ACER PROPOSAL 2022

How does nonconductive wood formation relate to sap and sugar yields across five maple species?

1) EXECUTIVE SUMMARY

Trees vary widely in both sap yield and sugar concentrations for reasons that are not well understood. Compartmentalization of taphole wounds is critical to long-term tree health. We hypothesize that trees yielding more sap are less effective at taphole wound compartmentalization because wood traits conducive to high flows do not allow efficient compartmentalization, while trees with greater sugar concentrations are more efficient at compartmentalization because the process requires carbohydrates. Better understanding these relationships would inform the selection of trees for breeding and planting. Nonconductive wood column (NWC) formation is under-studied in species other than sugar maple, so we will study four maple species in addition to sugar maple, to generate evidence-based tapping guidance for these under-utilized species. We will also validate the use of a non-destructive tomographic approach to measuring sapwood depth and nonconductive wood development, which if successful would greatly expand options for future studies. We will collaborate with Extension, NAMSC, and state producer associations to communicate our findings with producers in including talks, workshops, and various publication formats. Short-term benefits to producers will include improved tapping guidelines for red maple, silver maple, Norway maple, and boxelder. Longer-term benefits will derive from a better understanding of the tradeoffs between sap and sugar yield vs nonconductive wood formation for selecting improved trees, and from a validated non-destructive method to study NWC and heartwood formation. Our anticipated outcomes include greater, more sustainable, and optimized utilization of several maple species across a wide geographic range.

2) ALIGNMENT AND INTENT

This project is aligned with the Producer and Landowner Education project type. Our project will generate data and guidelines to help producers expand possibilities for sustainable maple tapping to currently under-utilized species across a wide geographic area. Our overarching objective is to understand potential tradeoffs between sap yield and nonconductive wood column (NWC) volume in multiple species of maples to enable greater geographic and taxonomic diversity of syrup production. We will employ and validate a novel, non-destructive approach for monitoring NWC formation in maples, and will communicate our findings in multiple venues with both large and small producers in several states using a variety of approaches. We will share our novel approach for non-destructively studying the formation of NWC, both as an educational tool and as a new method for future studies on high-value trees. Producers who are able to diversify production to additional species will benefit from not only greater production but also greater resilience in the face of climate variability.

Background

Nonconductive wood columns (NWC) and tapping sustainability

Trees respond to xylem wounds (such as tap holes) by forming nonconductive barriers that block vessels and render the tissue impermeable to both water and pathogens (Shigo and Hillis 1973, Shigo 1976, Walters and Shigo 1978, Smith 2015). Species differ widely in the physical and chemical processes that seal off this zone from the rest of the tree. Generally, the presence of oxygen in damaged vessels induces a programmed reaction resulting in the blockage of these and adjacent vessels with tyloses, gels, and/or hydrophobic lipids, and the generation of antimicrobial compounds such as polyphenols and suberin in parenchyma cells as they die (Shortle 1979, De Micco et al. 2016, Morris et al. 2020). Once complete, this zone of modified wood contains no living cells and serves as a physical barrier with the maximum protection the tree can afford between the wound and functional sapwood. The eventual closure of the wound via cambial growth forms the final barrier, sealing the NWC behind newly-formed living sapwood and bark.

The sustainability of sap collection for maple syrup production requires that the tree can replace sapwood at a greater rate than the losses incurred by tapping. For a tree to maintain (or ideally, to increase) its sapwood area or volume as it grows, ***the average rate of amount of functional sapwood loss to NWC formed around tapholes, NWC around other injuries, and the development of heartwood must not exceed the average rate at which new sapwood is formed***. Sapwood is a critical organ not only for the transport of water from soil to leaves (analogous to an animal's vascular system), but also for the storage of nutrients and carbohydrates (i.e. sugars and starches) in the part of the tree least susceptible to damage (analogous to an animal's body fat reserves) (Hacke and Sperry 2001). Carbohydrates are critical to tree health as a stored source of energy and building blocks for growth or defense in stressful times. Depth to heartwood in sugar maple varies widely among trees and sites, and likely has genetic, geographic, and microsite components (Yanai et al. 2009, Baral et al. 2013, Germain et al. 2015), but also relates to each tree's history of injury from ice damage, harvest of nearby trees, or skidders and other vehicles. Maintenance of apparently redundant sapwood poses a metabolic cost to the tree (Pruyn and Harmon 2003) and so must provide some benefit to the tree's long-term survival strategy, likely via reduced risk of catastrophic hydraulic failure during drought (Ewers et al. 2007).

Drilling a taphole generates nonconductive wood columns (NWC) as a compartmentalization response around the hole. The volume of NWC is highly variable – 50-150 times the volume of the taphole in sugar maples (van den Berg 2012), and forms a roughly vertical "column" around the taphole, in part because vessels are more interconnected vertically than horizontally (Hacke and Sperry 2001). Fortunately, healthy sugar maples over 10" diameter are known to generally put on more than enough radial growth each year to replace the amount of sapwood that became nonconductive, assuming tapping guidelines are followed (van den Berg 2012). Tapping guidelines for any species must consider the relative balance of growth and NWC formation but have not been studied in detail on most other maples, which represent an under-utilized potential resource across much of the Northeast and Midwest.

Is there a tradeoff between sap or sugar yield and NWC?

The drivers of variation in sap yield, sap sugar concentrations, growth rate, and amount of NWC formed around taphole remain poorly understood. Climatic, edaphic, and microsite factors (e.g. winter/spring temperature variation, stand density, slope position, soil texture, pH, and nutrients) may all contribute, but a substantial portion of this variation is likely driven by genetic variation as well (Wilmot 2011).

There are theoretical reasons to believe that a relationship between sap yield, sugar concentrations, and NWC formation may exist independent of climatic and other site-level effects. Eckstein et al. (1979), working in hybrid poplar, observed a strong relationship between wood anatomy and the amount of discolored nonconductive wood required to isolate a xylem wound (see also Smith and Shortle, 1993). Specifically, trees that were efficient compartmentalizers (i.e. those that formed the smallest NWCs around wounds) generally had fewer, smaller, and less-interconnected vessels than less efficient compartmentalizers which required a larger amount of NWC to isolate a wound.

