a high local water table. After testing seven different techniques to dry wood specimens, wrapping a specimen in string and allowing it to dry slowly was the method least likely to cause warping and cracking. Microscopic examination of wood cross sections reveal tree rings with distinct anatomical features, with implications for taxonomic identification. Tentatively identified taxa that are present at the Gray Fossil Site are similar to those present in pre-modern forests of northeastern Tennessee. Finally, loss on ignition tests indicate that the Gray Fossil Site wood lacks extensive permineralization or mineral replacement. The presence of alpha-cellulose, albeit stained with iron oxides, illustrates the potential for future stable isotope analyses.

Introduction

Well-preserved fossil wood that is potentially suitable for dendrological studies has been recovered from the early Pliocene-aged Gray Fossil Site in northeastern Tennessee. The Gray Fossil Site is considered to be 4.5-4.9 million years old based on mammalian biochronology (Samuels et al. 2018, Wallace and Wang 2004). To date, most paleontological research that has been performed on this assemblage has focused on vertebrate fossils (Samuels et al. 2018). Paleobotanical research has emphasized carpaceous seeds (Hermsen 2021, Siegert and Hermsen 2020, Quirk and Hermsen 2021) and pollen (Liu and Quan 2020, Ochoa et al. 2012, Ochoa et al. 2016, Zobaa et al. 2011). Wood is one of the most common paleobotanical materials excavated at the Gray Fossil Site, yet it remains understudied.

Fig. 1. Map showing the location of the Gray Fossil Site.

Wood specimens excavated from the Gray Fossil Site are saturated due to a locally high water table. Since saturated wood from the Gray Fossil Site is prone to cracking and warping there was a need to establish best practices for drying and preparing specimens for dendrochronological analysis. Cracking and warping that occurred during drying under seven different methods were visually evaluated. Wrapping a specimen in cotton string followed by slow drying was ideal for reducing cracks and spalling (Madsen and Widga, 2020). The results of this study also revealed the presence of preserved, visible tree rings.

Previous researchers have identified Gray Fossil Site wood as cypress or other thermophilic taxa (Brandon, 2013) based partly on climate reconstructions that relied on the presence of *Alligator* to infer a warmer climate. Plant micro and macrofossils from the Gray Fossil Site (e.g., pollen, seeds, leaves) indicate the presence of *Pinus, Quercus,* and *Carya,* as well as taxa with Asiatic origins, *Vitis* and *Sinomenium* (Baumgartner, 2014, Ochoa et al. 2016, Zobaa et al. 2011). Other early research suggested that the wood was charcoalized and thought to be a result of occasional fire events (Zobaa et al. 2011), though later analyses have found it to not be charcoal (Baumgartner, 2014).

Few annually-resolved records of climate variability at the Gray Fossil Site have been examined. Varved sediments have been suggested to represent a series of 24-year-long cycles (Shunk, 2009). These sequences were partially synchronized with nearby tree ring sequences from a large log (unspecified taxon) (Shunk, 2009). However, multiple wood samples were not examined to ensure redundancy, nor were any stable isotope analyses performed on the wood.

The current study characterizes the Gray Fossil Site wood in preparation for future stable isotope analyses. It includes identification of wood specimens (to family level), an estimate of

organic preservation based on loss-on-ignition (LOI) tests, and determines that traditional alpha-cellulose extraction methods are suitable for the Gray Fossil Site wood.

Methods

Drying techniques used on Wood from the Gray Fossil Site

Dendrological analysis of fossil wood requires that saturated samples be dried. Once dry, specimens are cut, wrapped in string or tape to prevent delamination, and stored in airtight plastic bags (Methods for Collecting Archaeological Wood for Dendrochronological Analysis, n.d.). Wood samples for dendro-related analysis may be dried under a fume hood, in a vacuum oven, or a microwave (Speer 2010). Refrigeration is also a known technique for slowly drying fossils, and alcohol replacement of pore-water can also facilitate drying. Another potential method to slow dry saturated fossils is bury undried fossils in sand and allow it to dry under low, but even, pressure.

The initial phase of this study was to evaluate different stabilization methods for wood from the Gray Fossil Site. Historically, Gray Fossil Site wood has been stored in sealed plastic containers to control the drying rate. Optimal drying methods were determined by experiment, testing for drying wood. A variety of drying methods were tested on Gray Fossil Site wood samples of relatively standard sizes and shapes.

To test different drying methods, wood sample blanks were set up for several different drying tests. First, a control sample with no treatment was placed under a fume hood to dry at room temperature (Speer, 2010). Specimens were also dried in a vacuum oven at 90 C for 3.5 hours or microwaved on high for 20 second bursts until steam was no longer released (Speer, 2010). Specimens were also placed in a 4°C refrigerator, and monitored for cracking and weight loss. Additional methods modified from techniques in use at underwater archaeolgoical sites were also tested. These included 1) wrapping with cotton string, 2) alcohol dewatering, and 3) controlled drying in a sandbox.

