TREE BIOLOGY AND DENDROCHEMISTRY

KEVIN T. SMITH and WALTER C. SHORTLE

USDA Forest Service, Northeastern Forest Experiment Station, P.O. Box 640, Durham
New Hampshire 03824 USA

ABSTRACT. Dendrochemistry, the interpretation of elemental analysis of dated tree rings, can provide a temporal record of environmental change. Using the dendrochemical record requires an understanding of tree biology. In this review, we pose four questions concerning assumptions that underlie recent dendrochemical research: 1) Does the chemical composition of the wood directly reflect that of the soil? 2) Can the analysis of individual rings pinpoint the specific year of environmental change? 3) Are differences in element concentration in the wood directly related to chemical differences in the sap? 4) Are samples selected for dendrochemistry free of the effects of tree injury and infection? We suggest methods to reduce the uncertainty from these assumptions.

INTRODUCTION

Environmental scientists use dendrochemistry, the interpretation of elemental analysis of dated tree rings, to reconstruct the timing of changes in the external chemical environment. Much of this research is to discover temporal markers of environmental change (Bondietti et al. 1990; Guyette, Henderson and Cutter 1992; McLennan and Vinnerstedt 1993) or to find direct evidence of phytotoxicity (Berish and Ragsdale 1985). Increment cores are appealing samples for such analyses. Sampling with an increment borer is comparatively nondestructive, environmentally benign and usually simple to accomplish. Increment cores appear to be durable, spatially and temporally determinate and linked to the external chemical environment.

Although dendrochemistry can provide useful environmental information, trees are not passive recorders of the external environment. Biological processes mediate the formation of the chemical record. The use of dendrochemistry to find markers of environmental change requires an understanding of tree biology. We should address specific questions during the interpretation of chemical trends in tree rings: 1) Is the measured element taken up preferentially, discriminated against, or does the element follow hydraulic mass-flow? 2) Is the element translocated in the apoplast or the symplast? (The apoplast is that portion of the tree that is outside of the cytoplasm and is the aggregation of cell walls and open lumens of xylem elements (Nobel 1991). Transport in the apoplast is passive, regulated by bulk hydraulic flow and ion displacement (Kunin 1960), and follows gradients of decreasing concentration. The symplast is the aggregation of cytoplasm, or living cell contents, interconnected by plasmodesmata (Nobel 1991). Transport of elements into and within the symplast is active and is frequently counter to gradients of decreasing concentration.) 3) Finally, is translocation mediated by the symplast of a colonizing pathogen?

The details of biological expression vary from species to species. Cutter and Guyette (1993) and DeWalle et al. (1991) considered the relative suitability of species for dendrochemistry. However, the basic biological principles of translocation, infection, and tree response to injury and infection have not been applied fully in the selection of species for analysis. Frequently, the method of chemical analysis is emphasized, without an examination of how variability in the chemical record can result from basic tree biology. This lack of examination can result in the rejection of either a particular tree species for investigation (Zayed, Loranger and Kennedy 1992) or rejection of dendrochemistry itself. Failure to apply basic principles of tree biology has led to the interpretation of results based on questionable assumptions. Here are some of these assumptions from research on the dendrochemistry of
metals. Resolution of the doubts around these assumptions will yield more convincing and useful research results.

**Questionable Assumptions**

The first assumption to question is that wood chemistry reflects the chemistry of the soil and soil solution. The chemical relation of soil and stem is a series of abrupt thresholds and equilibria. In this paper, sap refers to the liquid that flows in the apoplast, primarily in the conducting elements of the wood. Sap is not cytoplasm, the contents of living cells. Most elements do not follow simple diffusion or mass-flow kinetics from the soil to the stem (Prenzel 1979). Even very high concentrations of metals such as Pb in the soil may not be readily detected in the wood of fast-growing, insensitive trees, as Łukaszewski et al. (1993) showed for *Populus marilandica*.

European beech (*Fagus sylvatica*) discriminates against uptake and translocation of Al and Na, whereas Mn, Ca, K, and P are selected for preferential uptake (Prenzel 1979). Mechanisms of exclusion can involve the maintenance of steep pH gradients. The pH in the absorbing root tip increases more than one full unit over a distance of just a few cells, causing the bulk precipitation of certain metals, especially Al. Other metals, such as Ti and Mo, increase in solubility as pH increases; the effect of increased solubility in the root on the uptake and translocation of these elements has not been demonstrated.