We hypothesize that xylem anatomical traits related to high hydraulic conductance (greater vessel diameter, cross-sectional density, and interconnectedness) also increase the amount of sap extractable during the late-winter tapping season when positive pressure develops in maples (Hacke and Sauter 1996) and sap can be collected with or without vacuum**. *If true, this presents a tradeoff relevant to producers: wood that is more conductive to sap flow may also require a greater volume of nonconductive wood to effectively isolate the tap wound from living sapwood***. These variations in NWC may be detectable and associated with the considerable variation in sap yield among individual trees even on a single site, and we hypothesize that they will be even greater when looking at variation across maple species that vary widely in wood density (e.g. between sugar maple and "soft" maples; see "*Beyond sugar maple*" below).

Wound compartmentalization is an active physiological process that consumes carbohydrates for energy and the formation of secondary metabolites, competing with other processes requiring energy such as growth (Smith 2015). In fact, Copenheaver et al., (2014) attributed declines in growth following the onset of tapping as being at least partly due to the resources needed to close and isolate the taphole wound. We therefore hypothesize that trees with higher concentrations of sugar in the sapwood shortly after wounding will be able to more rapidly initiate an effective compartmentalization response and will therefore compartmentalize taphole wounds more efficiently and ultimately have less overall NWC per tap. This may or may not directly relate to trees which have greater reserves of carbohydrates (i.e. both sugar *and starch*) in the sapwood where sugar represents immediately available energy and starch represents longer-term storage. Relatedly, rates of taphole closure have been documented to vary significantly with stresses such as defoliation which affect the storage of carbohydrates (Wargo et al. 1972); and also with other factors affecting tree health, such as site fertility (Huggett et al. 2007).

The search for a "sweeter" sugar maple

Sugar maple, like many other trees, exhibits substantial genetic variation across its range, but also among individuals within a single forest stand (Jackson et al. 2021). It has long been recognized that some individual trees (or populations) have reliably high or low sugar concentrations and sap yield relative to neighboring trees in the same year (Taylor 1956, Larsson and Jaciw 1967, Marvin et al. 1967, Wilmot 2011), and sugar maple genetic selection and breeding programs have been aimed at improving sugar concentrations as well as tree vigor and stress tolerance (Kriebel 1957, 1989, Wild 2021). A number of projects established in the 20th century remain underway, with slow progress due to the long time it takes to grow sugar maple to reach reproductive maturity and achieve 10" diameter for tapping. The progeny of trees in such trials (e.g. from Cornell), as well as cultivars marketed in part for high sugar concentrations (e.g "Sweet Shadow", "Legacy", "Super Sweet") are available for purchase in the landscape trade. A clear majority of producers recently surveyed (62%) were in favor of sugar maple genetic improvement programs, particularly to safeguard against the decline of wild trees to a changing climate (Legault et al. 2019). ***If future improvement efforts are to succeed, a detailed understanding of the tradeoffs between sap yield, sugar content, growth rate, and NWC formation will be critical to producing genetic lines of trees with the optimal balance of traits to maximize syrup production without compromising tapping sustainability.*** Understanding these relationships may also provide additional rationale for the utilization of such improved trees.

Recent work in "sweet tree" plantations with grafted cuttings or seeds collected from trees with high sap sugar concentrations (Wild 2021) seems to indicate a tradeoff between high sap yield and high sugar concentrations, driven mostly by low yields in many of the sweetest trees (i.e. those averaging >3% sugar content across multiple years). There may therefore be an optimum target sugar content that maximizes the total syrup yield per tap. However, this optimum is only relevant if such trees are typical in both growth rate and the rate of NWC formation around each taphole, such that existing tapping guidelines remain sustainable. If unusually "sweet" trees also accumulate less nonconductive wood over time, this represents an additional but previously unappreciated benefit of developing and planting these genetic lines. These relationships are far from certain in the existing literature - earlier work study showed a *positive* correlation between average sugar concentration and average sap yield across trees when several years of data were averaged (Marvin et al. 1967), though Blum (1971) found little evidence of any correlations at the stand scale in a single year. Given the long time it takes a sugar maple to mature, planting improved cultivars, clones, or genetic lines is likely always to play a limited role in the industry, but smaller producers and landowners may still find it worthwhile to make such long-term investments on their property for a variety of reasons. Old farmsteads across the northeast often feature large sugar maples that were planted along a roadside or fence line over 100 years ago, and many of these trees still are still tapped annually by small-scale producers or hobbyists. Future efforts at selecting improved genetic lines of sugar maple may need to focus both on tolerance for the changing and more interannually variable climate as on sugar and sap yields, and to the extent that growth is less reliable in a more variable climate, trees that form small, efficient NWCs may also be highly desirable.

Beyond sugar maple

Acer is one of the most diverse genera of woody angiosperms, with well over 100 species, though only a few are native to eastern North America. Within the genus, the hard maples (sugar maple - *Acer saccaharum* and black maple - *A. nigrum)* are closely related to each other, but are distantly related to the soft maples (red maple - *A. rubrum* and silver maple - *A. saccharinum*; also closely related to each other),, while boxelder (*A. negundo)* is a fairly distant relative of both these groups (Li et al. 2006). Across these species, the hard maples generally grow more slowly and are more sensitive to soil characteristics than the soft maples (Burns and Honkala 1990). Norway maple (*A. platanoides*) is a non-native species with intermediate wood characteristics (Meier 2008), which has been planted widely in North America and has naturalized in many environments. Norway maple also grows quickly and has distinct physiological traits that help it to become established in new environments (Paquette et al. 2012). While we do not advocate planting or encouraging the spread of any non-native maple, utilizing existing trees where they are already established in mixed forest/developed landscapes might offer opportunities for new/small producers, or as educational projects (e.g. in school yards).

Syrup ***production involving a mix of maple species may offer greater climate resilience to the industry***. Moreover, maintaining mixed-species sugarbushes likely leads to a wide range of ecosystem benefits, and information on how to do so sustainably tap multiple maples may make syrup production more attractive to smaller producers utilizing existing woodlands on their farmsteads. Despite ecological differences, other native maples commonly co-occur with sugar maple across its range (Burns and Honkala 1990). There is currently broad consensus that diversification of agroecosystems enhances productivity, the sustainable provisioning of ecosystem services, and the system's resilience to extreme climate events, pathogens, and insect outbreaks (Altieri et al. 2015, Dymond et al. 2015, Spiegal et al. 2018, Fichtner et al. 2020). More diverse sugarbushes would provide a broad array of external benefits, ranging from greater hydrologic regulation and carbon storage capacity, wildlife habitat, recreational and cultural benefits, and greater ecosystem stability (Schwenk et al. 2012, Caputo et al. 2016, Pan et al. 2018).