The string method involves wrapping a specimen in cotton string with constant tension and density (Methods for Collecting Archaeological Wood for Dendrochronological Analysis, n.d.). This method provided stability and even pressure as the sample dried to limit warping, cracking and loss of sample pieces. Cotton string, cotton being a natural fiber, allowed water to evaporate, as opposed to plastic wrap which would retain moisture.

Another method that was tested was the use of alcohol to facilitate drying (Hillman and Florian 1985). A sample was placed in 30 mL water and 20 mL of 91% isopropyl alcohol. An hour later, 20 mL alcohol was added, and another hour later, 30 mL alcohol was added. The sample was soaked in this 70% alcohol solution for two hours before being removed to record weight and surface cracking. A lid was placed on the system after each addition of liquid to prevent evaporation.

The last method tested was a sandbox drying technique. In a standard 5 gallon bucket lined with garden mesh. A wood sample was placed on an 8-10 cm sand base, then covered with the sand. Sand should provide constant pressure on all sides of the sample to limit cracking, however, due to the nature of the drying technique, the sample was not able to be monitored throughout its drying time. The sample was weighed before being placed in the sand bucket, and again after three months of time in the sandbox.

The cotton string method resulted in specimens with the fewest cracks of the techniques tested. This technique was used for the remaining samples, where specimens were individually wrapped in cotton string, then allowed to dry under a fume hood (Madsen and Widga, 2020).

Wood identification

Phase two of this study was to identify the arboreal taxa represented by wood samples from the Gray Fossil Site. A dichotomous key (see Appendix A) of wood micro-anatomical features was developed from samples that had been sanded and leveled to display the rings and assorted ring structures (all samples were photographs from Speer, 2010). An example of the rings and identifying features is shown in Figure 2. Eight fossil specimens were examined, see Table 2 in Results.

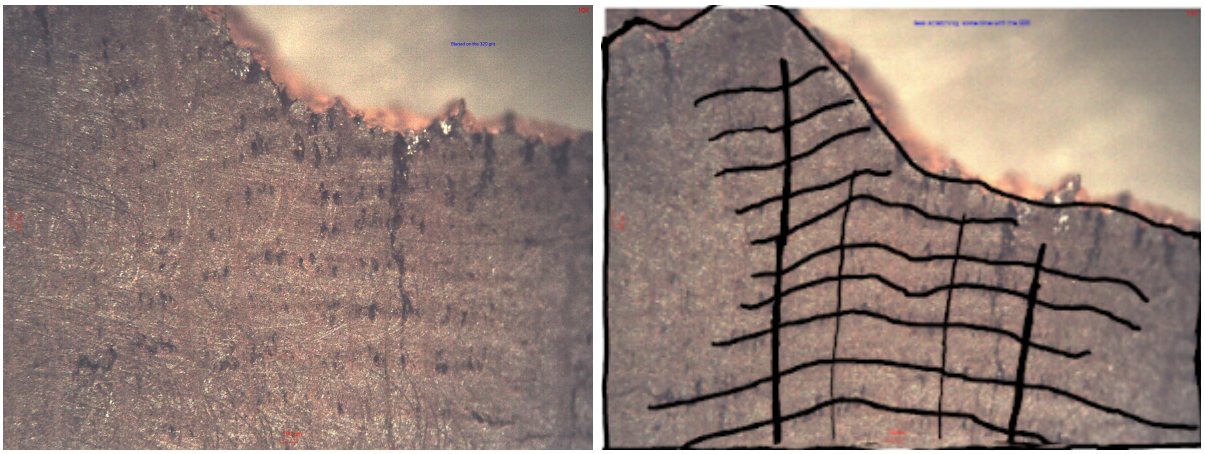

Fig. 2. *Quercus sp.* Left, original photo. Right, with lines outlining the edge of the specimen, rings (horizontal lines), and rays (vertical lines). Pictures by author.

Wood organic preservation

The last part of this study estimates the amount of organic carbon remaining in wood from the Gray Fossil Site. Loss on ignition (LOI) was used to measure the amount of organic material in a sample. These data offer insight into the impacts of diagenesis or the amount of mineralization the wood has experienced following deposition.

LOI of the Gray Fossil Site wood samples followed standard procedures (Mustoe, 2016). All LOI samples were smaller fragments that had broken off from larger specimens. Powder

samples were milled from multiple rings with a dremel tool. Sampling across rings provides an average of yearly variation instead of annually-resolved values. For the purposes of the LOI analysis, drilling into the sample also avoids contamination by clay particles from exterior surfaces.

Wood powder and fragments were ashed in a muffle furnace at 500°C for one hour, removed, and weighed to estimate the amount of volitized organic material. They were then combusted at 900°C and weighed a final time to estimate CO3 content. The following is the equation to calculate losses after each burn (from Mustoe, 2016):

(weight after burn-weight of crucible)/(original weight-weight of crucible)*100.