Ratios of the molar concentration of Al:Ca have been useful markers of environmental stress in the soil solution and in excised root tips (Shortle and Smith 1988; Schlegel, Amundson and Hüttermann 1992). However, the Al:Ca ratio is likely to be less useful in wood analysis because of the exclusion of Al from the sap within a very short distance from the tip of the absorbing root (De Visser 1992; cf. Bondietti, Baes and McLaughlin 1989). The small amount of Al that is not precipitated is associated with organic chelators and conducted in trace quantities in the sapstream. The accumulation of Al in foliage is the result of metal precipitation at the end of the sapstream and reflects the hydrological flux of transpiration. Similarly, trace amounts of heavy metals such as Pb can be detected in wood (Robitaille 1981). We suggest that the observed decrease in Pb in more recently formed wood of trees from an unpolluted site could be due to a briefer exposure of the wood to the sapstream, rather than a decrease in the amount of Pb in the soil (cf. Robitaille 1981).

Selectivity of uptake can be accomplished by active transport into the symplast, as for Mg and K. This active, symplastic transport can be further facilitated by mycorrhizae, especially for P. Selectivity of uptake in the apoplast, the cell wall network, is less directly controlled, but is maintained through the production of ion-exchange sites and pH gradients. For essential Ca, translocation is apoplastic through successive ion displacement. Nonessential Ba and Sr are similarly translocated in the apoplast. The translocation of Mg is both through active transport in the symplast and ion displacement in the apoplast. In European beech, concentrations of S, Fe and Mg were not increased or decreased relative to expectations based on simple bulk flow (Prenzel 1979). Some symplastic elements such as S may be taken up and translocated in quantities greater than needed for nutrition (Ohman and Grigal 1990). Because of their essentiality and symplastic transport, we expect S, Fe and Mg to be selectively favored for uptake under conditions of nutrient limitation.

Contrary to the apparent expectation of Long and Davis (1989), significant differences in trace metal concentrations are not always detected, even when the point source of metal pollution is well documented. The detection of apoplastic, nonessential elements has value as a qualitative marker of availability for uptake. Without knowing the hydrological flux, we believe that quantitative comparisons for time periods within a single tree or between trees may be difficult to justify.
The second assumption to question is that the analysis of individual rings can pinpoint the specific year of environmental change (Cutter and Guyette 1993). Sophisticated analytical techniques, such as X-ray fluorescence, laser mass spectroscopy and proton-induced X-ray analysis, can estimate metal concentration for walls of individual cells or small groups of cells. Consequently, the resolving power of the analytical technique appears to be quite high. However, the metals in wood are translocated in the symplast and apoplast of all of the rings of sapwood, the portion of tree stem and branch wood that contains the symplast, conducts water and stores starch. Many tree species contain an outer band of sapwood that surrounds an inner core of heartwood. The symplast, water-conducting vascular elements and starch are absent from heartwood. Sapwood varies within and between species in both radial width and number of rings. Therefore, the presence of an element in a specific ring dated to a precise year does not necessarily mean that the element was present in the tree environment in that year. Furthermore, Shigo and Hillis (1973), Shigo (1984) and many subsequent workers distinguish heartwood formed as a consequence of tree age from wound-initiated discoloration. The stems of some trees, such as species of maple, birch and poplar (genus Acer, Betula and Populus, respectively), do not contain heartwood, irrespective of age (Manion 1991). In those species, central columns of discolored wood are the result of wounding. Frequently, the discolored wood is infected by decay fungi or associated microorganisms. The chemistry of true heartwood is also altered in the infection and decay process. The distinction between heartwood and wound-initiated discoloration is important, because these internal transformations affect dendrochemistry (Shigo and Hillis 1973).

Translocation across the width of sapwood is responsible for at least some of the observations attributed to radial translocation (Łukaszewski et al. 1988; Zayed, Loranger and Kennedy 1992). Radioactive isotopes of nonessential elements such as $^{90}\text{Sr}$ and $^{137}\text{Cs}$ show that metal elements may be detected in wood formed prior to or following the period of metal uptake. $^{90}\text{Sr}$ is translocated in the apoplast, similar to essential Ca. $^{137}\text{Cs}$ is translocated in the symplast, similar to essential K. Metals newly introduced to the sapstream can be bound to wood formed years before the onset of altered sap chemistry. For example, the radioactive isotope $^{90}\text{Sr}$ was introduced to the biosphere through the atmospheric testing of atomic devices, with peak deposition in the mid-1950s. In red spruce (Picea rubens), significant concentrations of $^{90}\text{Sr}$ were found in wood formed in the mid-1920s (Bondietti et al. 1990). This is consistent with the fact that red spruce trees frequently contain 25–30 annual rings of sapwood, as determined by visual inspection (Smith and Shortle, in preparation). In American beech (Fagus grandifolia), the peak concentration of $^{137}\text{Cs}$ occurred in wood formed 18 yr prior to the peak deposition of the radioisotope (Brownridge 1984). Brownridge (1984) detected peak concentration of $^{137}\text{Cs}$ near the sapwood/heartwood boundary, despite differences in the number of rings of sapwood present. We agree with Brownridge (1984) that the $^{137}\text{Cs}$ was probably translocated into more recently formed rings as sapwood was transformed into heartwood. Essential K and the nonessential analog $^{137}\text{Cs}$ are retained in the symplast.