Table 1. Statewide estimates of other maple species per thousand 10"+ sugar maples *based on FIA data. There are substantially more red maples than sugar maples in most syrup-producing states (bold values). Boxelder and Norway maple are likely severely under-represented in these data, because these species are preferentially found in locations that don't meet FIA sampling criteria. For example, no 8"+ boxelder were tallied in NH by FIA, but we have already identified several possible boxelder study locations in Durham NH. States with the greatest potential to expand production using boxelder and silver maple include MN, IN, WI, and OH. From Norway maple, CT, MA, and PA would gain the most.*

	VT	NY	ME	WI	MI	NH	PA	OH	MA	CT	IN	MN	WV
Red 10"+	614	**1,145**	**1,891**	930	886	**4,351**	**2,947**	**1,287**	**5,143**	**6,689**	508	868	**1,614**
Silver 10"+	5	44	4	113	84	25	18	140	54	29	220	228	15
Boxelder 8"+	8	19	1	157	18	-	32	174	4	-	178	706	48
Norway 10"+	-	2	1	<1	1	-	10	-	13	39	-	-	1

Red maple (*A. rubrum*) is already an important species utilized by many producers and is recognized to be more adaptable to a variety of site conditions than sugar maple, particularly with regard to a warming climate (Abrams 1998, Legault et al. 2019). Even in Vermont, with the highest ratio of sugar to other maples (Table 1), it is estimated that 1 in 6 taps is in a red maple (Wilmot 2016). Maples in general have increased in abundance across the eastern US at the cost of fire-dependent species (Nowacki and Abrams 2008), and red maple in particular competes well on dry sites formerly dominated by fire-dependent species. While sugar concentrations are generally lower in red maple than sugar maple, they have similar overall syrup yields per tap, and contrary to common belief, their sap does not become buddy earlier than does that of sugar maple (van den Berg et al. 2020). With affordable reverse-osmosis systems becoming more widely used by even smaller producers, any additional cost of evaporating less-concentrated red maple sap is greatly reduced or eliminated, and finished syrup is not noticeably different from that of sugar maple (van den Berg et al. 2020).

Other native soft maple species including silver maple (*A. saccharinum*) and boxelder (*A. negundo*) can be used for syrup production (Heiligmann et al. 2006) and also represent potentially under-utilized resources for diversifying syrup production across a wider climate space within the US, particularly in the upper Midwest (Table 1). Syrup from boxelder reportedly has a different flavor and is marketed separately from other maple syrups, at least in Canada (Heiligmann et al. 2006). Boxelder has the westernmost distribution of eastern maples and is generally the smallest and shortest-lived of these species (Burns and Honkala 1990), and is often tapped upon reaching ~8” diameter (Agriculture Canada 2000). Both of these species occur naturally in floodplain forests but thrive in a wide variety of soil conditions, especially in disturbed and developed landscapes. Their faster rates of growth mean they may reach sufficient size for tapping much sooner than sugar maple, making them better candidates for planting, and their rapid growth may also more easily outpace the accumulation of nonconductive wood. As a fast growing and common but non-native species, the potential for syrup production from Norway maple may also be under-appreciated (MapleTrader 2013, ThisInspiredLife 2020, Kingston 2022).

The landscape trade already markets various cultivars of all five of our study maples, most of which have been selected for traits that make them particularly attractive or well-behaved landscape specimens, including *Acer x fremanii*, a hybrid between red and silver. Nonetheless, ***if the appropriate traits for selection can be identified for these species*** as we plan to do for sugar maple, ***this existing selection of diversity could serve as one base of variation from which to select varieties that are optimized for syrup production***. Faster growth rates and shorter time until reproductive maturity would speed this process relative to sugar maple. A combination of rapid growth rates and easy propagation by cuttings may make silver maple especially amenable to selection and improvement in the future (Preece et al. 1991).

The volume of NWC formed around tapholes in soft maples has not been studied in sufficient detail to understand whether standard tapping guidelines are truly appropriate for these species, or whether they should be modified. Shigo (1965) reported that taphole wounds were larger in red maple (or perhaps soft maples in general) than in hard maples. Red maples are also more variable than sugar maple in terms of depth to heartwood (Wilmot 2016), and they are also more vulnerable to vertical cracking when nails or spouts are driven past the cambium (Burns and Honkala 1990). Wilmot (2016) reported that the amount of NWC formed around tapholes was *not* any greater in red maple than in sugar maple but advised extra caution in tapping in vertical alignment with non-taphole wounds which might have induced a large NWC. This study, like several studies in sugar maple, did not explicitly consider the rate of growth or heartwood formation. van den Berg et al. (2021) noted that a taphole which intersects an existing NWC or other defect results in a drastically larger NWC than one that only intersects clean, healthy sapwood. We are aware of no formal studies into the formation of NWC in other maple species.

Wood anatomy within the maple family (Sapindaceae), and in particular between North American soft vs hard maples, varies in a number of properties including density, hydraulic conductance, vulnerability to embolism, and the arrangement of parenchyma (Lens et al. 2011, Meier 2011, Pace et al. 2022). ***Wood density is a master trait among tree species which relates to a wide range of ecological and physiological strategies*** such as growth rate and longevity (Umaña et al. 2022), drought resistance (Hacke and Sperry 2001), susceptibility to injury and infection (Romero and Bolker 2008), as well as to ecosystem services such as carbon sequestration (Babst et al. 2014, Chojnacky et al. 2014). The maples we propose to study differ in wood density by roughly 30% (Meier 2011), for example from 0.44 to 0.56 g/cm^3 (oven-dry mass/green volume;), which is a substantial portion of the entire range of wood densities found among temperate hardwoods (Chojnacky et al. 2014). Species with lower wood density and larger vessel size are less resistant to mechanical damage *and generally also less able to effectively isolate the wound from living sapwood* via the formation of a nonconductive wood zone (Larjavaara and Muller-Landau 2010). Wind and ice damage are the two most common causes of damage to sugarbushes (Legault et al. 2019), and the loss of limbs one of several types of xylem injuries that predisposes trees to fungal infection, loss of live sapwood to nonconductive wood, and eventually decline (Hesterberg 1957, Aho et al. 1983).