Extraction of Alpha-Cellulose from the Gray Fossil Site Wood

Finally, I attempted to extract alpha cellulose from the wood to further explore organic preservation at the molecular level. A variety of methods were considered (see Appendix B). Preliminary laboratory method development followed Brendel (2000). Three small samples (<15mg) of powdered Gray Fossil Site wood and three samples of modern, kiln-dried, red oak (*Quercus rubra*) were selected to test Brendel's methods. Powder samples were put into glass vials, treated with 2mL 80% acetic acid and 0.2mL of 69% nitric acid. All samples were vortexed and then placed in a dry-bath at 120°C for 20 minutes. Approximately 2.5mL 99% ethanol was added to each sample, samples were vortexed again, then centrifuged at 3000rpm for 5 minutes. After the centrifuge, the samples were decanted and dried.

Results

Conservation

Multiple drying methods limited cracking and overall destruction; future conservation efforts should keep in mind that an average of 42.5% of any one Gray Fossil Site wood specimen's weight is composed of water (Table 1). Such information (i.e., that drying status can be measured by monitoring specimen mass) is a productive measure of drying stage.

Table 1: Specimen Weights and Percent Water

Specimen	Initial Weight (g)	Dry Weight (g)	Water %
ETMNH 32028	482	316	34.4
ETMNH 33001-1	113	64	43.4
33001-2	571	358	37.3
33001-3	850	478	43.8
ETMNH 32914-1	642	340	47.0
32914-2	273	149	45.4
32914-3	412	216	47.6
32914-4	97	57	41.2

Physical restraints such as cotton string or a sandbox were used to minimize expansion during drying, while a vacuum oven, microwave, and refrigerator were used to control the rate of drying. An alcohol replacement test provided data on the rate of non-water evaporation. Drying methods were evaluated by measuring drying speed and the degree of surface cracking. Samples were weighed at regular intervals to monitor water weight loss, then were considered dry when weight stabilized. The different levels of surface cracking and destruction can be seen in Appendix D.

Wood Identification

To identify potential taxonomic categories present in the Gray Fossil Site wood flora, the first test was whether or not vessels are present (Appendix A). For specimen ETMNH 33208, vessels are present therefore the sample is from a hardwood species. As the wood is ring porous and there are narrow rays between thicker rays, the specimen is similar to oak. With identification based on Hoadley (1990), the wood belongs to the genus *Quercus*.

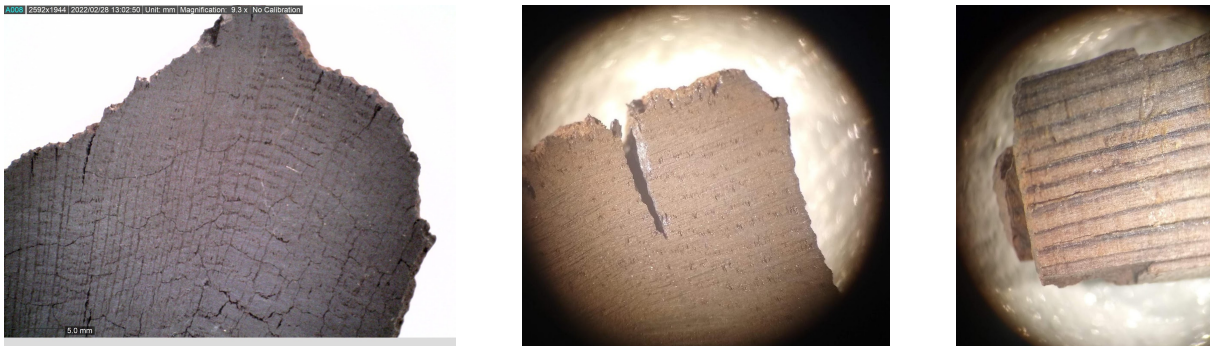

Fig. 3. Left: ETMNH 33208. *Quercus sp.* Center: ETMNH 32512. Right: ETMNH 32518. Images by author.

Other specimens included unspecified fir-like wood (ETMNH 32514), soft pine (ETMNH 32513, 32522, 32517, 32518, and 32519), unidentified ring porous hardwood (ETMNH 32516), and a chestnut or oak specimen (ETMNH 32512). 25% of examined specimens are likely hardwoods, while the remaining 75% of examined specimens are likely softwoods (Table 2). Overall, the sample consists of predominantly softwoods with further detail difficult to ascertain. Identifying features like resin tubes, pores, and rays were difficult to identify, and while rings were undoubtedly present, they were often compressed. This compression may be due to burial and diagenesis, and could result in misidentification.

Table 2: Identification

Specimen	**Identifying Features**	**Modern Trees with Similar Features**
ETMNH 32513	Small resin canals, few in number, one earlywood/latewood transition visible	Similar to Spruce or fir
ETMNH 32514	Distinctive banding between earlywood/latewood. No resin canals or vessel elements visible.	Similar to Eastern Larch
ETMNH 32519	Few small resin canals, wavy growth rings	Similar to Spruce or fir
ETMNH 32516	No distinctive rings, clear pores	Potentially a ring porous hardwood
ETMNH 32512	Ring porous	Chestnut or Oak
ETMNH 32522	Not ring porous	Softwood/gymnosperm
ETMNH 32517	Not ring porous	Softwood/gymnosperm
ETMNH 32518	Small resin canals	Similar to larch or fir

Organic preservation of Gray Fossil Site wood

Fossil wood samples (2020-13, 2020-14, 2020-15, 2020-16) had an average organic content of 84% (Table 3). By comparison, the modern red oak had 90% organic content.