Substantial amounts of P and K accumulate in the sapwood as a result of the normal functioning of protoplasts. The high concentrations of P and K are not "left over" from the process of wood formation as was interpreted for red spruce (Arp and Manasc 1988) and whitebark pine (Pinus albicaulis) (Peterson and Anderson 1990). Nor are the reduced concentrations of P and K in heartwood due to "leaching" from ion-exchange sites in the wood (Arp and Manasc 1988). As a ring of sapwood is transformed to heartwood, the symplast containing the P and K withdraws into more recently formed sapwood. Similarly, in English oak (Quercus robur), concentrations of P, K, Mg and Mn are greatest in the inner sapwood rings (adjacent to the heartwood). This enrichment may be due to the importation of the elements from rings converted to heartwood. Concentrations also
tend to decrease from inner sapwood to outer, more recently formed, sapwood (De Visser 1992). In Scots pine (*Pinus sylvestris*) grown in the vicinity of a Zn smelter, elevated concentrations of Zn occurred in wood formed several decades prior to the onset of emission (Lukaszewski *et al.* 1988). We suggest that this is due to the width of functional sapwood and not, as Lukaszewski *et al.* (1988) suggest, to radial translocation.

The third assumption to be questioned is that differences in element concentration in the wood are due to differences in concentration in the sap. For symplastic elements that are accumulated against steep concentration gradients, such as P and K, this assumption seems especially unlikely. Momoshima and Bondietti (1990) and Bondietti *et al.* (1990) investigated ion-exchange properties of Ca in the apoplast of red spruce. In concept, the tree stem is a renewable ion-exchange column in which sap cations are in equilibrium with the cell-wall system. The pattern of cation mobility can be modeled using the Donnan equilibrium (Kunin 1960). The Donnan system predicts the binding distribution of cations of different valences that are associated with fixed and mobile anions. Chromatographic fractionation and changes in binding capacity and sap pH all determine the vertical and radial trends in cation concentration. The alkaline earth elements were fractionated as they moved up the stem in the sapstream, in order of decreasing mobility: Mg$^{2+} >$ Ca$^{2+} >$ Sr$^{2+} >$ Ba$^{2+}$ as predicted from ion exchange theory (Momoshima and Bondietti 1990). Ag$^+$ was retained in the lower bole, apparently bound to sulphhydryl groups. Consequently, samples for comparison must be taken from the same height on the stem.

In red spruce, the number of potential ion-exchange sites decreases in more recently formed wood as the tree increases in diameter (Bondietti *et al.* 1990). Tentative explanations for this observation include the reduction in amounts of pectic materials and hemicellulose due to increases in tracheal cell size with age (Bondietti *et al.* 1990) and increased crystallinity of cellulose and decreased lignification with age. Under conditions of uniform sap cation concentration and pH, the concentration of Ca$^{2+}$ will decrease due to decreased binding capacity in more recently formed wood (Momoshima and Bondietti 1990). The number of binding sites at the same relative position in the stem also varies among individual trees. The expression of Ca$^{2+}$ concentration as a percentage of total possible concentration may be more useful than the expression of absolute concentration. This is analogous to the determination of percent base saturation, which is commonly expressed in soil analysis. Previous research notes the decrease in concentration of Ca in more recently formed wood, but attributes the decrease to reduced availability (Arp and Manasc 1989; Frehlich, Bockheim and Leide 1989) rather than to a reduced capacity for binding.

The fourth assumption to be questioned is that samples selected for dendrochemistry are free of the effects of tree injury and infection. Until recent years, most of the research on metals in wood was related to the pathology of trees and forest products (Scheffer 1939; Good, Murray and Dale 1955; Ellis 1959). As wood is altered due to injury and infection, the concentration of mobile ions tends to increase (Tattar, Shigo and Chase 1972; Safford, Shigo and Ashley 1974). The mobilization of ions occurs in advance of visual changes in the wood or changes in structural properties (Smith and Shortle 1988). K$^+$ and to a lesser extent, H$^+$, are the principal cations that initially mobilize following injury and infection. This increase in wood ionization, especially of K$^+$, is detectable using non-destructive electrical measurements (Ostrofsky and Shortle 1989). Increased concentration of H$^+$ also affects the Donnan binding equilibrium of other cations, potentially altering their concentration in the wood.

