7. Tree Health and Physiology in a Changing Environment

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A tree is a large, long-lived, perennial, compartmented, woody, shedding, walling plant. This definition is based on new tree biology concepts (Shigo, 1986a,b, 1991) and explains much about how mature trees function through their unique structure. When the tree begins its life, it is mostly leaf in mass (Fig. 7.1a). As a tree grows in stature, it becomes mostly stem in mass and the foliage represents only a few percent of the total mass. Roots remain relatively constant at about one-fifth the total mass as a tree grows from a small sapling to a mature standard in the forest canopy. Branches represent only a small fraction of total mass, which decreases over time, as older branches are shed. Also shed are leaves, roots, and outer bark. However, aging wood cannot be shed, but dies internally as sapwood is transformed into a core of protection wood, often called "heartwood" (Fig. 7.1b,c; Table 7.1).

Annual rings are formed each growing season in the wood of trees in the temperate zone (Fig. 7.1b). The rings can be used in dendrochronology and dendrochemistry as a record of a changing external environment. These rings are further compartmented into cells. Large, dead, thick-walled cells function for transport and support. Small, live, thin-walled cells store food and defend against the spread of infection (Table 7.1). The

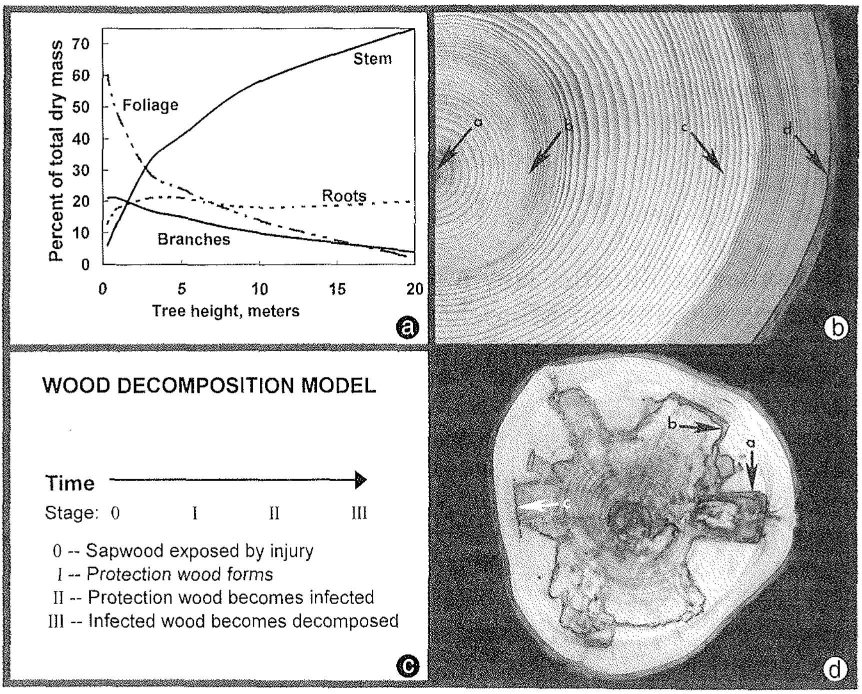

Figure 7.1. Development of individual trees. (a) Change in dry mass fraction of F = foliage, S = stem, R = roots, B = branches associated with increasing height growth in red spruce. (b) Record of diameter growth seen as annual growth rings in red spruce from pith (a) to cambium (d); see also outer band of living sapwood and inner core of protection wood. Note that segment a–b contains wood formation during the sapling stage of tree growth, b–c contains wood formed during the pole stage, and c–d wood formed as a mature standard. (c) Wood decomposition model (see Table 1). (d) Tree survival model illustrated in red maple, a = protective zone, b = protective zone incorporated into protective wood, and c = barrier zone (see Table 1).

small cells make up over 90% of the cell number, but only 10% of the mass. Most of the mass is composed of large, dead, woody cells that give stemwood its commercial value. When the live cell network dies due to age, injury, or infection, a protective tissue is formed to reduce the internal rate of infection and decomposition following exposure to the external environment by injury. The protective tissue may be of the protection wood type, which forms in the core of the stem, or it may be a protective zone, which forms in the peripheral sapwood to stop the spread of infection into the outer living wood, vascular cambial zone, and inner living bark (Fig. 7.1c,d; see Table 7.1). Infection in living trees will spread at highly variable rates through the dead core of wood exposed by

Table 7.1. Summary of Properties of Major Types of Wood Formed in the Stem of Living Trees

Wood type	Derivation	Composition	Characterization
Sapwood	Cell division by the vascular cambium	Mostly live cells	Stores food Defends against infection Conducts sap Supports crown
Protection wood	Forms from sapwood	Mostly dead cells	Preservatives formed from stored food Reduced rate of infection Dehydrated and deionized Partial support of crown
Infected wood	Forms in protection wood	Live cells of bacteria and fungi	Preservatives degraded Onset of decay process Ionized and rehydrated Partial loss of support
Rotted wood	Final stage of infection	Complex living community	Preservatives lost Cell wall structure lost Retains water and nutrients Humus-like
Protective zones	Forms from sapwood as a column boundary layer, or From the vascular cambium as a barrier zone and woundwood	Dead cells: hypersensitive reaction	Layer of highly preserved cells Blocks infection spread Low permeability High mineral content Results in tree survival, not disease resistance

shedding and injury. Trees may live a very long time with internal infections due to the formation of protective "walls" of altered tissue by a process called compartmentalization.