Table 3: Loss on Ignition Data

Sample Number	Type of Wood	% Organic	% CO3
2020-13	Fossil	86.58%	12.46%
2020-14	Fossil	90.61%	1.73%
2020-15	Fossil	79.1%	2.25%
2020-16	Fossil	79.85%	17.08%
2021-9	Modern red oak	90.36%	0.0057%

The methods used to extract alpha-cellulose were a qualified success. Following the acetic and nitric acid steps, the modern wood samples were a bright orange color, while the Gray Fossil Site samples were dark brown. After boiling and cooling, the color of the liquid changed. The modern samples were a pale yellow, and the Gray Fossil Site samples were an amber color. Alpha-cellulose extraction from modern wood was successful in that a pale white material settled out (cellulose). Gray Fossil Site wood had no material settle out. For that reason, only the modern wood received the rest of the Brendel treatment with rinses of 99% ethanol, deionized water, and acetone.

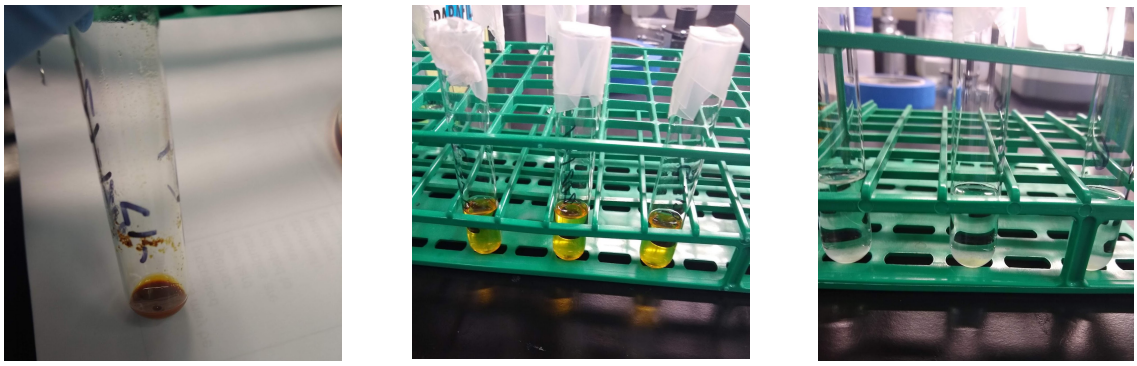

Fig. 4. Left: orange, rust-colored Gray Fossil Site sample following ethanol. Center: Modern samples after boiling. Right: Modern sample cellulose. Images by author.

As the Gray Fossil Site samples were completely dissolved by the first Brendel methods test, a larger Gray Fossil Site sample of 110.82 mg of powder was measured and prepared using the same process. Prior to boiling, the color of the liquid was a deep chocolate brown, and after boiling the color was like medium coffee. Following the ethanol step, the sample was reduced to an orange smear, whereas the cellulose from the modern wood sample became visible. None of the later steps changed the orange color. This is likely the limitation to Brendel's method as the Gray Fossil Site wood would have diagenic changes to account for when extracting cellulose.

Discussion

Conservation

The most successful drying method, as defined by minimal surface cracking, no fragmentation, and relatively fast drying time, was the string method. The only cracking that occurred with this method was restricted to the surface. The sample could be rotated to aid even drying, and while the sample could not be visually monitored, sample mass stabilized after 14 days. The removal of the cotton string left behind a few cotton threads, but those were easily removed. The Control sample provided a baseline of what to expect for simple fume hood drying, and was the next most successful drying method. Some cracking occurred, as expected based on other dry specimens. The sample could not be rotated, as wet wood samples are mushy and unstable, though it could be visually monitored as well as monitored with weight measurements.

Water loss was relatively easy to monitor on refrigerated samples, but it did not effectively remove moisture from the sample. The control sample indicated that significant weight loss occurred as samples dried, and the refrigerated sample did not show this. The refrigerator slows drying, but it did not stabilize the sample. Surface cracking during alcohol replacement was present, likely due to the rapid evaporation of alcohol.

Both the vacuum oven and microwave method resulted in many cracks and total separation of sample pieces. The sandbox sample was difficult to monitor. The sample went in and was removed after 3 months. Two large cracks occurred suggesting that drying in a sandbox is both ineffective and impractical. However, should time not be of consequence and bigger cracks be preferable, this method would be worth considering.

Overall, most samples were dry within 14 days of being removed from a wet environment. Samples that were placed in rapid drying environments (e.g., vacuum oven, microwave), tended to have more cracking. Slower drying samples showed the least cracking.