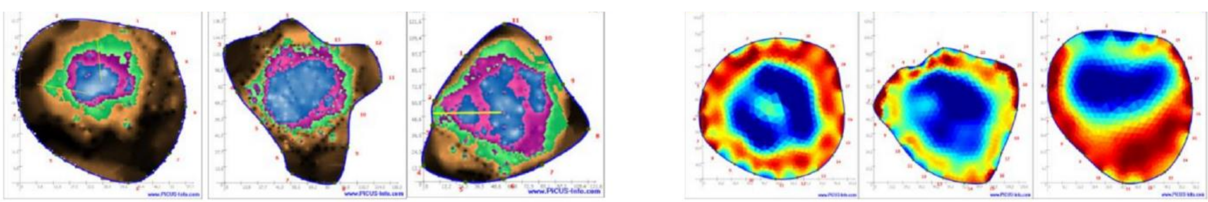
Looking inside the tree non-destructively

One reason that the process of NWC formation is under-studied is that measuring the volume and maximum cross-sectional area of sapwood lost to each taphole's NWC requires felling the tree and sectioning it into many cross-section "cookies", each of which must be processed and measured either directly or via image analysis software. A non-destructive approach would allow such work to be done non-destructively on a living tree, enabling repeated measurements on the same tree, study of high-value trees such as potential genetic stock for breeding or cloning programs, or large numbers of trees across environmental gradients. Tomographic methods for use on living trees have been recently developed and are used to detect the size and location of cavities, incipient decay, heartwood, and other defects (Brazee et al. 2011, Marra et al. 2018, Brazee and Marra 2019, Burcham et al. 2019), but have not yet been validated specifically on NWCs that form around tapholes.

Sonic tomography is performed by inserting minimally invasive steel pins through the bark on all faces of the tree so the pins just barely make contact with the wood (Göcke et al. 2010). A signal is then sent through each pin in the form of an acoustic wave by tapping with a specialized hammer. The other pins receive the soundwave and record the elapsed travel time through the wood tissues. The number of measuring points, ranging from 8 to 50 or more, directly influence the resolution of the tomograph as the images generated are created via triangulation of the elapsed travel times. Travel times are affected by changes in wood density as well as cracks, incipient decay, and hollow cavities (Göcke et al. 2010).

Electrical resistance tomography is performed by using the same minimally invasive steel pins as the sonic tomography measurement. Electrical resistance tomography is performed by passing a current from one pin to another on the opposite face of the tree. The remaining pins are then measured in adjacent pairs to measure differences in the electrical field indicating anomalies in the wood tissue (Göcke 2017). The primary factor that electrical resistance tomography measures is differences in conductivity of the wood which is influenced by wood moisture content or tissue separation (Göcke 2017).

Figure 1. *Examples of three sonic tomograms (left) and three electrical resistance tomograms (right; on different trees), showing highly-resolved spatial detail of wood density and water content within each cross-section. Adapted from Göcke (2018).*



These technologies work in synergy with one another since they utilize the same minimally invasive measuring points and produce two complementary models of wood properties across a tree's cross-section, which complement one another when interpreted together (Göcke 2018). Sonic tomography provides insight into the physical density of the wood while electrical resistance tomography provides insight into the moisture content of the wood allowing images to be produced displaying the internal conditions of the tree. As with the destructive approach, a 3-D model of the tapping zone (or any other volume of interest) can be assembled by interpolating between multiple 2-D "slices". Since all of these measurements are non-destructive and relatively quick (less than 45 minutes per tree), repeat measurements on a single tree are feasible, and a larger population of study trees may be sampled increasing the statistical power of analyses performed. We anticipate that tomographic methods are suitable for detecting taphole-related NWC, which will enable this and future studies of NWC to be conducted efficiently, non-destructively, on high-value trees, and in a temporally explicit manner. This methodology would lead to future opportunities for partnerships between producers and researchers to conduct applied on-sugarbush studies of trees, and for such studies to more efficiently expand their sample size in a way that encompasses variation in both site factors and tree genetics.

Project Objectives

This project will address key knowledge gaps concerning the formation of NWC around tapholes in order to facilitate the development of more diverse and climate-ready sugarbushes on suitable lands within the existing land base, expansion of production to a wider land base, and the potential development of improved genetic lines of maples that optimize tradeoffs between NWC and syrup yield.

Objective 1: Determine whether NWC volume around tapholes correlates with sap yield and sap sugar content across individual sugar maple trees, including trees selected for increased sugar concentration.

Objective 2: Determine how these variables correlate in other maple species including red, silver, boxelder, and Norway maple.

Objective 3: Validate a non-destructive approach to measuring changes in NWC in real time, to enable future research on this topic to be done on high-value trees and on larger numbers of trees.

Objective 4: Communicate results and recommendations with syrup producers in a variety of venues and media, with the goal of educating existing and new producers on the potential value added by tapping soft maples, and best practices for doing so. This includes more specific selection targets for traits in breeding improved maples, as well as evidence-based tapping guidelines for species that do not currently have any.

3) TECHNICAL MERIT

Work Plan

To achieve objectives 1-3, we will use a combination of destructive (tree-felling and cross-sectioning) and non-destructive (tomographic) approaches to measure the NWC that forms around tapholes for two successive years. A subset of at least 5 tomographically scanned trees per species will eventually be felled and sectioned to validate the results of the tomography against direct destructive measurement.

For the main study, we will study a total of 12-15 previously untapped trees of each of the five maple species we are studying. Selected trees will be at least 10 inches in diameter at breast height (8 inches for boxelder; Agriculture Canada, 2000), in codominant or forest-edge canopy position relative to neighboring trees, with healthy crown condition and no major defects in the bottom 16 feet of the bole. The sites of two future tapholes will be marked with paint in line with the breast-height cross-section at 4.5 feet height. A pre-tapping tomography scan will be conducted on each tree this height, and four additional cross-sections 6 and 12 inches above and below this height will be scanned as well. This will be accomplished by hammering stainless steel pins through the bark until they are just touching the sapwood at each height. Pins will remain in the tree for the duration of the study (flagged for safety). Depth to heartwood and the location of any internal defects will be noted on the scan data prior to proceeding with drilling the tapholes. At least two sites will be used for each species. Possible study sites include UNH woodlands properties, town forests, land trust properties, and active sugarbushes. We will prioritize locations will be within ~30 minutes travel time of UNH to facilitate daily sap collections. We have already identified a large proportion of the required trees on UNH lands alone.