The use of dated stemwood tissue to study dendrochemistry as a record of external change requires knowledge about the internal development of sapwood, protection wood, infected wood, decomposing wood, and protective zones because these processes can alter the pattern of external chemical change recorded in functioning sapwood. Furthermore, differences in anatomical structure associated with the sapling, pole, and mature stages of trees (Fig. 7.1b) result in a greater density of binding sites in the core of the tree than in the periphery. The consequence of this structural change is a decreasing concentration of exchangeable divalent base cations from the core to the periphery under constant uptake conditions.

Taking a standard stocking chart for northern conifers as a model of how populations of trees develop (Fig. 7.2a), the number of trees per unit

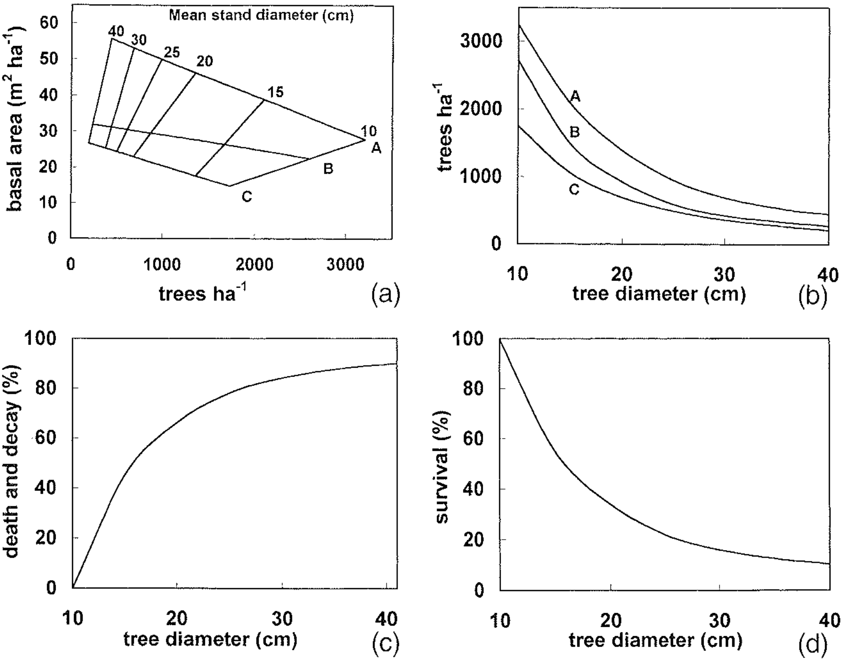

Figure 7.2. Development of tree populations as derived from a forest stocking table. (a) Standard stocking table for northern coniferous species. The A-line indicates overstocked stand, B-line indicates fully stocked stand, and C-line indicates understocked stand. (b) Decreased stand density associated with increased stem diameter (dbh). (c) Cumulative frequency of dead and decaying trees as trees progress from saplings (<10 cm dbh), poles (10 to 30 cm dbh), and mature standards (>30 cm dbh). (d) Survival frequency of individual trees through stand development. The time scale for stand development is highly variable from several decades to centuries.

area decreases at all stocking levels as trees increase in size (Fig. 7.2b). The greatest rate of loss of trees takes place during the early pole stage (trees 10 to 20 cm dbh). Losses continue through the late pole stage (20 to 30 cm dbh), but at a decreasing rate. Further decreases in mature standard trees (>30 cm dbh) will occur when various disturbances take place in the forest. Pests, pathogens, and declines caused by multiple stressors can cause tree loss to occur at rates observed in earlier stages of forest development.

By the time the average tree diameter reaches 40 cm dbh, the death and decay of 90% of the large sapling population (10 cm dbh) will have occurred (Fig. 7.2c). As in the individual tree, death and decay always accompany population growth. Forests are sustained through the survival to maturity of 10% of the initial sapling population (Fig. 7.2d). Survival occurs because of a highly developed defense system in these remarkable plants that sustain the forests of the terrestrial ecosystem. Trees cover only 10% of the land area, but produce 90% of the terrestrial biomass.

Human activities of forest clearing, tree harvesting, and air pollution have added a new dimension to the delicate balance of life and death that sustains forest life. Will trees adapt to modern environmental change to sustain forest health and productivity?

We have investigated the response of the northern spruce forests to environmental change during the 20th century, which has experienced marked increases in acidic deposition. We have looked for records of changing tree growth and changes in the base cation nutrition of the root zone in the stemwood of mature trees. We have related the current status of tree stress from below ground change to biochemical markers in the foliage. What have we learned?