Identification

Identification of the wood from the Gray Fossil Site provides an opportunity to examine Pliocene landscapes. In order to do so, a baseline understanding of modern forests in eastern Tennessee must be established. According to James (1955), northeastern Tennessee is composed of three notable tree groups, evergreen trees, deciduous trees, and ornamentals. For the full list, please see Appendix C. Common taxa of each group include the following genera: *Pinus, Tsuga,* and *Picea* (pine, hemlock, and red spruce), *Magnolia, Quercus,* and *Acer* (magnolia, oak, and maple), and *Staphylea, Prunus,* and *Menispermum* (bladdernut, mountain ash, and moonseed) respectively. Modern forests at the elevation of the Gray Fossil Site occupy a climate envelope with an average temperature of 13.67°C and 44.4 inches of rainfall, annually (Elder, 1958). With seasonal variations, the climate of modern East Tennessee is humid and temperate (Elder, 1958).

The Pliocene climate of the Gray Fossil Site was likely less seasonal and slightly warmer than modern, based on the vertebrate fauna. The presence of alligators indicates a warmer environment (Baumgartner, 2014), as do the crown heights of rodents (Schap et al., 2021). Although non-arboreal floral remains suggest a warmer-than-modern climate (Baumgartner, 2014), the wood specimens examined as part of this study are not different from taxa found on the modern landscape. Even for the partially identified specimens, all families are present in the modern day. Distribution and count of the number of trees is not possible at this time, as so few samples have been examined.

Composition

Gray Fossil Site wood has experienced minimal diagenetic alteration based on LOI analyses. With an average of 80% organic content in comparison to 90% modern wood organic content, any further information about mineralization of Gray Fossil Site fossil wood will require XRD or XRF analyses, which are outside of the scope of this study.

As the Gray Fossil Site wood has most likely experienced some mineralization during diagenesis, as indicated by LOI, the lack of unaltered alpha cellulose is unsurprising. The fossil site itself is situated in Knox Dolomite, the unified northwestern section of the Knox Group (Whitelaw et al. 2008). The Knox Group is known to weather to an orange-red clay, indicating the presence of iron oxides (Rodgers, 1953). The Gray Fossil Site wood had a comparable orange-red color during alpha-cellulose extractions, also indicating the presence of iron oxides. Richter (2008) based his work off of Brendel, with a key addition for sub-fossil and fossil wood. In addition to Brendel's method, 30% HF eliminated iron oxides (Richter, 2008). For future stable isotope studies on the Gray Fossil Site wood, such a technique should be considered, if not employed to isolate alpha cellulose.

Implications of this study for future stable isotope research

When examining tree rings for stable isotope analysis, the first consideration is what isotopes should be examined. The primary elements in trees are carbon, oxygen, and hydrogen, all have climate-sensitive stable isotopes (McCarroll and Loader, 2004). The carbon in trees comes from carbon dioxide, associated with respiration, while the oxygen and hydrogen are primarily deposited in the rings and correlated with soil moisture (McCarroll and Loader, 2004). McCarroll and Loader (2004) emphasize that no single stable isotope perfectly records any one climatic signal. Instead, each stable isotope data point is correlated with certain climate

indicators. Due to the fine resolution of a year-to-year sampling with tree rings, annual climate variability is apparent, despite the complexity of recording exact climate conditions.

McCarroll and Loader (2004) primarily examined modern tree rings, however, fossil and subfossil tree rings are also useful, but require different methodological approaches. Focusing on stable oxygen isotopes, Richter et al. (2008) found that subfossil wood can have climate sensitive isotopes. Their analysis of fossil wood from nine different localities found that oxygen isotopes are related to mean average temperature, and that precipitation data may still be preserved in cellulose for millions of years (Richter et al., 2008). Richter et al. (2008) studied Eocene to Holocene wood, bracketing the age of the Gray Fossil Site. Stable oxygen isotopes from the Gray Fossil Site adds an additional comparison point to other already studied localities.

Stable oxygen isotopes are not the only isotopes of importance when it comes to studying fossil wood. Van Bergen and Poole (2002) examined fossil and archeological wood samples to understand the effects of fossilization on carbon isotope ratios. They found that some diagenetic changes occurred, particularly in Cretaceous samples (Van Bergen and Poole, 2002). However, as long as FTIR techniques were used to identify potential contaminants in individual tree samples, they found that the stable carbon isotopes for Tertiary specimens would provide appropriately accurate ratios for further paleoclimate analysis (Van Bergen and Poole, 2002). Again, stable carbon isotope data from the Gray Fossil Site expands the body of knowledge concerning past climates.

Conclusion

While sediments and identified vertebrate and plant fossils provide some paleoclimate information, stable isotope analyses have the potential to provide high resolution climate data at an annual scale. No stable isotope analyses of sub-fossil wood have been performed at the Gray Fossil Site, so this study opens the door to previously unknown information. Additionally, the time period of the wood means that the data can be compared to previous studies of Tertiary fossil and subfossil wood, as well as a comparison to some other isotope analyses on mammals, reptiles, and sediments at Gray Fossil Site.

Future steps include revisiting alpha-cellulose extraction, individual ring sampling, and assembling a floating chronology of precipitation and temperature information from stable isotopes.