In early February of year 1, when the forecast shows several consecutive days of freeze-thaw cycles, a 5/16” taphole will be drilled to a depth of 2” in each study tree at the pre-determined location. A tap and dropline will be installed, draining to a vented 5-gallon bucket at the base of each tree. Collection will be done under gravity only. Sap buckets will be collected after every 1-2 days of flow and weighed to calculate sap yield. Each bucket will be completely thawed (if necessary) and sugar content measured using a Mettler-Toledo Refracto 30PX temperature-compensated refractometer. Sap will not be processed into syrup for this project, but may be given to a local small-scale producer if one is interested. Sap collection will be discontinued when the leaf buds swell to mark the onset of unmarketable “buddy” sap chemistry. Total yield in gallons of sap per season and total sugar yield in grams per season will be used to calculate syrup yield (at 66 Brix) for each tree. This entire process will be repeated in year 2, because there is substantial interannual variability in sap yield driven by differences in spring conditions and in particular the frequency of freeze-thaw cycles during the sap collection season (Taylor 1956, Duchesne and Houle 2014). Tomography scans will be repeated every 2-3 months on trees that will eventually be felled, and every 6 months for all other trees in the study. The closure of each taphole will be monitored at each visit using calipers (Huggett et al. 2007).

An increment borer will be used to collect a 2" deep sapwood sample from ~18" height (i.e. well below the tapping zone) in November the year prior to each round of tapping. The core sample will be freeze-dried and analyzed for non-structural carbohydrates (Quentin et al. 2015, Landhäusser et al. 2018). This sample timing ensures that the core hole will not actively lose sap during the sap collection season, but still represents the same dormant season. A second nearby core sample (in vertical alignment with the first) will be used to measure the mean radial growth rate over the previous 5 years via annual rings.

At the end of the 18-month monitoring term, the subset of 5 trees per species will be felled by cutting at the root flare. A slab 8 inches thick will be ripped from each face of the tree containing a tap hole using a portable chainsaw mill. Each of these 10-foot length slabs will then be halved in length at the tap hole providing an upper and lower bole slab. Each of these half slabs will then be cut into 2-inch increments using a miter saw beginning at the tap hole and ceasing when the NWC is no longer detectable. These incremental cuts will then be photographed for processing. ImageJ image analysis software will be used to quantify the area of nonconductive wood above and below each tap hole, both in both photographs of physical cross-sections, and in tomographic images.

In the event that this validation fails to demonstrate a clear and reliable relationship between the nonconductive wood column volume identified in the tomography scan and that observed directly in felled tree cross-sections for a given species, at least 5 additional tapped trees of each species will be felled and sectioned to ensure a sufficient dataset to successfully complete objectives 1 and 2.

We will conduct a parallel but non-destructive (i.e. tomography only) study of high-value trees at the Cornell Maple Program's plantation in Grand Isle ,VT, where grafted "sweet trees" were planted between 1967-1973 (Demeritt 1985), as well as in a separate 1983 plantation in Lake Placid, NY that includes progeny of the Grand Isle trees (Staats 1992). Trees at both sites have been repeatedly monitored individually for total sap yield and sugar content (Wild 2021). Sampled trees will be selected to span a range of both sap yield and sugar concentration in existing data. Prior-year tapholes are marked on these trees and will be used to locate the optimal placement for tapholes in years 1 and 2. As with trees in New Hampshire, a 5/16" taphole will be drilled to 2" depth, and a tap and dropline will be installed into a single-tree collection bucket. Sap yield and sugar content at Lake Placid will be monitored daily as with our NH study trees, and the Grand Isle trees will be visited several times per season for similar measurements (no technician is onsite to make daily collections on Grand Isle). These sap and sugar data will supplement existing data from several recent years for these trees. These trees will not be felled due to their continued high research value and irreplaceability.

Table 2. Summary of study design *(# trees by level of study)*

Species:	sugar	red	silver	boxelder	Norway
Intensive (tap, tomography and fell/section)	5	5	5	5	5
Supplemental (tap, tomography)	7-10	7-10	7-10	7-10	7-10
High-value "sweet" trees (tap, tomography)	20-40	-	-	-	-

Data analysis

Objectives 1-2: *Correlations between NWC, sap yield, sugar and growth rate*

Unlike most previous studies, this study design will enable us to monitor the time course of development of NWCs around tapholes in two ways. Direct measurements on cross-sections will be made when the first of the tapholes is ~18 months old and the second is only 6 months old. The non-destructive method will provide additional information – in the subset of trees that will be felled and sectioned, tomographic scans will be conducted every 3 months to allow the development of each NWC to be monitored non-destructively. NWC size will be interpolated as a 3-dimensional volume between cross-sections.

Across all sugar maples studied, we will use a mixed effects model to determine the effects of annual sap yield and weighted-average sugar concentration on NWC volume and maximum NWC cross-section. All data will be averaged across the two study years to better reflect differences among trees rather than random variation among years. Site will be included as a random factor, and growth rate and carbohydrate measurements will be included as covariates. The analysis will be run separately both for directly sampled trees and for the far larger number of tomographically sampled NWCs (see objective 3).

For each species, the loss of functional sapwood cross-sectional area in the tapping zone will be compared with the average growth rate and a range of simple allometric assumptions about the formation of heartwood. This will be done for the full range of tree sizes analyzed for each species. Together, these calculations will be used to estimate risk of net loss of sapwood by size class, to determine whether the use of sugar maple tapping guidelines is equally appropriate for these other species.

Our results relating sugar content, sap yield, and NWC formation in sugar maple particularly (and especially in Cornell's high-value maple orchards) will provide critical information about possible tradeoffs associated with the selection and planting of high-yield and high-sugar trees. If both of our hypotheses about NWCs are correct, then high-sugar trees have additional benefits beyond simply lower cost of processing (i.e. maintaining greater amounts of functional sapwood and reduced risk of each new taphole intersecting NWC from a previous taphole), even if they don't yield more syrup overall. If both of these hypotheses are not fully confirmed, a more careful balancing of sap yield vs sugar content vs other tree traits may be optimal for selecting traits of future plantation trees. We will analyze data for the additional 4 maples examined under objective 2 in the same way, as these species are potential future targets for selecting more optimal trait combinations for syrup production – they have already been subject to substantial selection and breeding as landscape trees but to date not for syrup production.