We expect that samples selected for dendrochemical analysis would be visually uniform and free of discoloration and decay due to injury and infection. However, wood altered by injury and infection
may not be readily evident, especially in narrow increment cores. Certain tree species, especially conifers, do not markedly discolor early in the infection and decay process. Yet the chemical differences caused by wounding and infection are just as great. Trees are wounded throughout their lives through the natural shedding of branches and roots as well as pathogen activity and abiotic mechanical damage. The boundary-setting process within living trees that limits the loss of normal function following wounding is called compartmentalization (Shigo 1984). As part of the compartmentalization process, boundary layers may form between columns of infection and healthy wood. Relative to the surrounding healthy sapwood, the column boundary layer in red maple (Acer rubrum) is enriched in soluble K 7-fold, Ca 6-fold, and Mn 5-fold. In red maple, wood discolored through wounding and infection contains twice the K and 20% the Mn of healthy sapwood (Shevenell 1984). Wood that is chemically altered from vascular disease in the living tree often takes on irregular or fan-like patterns (Smith and Houston 1994). Consequently, altered wood may be inadvertently added into a sample for dendrochemical analysis. A small amount of highly altered wood can affect the results from a much larger total sample. For example, the contamination of a sample of red maple sapwood with 5% of a boundary layer would result in an apparent increase of K 36%, Ca 31% and Mn 25%, compared to uncontaminated sapwood. Also, the early stages of brownrot, which may not be visible in core samples, can be associated with a significant reduction in pH (Shortle 1982). The pH reduction can result in large changes in ion mobility and binding, without a change in absolute concentration.

**Problem Resolution**

Researchers can take several steps to minimize the effect of the questioned assumptions on research to find temporal markers of environmental change. Because analytical techniques will always provide data, and data arrays will almost always contain trends, explicit hypotheses should be tested. We contend that radial trends of chemical data are not good places to “go fishing” for research topics.

Entire investigations based on a single tree, or worse yet, a single core, should be discouraged (Scherbatskoy and Matusiewicz 1988). The effects of external microsite or internal tree condition on a single set of radial observations are just too great. Researchers should be alert to external signs on the trunk such as pitch and stem cracks that indicate a specific tree may not be suitable for sampling. Slight discolorations of the extracted core can indicate unsuitability for dendrochemistry, although the core sample may still have value for dendrochronology.

Modern techniques that analyze extremely small samples may not be a cost-effective means of determining temporal trends in environmental change (McClenahan and Vimmerstedt 1993; cf. Cutter and Guyette 1993). Analysis of a very small portion of an individual ring may introduce variation due to anatomical features such as latewood or earlywood composition (McClenahan, Vimmerstedt and Scherzer 1989). The high cost of an individual observation discourages the breadth of sampling necessary to validate the utility of a marker of temporal change. Minocha and Shortle (1993) have developed methods to easily, rapidly and inexpensively extract cations from wood. Pooling samples or data over decades or bidecadal periods may be a realistic way to consider data, especially given that elements are transported in previously formed wood.

The decrease in cation binding capacity as an individual tree grows, as shown for red spruce, indicates that analysis of data as a proportion of saturated binding may be useful (Momoshima and Bondietti 1990). Determining the direction and magnitude of change in concentration for successive periods of wood formation for an individual tree-ring series may be more useful than simple comparisons of concentration. Calculating the frequency of net change is a way to combine observations
taken from a number of different trees, without diluting the trend by averaging (Bondietti et al. 1990).

Dendrochemistry researchers know that it is important to avoid the simple chemical contamination of increment cores and other wood samples. However, the stringent cleaning or surfacing of cores may not be enough for dendrochemistry. Dendrochemical samples need to be handled carefully, because fungi can rapidly grow on the surface and through the wood of increment cores. Fungi are very effective in moving elements, especially metals, across ring boundaries. Controlling fungal growth requires the refrigeration of cores the same day they are collected, followed as soon as feasible by air-drying.

CONCLUSION

Tree rings provide a convenient record of tree growth. However, trees are not passive observers of the environment. Much tree ring research assumes that the external chemical environment is reflected in the dendrochemical record. This assumption is only partially correct. The biology of living organisms, including both its constitutive and induced features, greatly affects dendrochemistry. Valid interpretation of the dendrochemical record requires an understanding of how trees preferentially transport and exclude chemical elements and how wood chemistry can be affected by injury and infection.

REFERENCES


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