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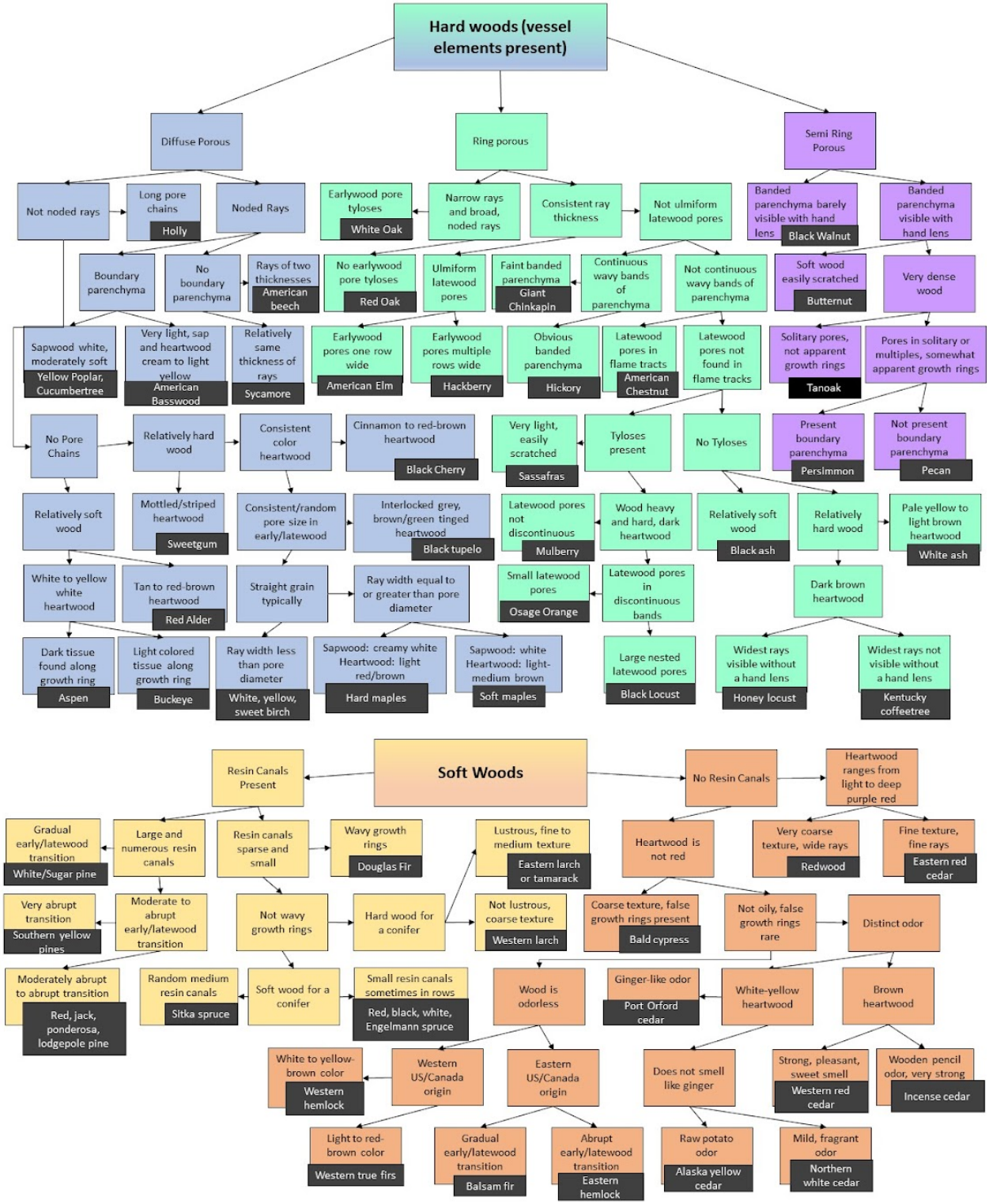
Appendix A