Both within and across species, ***this research may provide important clues to fundamental physiological relationships driving differences in sap and sugar yields among species and individuals within species.*** For example, correlations between starch storage and sap and sugar yield might provide insight into the physiological mechanisms behind "sweet trees" – are these trees that store more of their total carbohydrates as sugar rather than starch during the dormant season? Or are they trees which prioritize storage of carbohydrates over growth? Or perhaps which store more of their total carbohydrates in sapwood relative to roots and twigs during the dormant season? These clues could again be useful in understanding the tradeoffs inherent in selecting various traits in improved genetic lines of maples.

Objective 3: *Validation of non-destructive tomography as a method to study NWC formation*

For the tapped trees selected in both objectives 1 and 2, we will non-destructively scan each tree prior to tapping in a pre-determined, marked location. The development of NWC around each taphole will be measured using the combined sonic and resistance tomographs at each time step. Five trees per species will be felled to validate the tomography data, and a final scan conducted immediately prior to the harvest of the tree. Upon harvest, the area of the NWC at each cross-section will be directly compared with the final sonic and electrical resistance tomographs from these same cross-sections, and used to adjust our interpretation of all tomography data if necessary. A regression model will be used to correlate cross-sectional area of each NWC in each scan with that measured directly on the corresponding cookie in the calibration dataset. A separate validation model will be generated for total NWC volume.

The tomographic time series for each tree section will be compared (by difference) to the initial scan of each tree section in order to generate a time series of taphole NWC development in both the horizontal and vertical directions from each taphole. This will be validated against the difference in size between the 6-month old and 18-month old taphole NWCs on the harvested trees. In the event that tomography does not reliably show a reliable relationship with the destructively measured NWC area and volume, additional trees will be felled and sectioned in order to accomplish objectives 1 and 2 with satisfactory sample size.

Objective 4: *Stakeholder communication and Producer Education*

This project is aimed at generating results and recommendations for two key stakeholder audiences: producers who are unsure about how existing tapping guidelines apply to maple species beyond sugar maple, and also researchers who have been selecting and breeding improved sugar maple varieties. We commit substantial time and travel budget in year 3 to generating a variety of written products describing our results and recommendations and communicating these directly with the producers who could benefit from implementing them. See "Dissemination of Results" and "Data Sharing" sections below for a detailed list of our planned products and communication venues. ***These events will provide excellent opportunities for conversations with producers who already make use of either improved maples or trees other than sugar maple***, allowing us to incorporate their perspective in subsequent written products.

This project will also generate important data (particularly sap volume and sugar concentrations) from additional species that are relevant to communications with producers about processing costs and the return on investment associated with expanding tapping to species other than sugar maple. These data will be shared publicly and summarized in presentations and written products.

Furthermore, we anticipate that the tomographic methodology will not only be a useful research tool but will also have substantial outreach and educational value in and of itself. The methodology as described above is quick to set up, and data are generated in real-time for immediate viewing. A live demonstration of heartwood, taphole-associated NWCs, and other internal defects (which may or may not be externally obvious) on a small number of individual trees could be used as important teaching examples that would make a greater educational impact than using tree cross-sections as the only examples of the size, shape, and importance of nonconductive wood columns to tree health and tapping sustainability.

Table 3. Timeline *(on an academic calendar; spring=Jan-Apr; summer=May-Aug; fall=Sept-Dec).*

		2022	2023			2024			2025	
Obj.	**Activity**	**fall**	**spring**	**summer**	**fall**	**spring**	**summer**	**fall**	**spring**	**summer**
1,2,3	select trees, initial tomography	X								
1,2,3	increment cores	X			X					
1,2,3	tap trees		X			X				
1,2	collect sap/sugar data		X			X				
1,2,3	tomographic monitoring		X	X	X	X	X			
1,2	harvest trees						X			
1,2	process tree cookies						X	X		
1,2	carbohydrate analysis							X	X	
all	analyze data, generate recommendations						X	X	X	
4	present results					X			X	X
4	prepare publications							X	X	X

Relationship to other Federal proposals and current/past projects

The work described in this proposal has not been submitted as a proposal to any other Federal or non-Federal funding program. This work builds upon but does not duplicate a current ACER project at the University of New Hampshire in which Dr. Asbjornsen and PhD student David Moore are testing various new tap designs to optimize taphole length and width, producing the maximum sap yield (under gravity or vacuum) in sugar maple while minimizing the consequent loss of functional sapwood to NWC. Dr. Asbjornsen also has projects funded by NE-SARE and AFRI to study sap yields, sap properties, and nonconductive wood formation in potential syrup producing species other than maples, so the species used do not overlap with those planned for study in this project. However, data and experience gained in the above studies will inform our field and lab procedures and provide important context for interpreting our results from each of our 5 maple species. Specifically, unlike prior/current work at UNH, we will:

- Focus on "sweet" sugar maples with implications for selecting future high-production genotypes.
- Include *5 maple species*, most of which are under-utilized by producers and also under-studied.
- Validate a novel non-destructive method to study NWC volume which would greatly enhance possibilities for future research on NWC.
- *Directly observe the time course* of NWC formation after tapping.

4) ACHIEVABILITY

Outcome Indicators

We anticipate outcomes under **3.1**, **4.3**, and **5.1** that 1,500 producers will directly learn about findings relevant to maple production in at least 10 presentations and workshops, with benefits for to ecological and economic stability. Under outcomes **3.2**, **3.8**, **3.9**, **5.2**, and **5.3** we anticipate that 150 producers will have the opportunity and interest to increase production using our recommendations in the short term, initially, **(5.4)** adding ~10,000 additional taps. Under **4.1**, we aim to accomplish three research goals (i.e. objectives 1-3). Under outcome **4.4**, we plan at least 5 distinct publications arising from this research. Under **4.4a**, we anticipate approximately 1,000 views in the first year after results are communicated.

To **measure outcomes** 3.2, 5.2, and 5.3, at workshop and training events in which we participate, we will conduct brief surveys before and after our presentation with questions about:

- Current utilization of species other than sugar maple, or any improved varieties.
- Opportunities to do on their existing land base or additional lands, and at what scale.
- Perceived the barriers and benefits of doing so.

Outcome Indicator Measurement

Table 4. *Measurement plans.*

Indicator	How we estimated	How/when we will evaluate	Key factors for outcome
3.1, 4.3, 5.1	conservative estimate based on pre-2020 attendance of the largest (NY) event (~1000) and assuming not everyone at each event we present at will attend all talks	we will request attendance data from event organizers and also make visual estimates of the attendance of each presentation we give	event attendance varies by year and format, as shown by recent experience (e.g. 2020)
3.2, 3.8, 3.9, 5.2, 5.3	we estimate that 10% of producers reached will have interest and ability to expand production using our recommendations	surveys as described above	producers have varying ability to expand based on their existing land base and other logistics of their operations

4.1	these are laid out as objectives 1-3 above	upon completion of the project based on our ability to generate recommendations from the data we collect	NA
4.2a	We anticipate that Obj 1&3 will lead to additional questions and opportunities	upon completion of the project	contingent on variability in data and the extent to which our hypotheses are confirmed
4.2b	We anticipate that Obj 2 will result in reasonably final recommendations		
4.2c	we anticipate all 3 objectives will lead to implementation of new techniques		
4.4	see *Dissemination of Results*		
4.4a	view rate estimate based on prior publications and datasets in repositories	upon completion of the project, based on repository downloads, plus circulation of any print publications	publication availability often takes several months after the final version is accepted
5.4	we anticipate that producers expanding production will start small, averaging <100 additional taps at first	*see 3.2*	*see 3.2*

Dissemination of Results

Our project is aimed at generating information that will help producers more confidently make sustainable use of several under-utilized maple species, and also in understanding potential tradeoffs between tree traits which are under selection in long-term sugar maple improvement programs such as Cornell's. As such, the results are only useful if they are communicated effectively with the appropriate producers and other stakeholders. Our plan is to disseminate results in a variety of formats and venues, including both large and small in-person events. Maple producers will be our primary target audience but we will also disseminate results to other researchers and to forest management professionals, due to the implications for managing sugarbushes and mixed stands with potential as future sugarbush development.

Specific <u>in-person communications</u> will include:

- Presentations at 8 maple producers' association meetings (see letters of commitment from NAMSC, Cornell, and state organizations that already invite us to 5 such meetings).
- Presentations and demonstrations at two or more Maple Schools or other educational workshop events for new and established producers. This will include the Wisconsin Winter Institute to which we have already been invited (see letter of support).
- One presentation at a New England Society of American Foresters annual meeting

We will also publish our results and recommendations in a variety of formats and venues targeting a wide audience. **Specific <u>written products</u> will include:**

- An extension document describing modified tapping guidelines for red maple and other maple species besides sugar maple.
- Master's thesis which will be available to the public on scholars.unh.edu
- At least one producer-focused article (e.g. in *Maple Syrup Digest*)
- At least one forester-focused publication (e.g. in *Northern Woodlands*)

- At least one peer-reviewed scientific article (e.g. *Forest Ecology and Management*, *Canadian Journal of Forest Research* or *Tree Physiology*), with open access to maximize its impact and availability to producers and other non-academic users.
- Open sharing of all primary collected data upon completion of the project in a public repository; see “Data sharing” section below.

In years 2 and 3, we will seek additional low-cost opportunities to communicate our results in other formats as well, to maximize our audience (interviews, news articles, podcasts, etc).

5) EXPERTISE AND PARTNERS

Key Staff Roles and Qualifications

Table 5: *Key Staff, Partners, and Collaborators*

<table>
<tr><th>Name/Title or Orgnaization</th><th>Role</th><th>Relevant experience / successes</th></tr>
<tr><td>Dr. Matthew Vadeboncoeur
Research Scientist</td><td>Project lead. Will supervise grad student, manage data collection, logistics/safety, data QC, conduct outreach, and lead at least 2 written products</td><td>61 scientific publications, 10 as lead author. Experience presenting to maple producers, foresters, general public, and other forest stakeholder groups.</td></tr>
<tr><td>Dr. Heidi Asbjornsen
Professor</td><td>Academic advisor, data interpretation, outreach and communication</td><td>3 ongoing syrup-related research projects and other agroforestry projects, and has advised 35 graduate students in her career</td></tr>
<tr><td>Steven Roberge
Extension State Specialist</td><td>Will help develop recommendations and contribute to outreach and communication efforts to ensure maximum clarity for producers</td><td>15 years of experience working in extension, specializing in outreach, sugarbush management, and syrup industry</td></tr>
<tr><td>Tanner Frost
Graduate Student</td><td>Will collect data in the field and lab, lead at least 2 written products</td><td>Has worked in syrup production, current field and lab technician. Conducted syrup-related public outreach at a small farm</td></tr>
<tr><td>Adam Wild
co-director
Cornell Maple Program</td><td>Partner- will provide access to Cornell trees for study and previous data collected on these trees. will facilitate outreach and communication with producers</td><td>Past and current research on maple production and specifically on sap sugar content. Ongoing and continual outreach to producers, consumers, and other stakeholders</td></tr>
<tr><td>North American Maple Syrup Council</td><td>Collaborator - will facilitate communication with producers via events and written articles, e.g. in Maple Syrup Digest</td><td>Represents many industry stakeholders and promotes education and communication between producers and researchers</td></tr>
<tr><td>Maine Maple Producers Association</td><td rowspan="3">Collaborators - will facilitate communication with producers via meetings, workshop events, and electronic communications</td><td rowspan="3">These organizations represent and serve the producers of each state directly and board members have years of experience educating members via meetings, trainings, and publications</td></tr>
<tr><td>Massachusetts Maple Producers Association</td></tr>
<tr><td>Wisconsin Maple Syrup Producers Association</td></tr>
</table>

Dr. Matt Vadeboncoeur is a Research Scientist at the University of New Hampshire. He has two decades of experience managing forest ecology and forest management research projects, including field crew supervision, lab management, study design, data collection, statistical analysis, publication, and outreach to stakeholders. His training includes forest ecology and plant physiology among other disciplines. His research has mostly focused on how northeastern tree species respond to stresses ranging from nutrient limitation to extreme climate events. His current interests include applied research towards developing a more sustainable, diverse, and local food system while maintaining ecosystem services. He taps a mix of red and sugar maple in his Nottingham, NH backyard every year.