Hard woods (vessel elements present)
Diffuse Porous
Ring porous
Semi Ring Porous
Not noded rays
Long pore chains
Holly
Noded Rays
Earlywood pore tyloses
White Oak
Narrow rays and broad, noded rays
Consistent ray thickness
Not ulmiform latewood pores
Banded parenchyma barely visible with hand lens
Black Walnut
Banded parenchyma visible with hand lens
Boundary parenchyma
No boundary parenchyma
Rays of two thicknesses
American beech
No earlywood pore tyloses
Red Oak
Ulmiform latewood pores
Faint banded parenchyma
Giant Chinkapin
Continuous wavy bands of parenchyma
Not continuous wavy bands of parenchyma
Soft wood easily scratched
Butternut
Very dense wood
Sapwood white, moderately soft
Yellow Poplar, Cucumbertree
Very light, sap and heartwood cream to light yellow
American Basswood
Relatively same thickness of rays
Sycamore
Earlywood pores one row wide
American Elm
Earlywood pores multiple rows wide
Hackberry
Obvious banded parenchyma
Hickory
Latewood pores in flame tracts
American Chestnut
Latewood pores not found in flame tracks
Solitary pores, not apparent growth rings
Tanoak
Pores in solitary or multiples, somewhat apparent growth rings
No Pore Chains
Relatively hard wood
Consistent color heartwood
Cinnamon to red-brown heartwood
Black Cherry
Very light, easily scratched
Sassafras
Tyloses present
No Tyloses
Present boundary parenchyma
Persimmon
Not present boundary parenchyma
Pecan
Relatively soft wood
Mottled/striped heartwood
Sweetgum
Consistent/random pore size in early/latewood
Interlocked grey, brown/green tinged heartwood
Black tupelo
Latewood pores not discontinuous
Mulberry
Wood heavy and hard, dark heartwood
Relatively soft wood
Black ash
Relatively hard wood
Pale yellow to light brown heartwood
White ash
White to yellow white heartwood
Tan to red-brown heartwood
Red Alder
Straight grain typically
Ray width equal to or greater than pore diameter
Small latewood pores
Osage Orange
Latewood pores in discontinuous bands
Dark brown heartwood
Dark tissue found along growth ring
Aspen
Light colored tissue along growth ring
Buckeye
Ray width less than pore diameter
White, yellow, sweet birch
Sapwood: creamy white Heartwood: light red/brown
Hard maples
Sapwood: white Heartwood: light-medium brown
Soft maples
Large nested latewood pores
Black Locust
Widest rays visible without a hand lens
Honey locust
Widest rays not visible without a hand lens
Kentucky coffeetree
Soft Woods
Resin Canals Present
No Resin Canals
Heartwood ranges from light to deep purple red
Gradual early/latewood transition
White/Sugar pine
Large and numerous resin canals
Resin canals sparse and small
Wavy growth rings
Douglas Fir
Lustrous, fine to medium texture
Eastern larch or tamarack
Heartwood is not red
Very coarse texture, wide rays
Redwood
Fine texture, fine rays
Eastern red cedar
Very abrupt transition
Southern yellow pines
Moderate to abrupt early/latewood transition
Not wavy growth rings
Hard wood for a conifer
Not lustrous, coarse texture
Western larch
Coarse texture, false growth rings present
Bald cypress
Not oily, false growth rings rare
Distinct odor
Moderately abrupt to abrupt transition
Red, jack, ponderosa, lodgepole pine
Random medium resin canals
Sitka spruce
Soft wood for a conifer
Small resin canals sometimes in rows
Red, black, white, Engelmann spruce
Wood is odorless
Ginger-like odor
Port Orford cedar
White-yellow heartwood
Brown heartwood
White to yellow-brown color
Western hemlock
Western US/Canada origin
Eastern US/Canada origin
Does not smell like ginger
Strong, pleasant, sweet smell
Western red cedar
Wooden pencil odor, very strong
Incense cedar
Light to red-brown color
Western true firs
Gradual early/latewood transition
Balsam fir
Abrupt early/latewood transition
Eastern hemlock
Raw potato odor
Alaska yellow cedar
Mild, fragrant odor
Northern white cedar

Appendix B

Method	Speer	McCarroll	Richter	Van Burgen	Brendel
Type of wood?	Modern	Modern	Sub fossil and fossil	Fossil and subfossil	Modern
Biomolecule	cellulose	cellulose	cellulose	Cellulose or lignin	Cellulose
Stable Isotope	Any	Carbon and Oxygen	Oxygen	Carbon	Any
Sample Size	Not listed	Single sample or small batches	>100 samples	12 wood specimens, many smaller samples	10-100mg in glass tubes
Procedure	Lignin removal through oxidation in acidified sodium chlorite Hemi-cellulose removed by reaction w sodium hydroxide	Carbon isotopes: Combust sample in a sealed tube with copper oxide. Tube is under vacuum, then heated to 450C for 18 hours. Include standard samples for consistency. Oxygen isotopes: mercury (II) chloride or nickel tube pyrolysis.	Rinses of NaOH to get rid of hemicellulose, HF (30%) to remove iron oxides	Crush samples with mortar and pestle, extract with ultrasonication, centrifuge sample. Residues to be dried in the dark until chemical analyses.	Step 1: Add 2 mL 80% acetic acid. Add 0.2 mL 69% nitric acid. Seal and vortex. Boil gently at 120C for 20min Step 2: Allow to cool. Decant. Add 2.5 mL 99% ethanol Step 3: Seal and vortex. Centrifuge for 5 min at 2000 rpm. Decant supernatant Step 4: Add 5 mL 99% ethanol. Repeat step 3 Step 5: Add 5 mL deionised water. Repeat step 3 Repeat Step 4 Step 6: Add 6 mL acetone. Transfer sample to 1.5 mL microfuge tube. Vortex, centrifuge, then decant. Dry under vacuum Estimate cellulose quality by color and structure: fibrous/white

Appendix C: Ornamental Group James (1955)

Family	Common Name
Phoradendron	Mistletoe
Aristolochia	Dutchman's pipe
Xanthorhiza	Yellow root
Berberis	Barberry
Menispermum	Moonseed
Cocculus	snailweed
Calycanthus	Sweet shrub
Lindera	Spicewood
Bignonia	Cross vine
Catalpa	Catalpa
Cephalanthus	Buttonbush
Diervilla	Diervilla
Lonicera	Coral Honeysuckle
Symphoricarpos	Coralberry
Viburnum	Hobblebush, witherrod, black-haw

Family	Common Name
Philadelphus	Mock orange
Itea	Willow
Hydrangea	Hydrangea
Physocarpus	Ninebark
Rosa	Rose
Pyrus	Ash
Prunus	Cherry
Euonymus	Strawberry bush
Amorpha	Indigo
Robinia	Locust
Rhus	Sumac
Ilex	Holly
Celastrus	Bittersweet
Sambucus	Elder

Family	Common Name
Staphylea	Bladdernut
Parthenocissus	Creeper
Dirca	Leatherwood
Stewartia	Camellia
Hypericum	St. John's Wort
Aralia	Hercules' club
Clethra	Alder
Rhododendron	Azalea
Kalmia	Lambkill
Oxydendrum	Sourwood
Epigaea	Arbutus
Gaultheria	Teaberry
Chionanthus	Fringe tree
Campsis	Trumpet creeper

Appendix C: Deciduous Group James (1955)

Family	Common Name
Arundinaria	Cane
Salix	Willow
Carya	Hickory
Corylus	Hazelnut
Castanea	Chestnut
Morus	Mulberry
Asimina	Pawpaw
Hamamelis	Witch hazel
Spiraea	Hardhack
Crataegus	Hawthorn
Gleditsia	Honey Locust
Aesculus	Buckeye
Ascyrum	St. Andrews Cross
Menziesia	Menziesia
Gaylussacia	Huckleberry
Fraxinus	Ash
Magnolia	Magnolia
Potentilla	Cinquefoil
Rhus	Sumac
Cornus	Dogwood
Rhododendron	Azalea

Family	Common Name
Yucca	Grass (bear)
Populus	Cottonwood
Carpinus	Beech
Alnus	Alder
Ulmus	Elm
Maclura	Osage Orange
Sassafras	Sassafras
Liquidambar	Gum
Pyrus	Chokeberry
Rubus	Dewberry, blackberry, raspberry
Robinia	Kelsey locust
Vitis	Grape
Nyssa	Black gum
Leucothoe	Leucothoe
Vaccinium	Cranberry, deerberry, blueberry
Viburnum	Viburnum, arrowwood
Quercus	Oak
Leiophyllum	Myrtle
Buckleya	Sapsuck
Cercis	Redbud

Family	Common Name
Smilax	Greenbrier
Juglans	Walnut
Ostrya	Hornbeam
Betula	Birch
Celtis	Hackberry
Calycanthus	Sweet shrub
Ribes	Currant, gooseberry
Platanus	Sycamore
Amelanchier	Serviceberry
Prunus	plum
Rhus	Poison ivy
Tilia	Basswood/linden
Cornus	Dogwood, cornel, osier
Lyonia	Maleberry
Diospyros	Persimmon
Liriodendron	Poplar
Acer	Maple
Spirea	Meadowsweet
Pyrularia	Buffalo-nut
Halesia	Silverbell

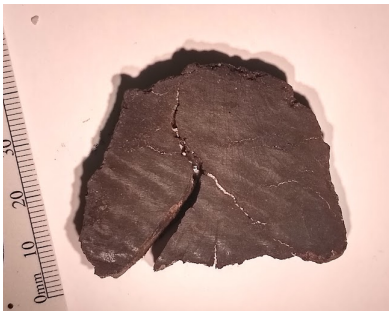
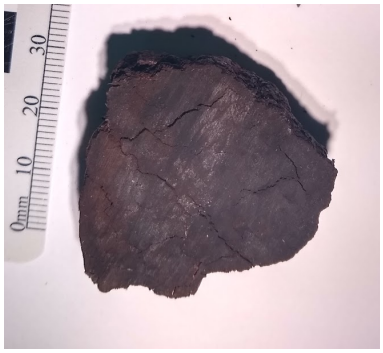
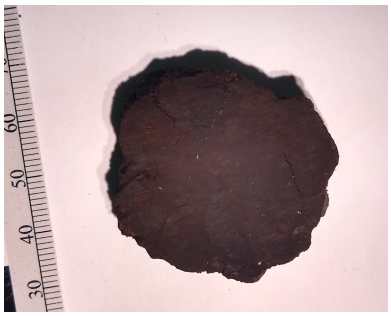
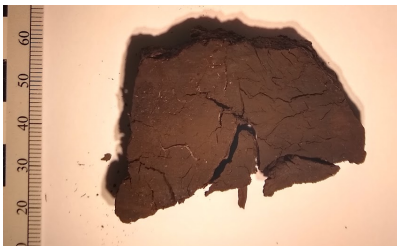
Appendix C: Evergreen Group James (1955)

Family	Common Name
Pinus	Pine
Picea	Spruce
Thuja	Arbor vitae
Leucothoe	doghobble/switch-ivy

Family	Common Name
Tsuga	Hemlock
Abies	Fir
Fagus	Beech
Ilex	Holly

Family	Common Name
Liquidambar	Gum
Juniperus	Cedar
Rhododendron	Laurel, rhododendron

Appendix D

Treatment	Images	Treatment	Images
1. String Sample		5. Sandbox	
2. Control		6. Vacuum Oven	
3. Refrigerator		7. Microwave	
4. Alcohol Replacement