Dr. Heidi Asbjornsen is a Professor of Natural Resources at the University of New Hampshire. She has current syrup-related projects, and past projects related to agroforestry. She has served as primary advisor for 35 past and current graduate students in her career.

Steve Roberge is an Extension State Specialist at UNH. He has over 15 years of experience working in extension, specializing in outreach, sugarbush management and the syrup industry.

Tanner Frost is a 2021 graduate of the Forestry program at the University of New Hampshire and would be supported by this proposal to complete a MSc degree in Natural Resources, with components of this project comprising his thesis. Tanner worked at Old Orchard Farm in Madbury, NH where he helped build the sugar house and assemble the evaporator, RO system, and plumbing. He was the primary installer and collector for 500 taps and was involved in the syrup making process. He also gave public tours of the production process during community outreach. Currently he is a research technician working projects relevant syrup production and tree physiology. ***He has been involved in all aspects of developing this project plan.*** Importantly, it was he who had the key insights that led to both the development of our hypotheses and the methods we propose to use. ***His education as an expert in aspects of maple production sustainability <u>would be an important secondary outcome of this project</u>.***

Partners: Adam Wild is a Senior Extension Associate and co-Director of Cornell University's Maple Program, which is a world leader in maple research, education, and outreach.

<u>Project Management Plan</u>

This project represents a close collaboration between researchers, extensionists, and producer associations. Our management structure is organized to facilitate frequent and effective communication among participating researchers and stakeholders and identifies clear responsibilities to ensure the successful accomplishment of project goals. Project calls of the core team will be held monthly to quarterly, to allow for regular exchange of information regarding progress on specific research tasks, coordination of project activities, and troubleshooting to address any issues. All written products and presentation materials will be made available to the entire team for discussion and feedback to ensure that results and recommendations are presented and communicated clearly. ***Vadeboncoeur*** will have primary responsibility for project coordination, maintaining communication among the team, ensuring that tasks are completed according to the timeline (Table 1), and production of data and results. He will work closely with ***Frost*** on field data collection, testing and cross-calibration of methods, quality control, data analysis, lab analysis, and preparing written products. ***Asbjornsen*** and Vadeboncoeur will co-mentor Frost; Asbjornsen will serve as academic advisor and will contribute to written products and outreach materials. ***Roberge*** will apply his expertise in adapting our findings into revised sustainability or tapping guidelines and will aid in the development of outreach presentation materials to ensure they communicate effectively with producers and other stakeholders served by cooperative extension. ***Wild*** will share data from the sweet sugar maple trees in the plantations we'll study and will provide access to these trees for non-destructive study. He will also facilitate outreach to producers in NY state and provide feedback and context for how our sugar maple results inform Cornell's existing study of genetically selected sweet trees. ***Cooperating Producer Associations*** (NAMSC and state associations) will facilitate the communication of our results with producers in their routine meetings, trainings, workshops, newsletters, and/or electronic communications. <u>Producer associations are not expected to incur additional costs as a</u>

result of this project. We have already received commitments from three state associations who are excited for their members to hear about our eventual results and recommendations (see letters). However, because the bulk of proposal development occurred during a very busy time of year for producers, we will reach out again to other associations in year 2 with offers to present results directly at an annual meeting or workshop event.

Data Management

All electronic data generated for this project will be backed up at least weekly by each data generator using Microsoft OneDrive cloud storage and shared among all project personnel. UNH has a site license for OneDrive, with no storage limit. Version control and document check-out features will be used to preserve data integrity. Regardless of cloud backups, project personnel will each maintain separate backups of their own documents. Project-critical field and lab notebook pages will be scanned for cloud storage as well. Draft manuscripts and outreach materials will also be shared internally in this folder. All materials will be retained for at least 5 years beyond the end of this project.

Data Sharing

Upon publication of our findings, all quality-controlled primary datasets will be made freely and permanently available on the Environmental Data Initiative (EDI). To enable discovery and usability of these data, EDI follows Best Practices for Dataset Metadata, fully describes all data table attributes, and includes temporal, geographic, and taxonomic coverages. These features align with FAIR data practices (Findable, Accessible, Interoperable, Reusable; Wilkinson et al., 2016). The EDI repository supports immutability and strong versioning of datasets. Each dataset version is provided its own unique DOI.

Publications and presentation materials will be made available on MapleResearch.org and/or UNH Cooperative Extension, to the fullest extent allowable by publisher copyright agreements. We have included funds in the budget for the publication of at least one open-access paper in a peer-reviewed academic journal. The thesis produced will be available to the public in the UNH Scholars Repository.

Sustainability of Plan

We anticipate that project results leading to the development of evidence-based tapping guidelines for red, silver, boxelder, and Norway maples will encourage and enable the more confident utilization of these other maples both in existing sugarbushes (especially red maple), and also on other lands not currently being used for syrup production. Our products include both direct in-person communication with producers at meetings and workshops, but also publication of our results in a variety of formats which will make our results and recommendations permanently available to a wide audience. Our partnership with cooperative extension in particular is an ideal means for ensuring that new knowledge is available and consistently included in advice to producers and other stakeholders. In particular, we have partnered with the Cornell Maple Program, which has over five decades already invested in selecting and studying “sweet” sugar maples, making it a leader in this pursuit. Their direct involvement will ensure that our findings will influence the future of this line of research to the extent appropriate.

The data generated by this project and the publication and educational outreach efforts to disseminate these findings will both promote the consideration of tapping a wider variety of maples. We anticipate that this work may have its greatest impact in parts of the US where sugar maple is less abundant than other maple species (Table 1) and perhaps even in states that do not currently produce substantial amounts of syrup. We also anticipate that while it will not be cost-effective for individual producers to deploy at scale, our novel approach for examining nonconductive wood columns and heartwood has tremendous potential educational value, and will enable a wide variety of more detailed research studies to be conducted on variation in NWC, e.g. under different collection conditions. It will also enable future studies on variation in sapwood depth, and other wood properties on high-value live trees, such as genetic improvement stock or large landscape specimens.

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