Tree Growth and Dendrochronology

Assessments of tree growth are part of most investigations of tree health and environmental change. Increases in the number, girth, and extent of stems, branches, and roots occur as a tradeoff. Tree energy is partitioned between growth and defensive investments (Herms and Mattson, 1992; Loehle, 1988). A growth investment such as annual ring width is far easier to measure than any defensive investment. Constitutive defensive investments include the programmed cell death and chemical enrichment associated with the transformation from sapwood to heartwood. Induced defensive investments include the enrichment of wound-altered sapwood with phenolics and resins (Hillis, 1987; Smith, 1997). Enhanced wood production can be an induced defensive feature through the formation of abnormally thick woundwood or "callus" ribs near a wound (Mattheck et al., 1992).

Dendrochronology, the interpretation of patterns of precisely dated tree rings, can provide a reliable temporal record of tree growth as affected by environmental change. The decline of red spruce in the northeastern U.S. prompted a detailed application of dendrochronology to tree health and forest disturbance (Cook and Zedaker, 1992; Johnson et al., 1995). Primarily through research on climate and the dating of wooden cultural objects, basic principles of dendrochronology have been developed (Fritts, 1976). With some adaptation, these principles may be applied to investigations of tree health and physiology in a changing environment (Table 7.2).

The principle of integrative growth states that tree-ring characteristics, such as width and density, are a composite response to intrinsic and extrinsic factors. Intrinsic factors include the genetic capacity for growth of the individual tree, provenance, and species. Extrinsic factors that limit

Table 7.2. Revised Principles of Dendrochronology[a]

1.	Integrative growth
2.	Limiting factors
3.	Range sensitivity
4.	Site selection
5.	Crossdating
6.	Uniform linkage
7.	Signal and noise

[a] Modified from Fritts (1976).

growth include competition from other plants, biotic and abiotic stressors, edaphic conditions, and climate. This integration is rarely linear and additive, although it may be approximated with a linear model (Van Deusen, 1989).

To characterize growth in red spruce forest stands across the northeastern United States, we extracted two core samples from each of 36 trees at nine locations (Fig. 7.3). Although sampling was restricted to apparently healthy, canopy-dominant individuals, trees varied with respect to age and canopy crowding. The measured ring width series

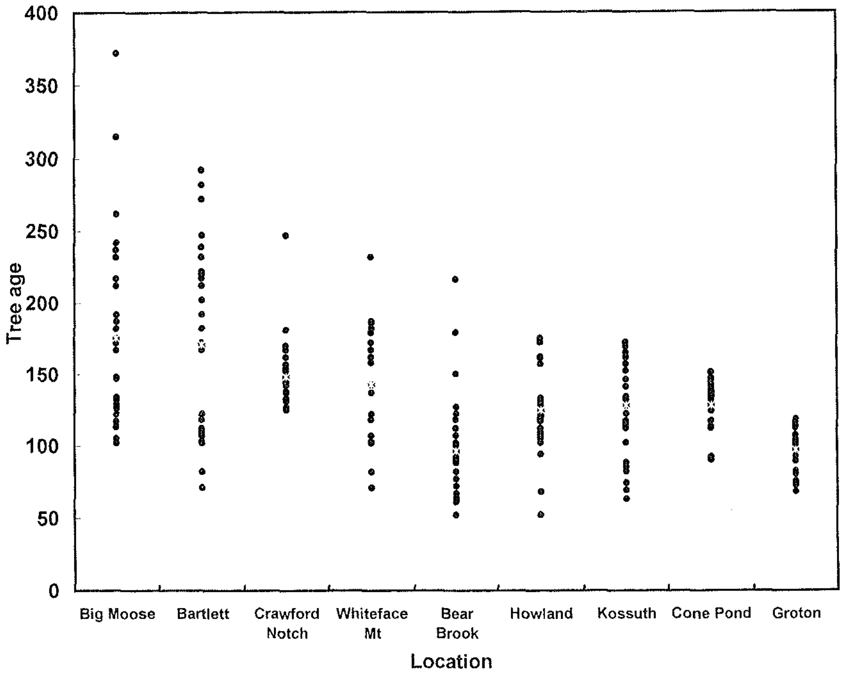

Figure 7.3. Tree age at breast height (solid circles) and location means (white crosses) for bored red spruce trees at the sampled locations across the northeastern U.S.

frequently appeared quite different from one another in general outline (Fig. 7.4a,b). Ring series were analyzed by two separate processes to examine (1) the high-frequency variation that may be attributable to annual climate variation and (2) the long-term growth trend.

In order to remove the medium- and long-term growth trend and to maintain as much as possible of the annual growth variation that may contain a year-to-year climate signal, the individual ring series were standardized. For standardization, each ring width series was fitted with a trend line calculated as a cubic spline (50% frequency cut-off of 30 years) using ARSTAN software (Cook, 1985; Holmes et al., 1986) (Fig. 7.4a,b). A standard index series was calculated by dividing the measured ring width by the trend line value for each year.

Patterns among index series (Fig. 7.4c) are more similar than they are among ring width series (Figs. 7.4a,b). The averaging of index series

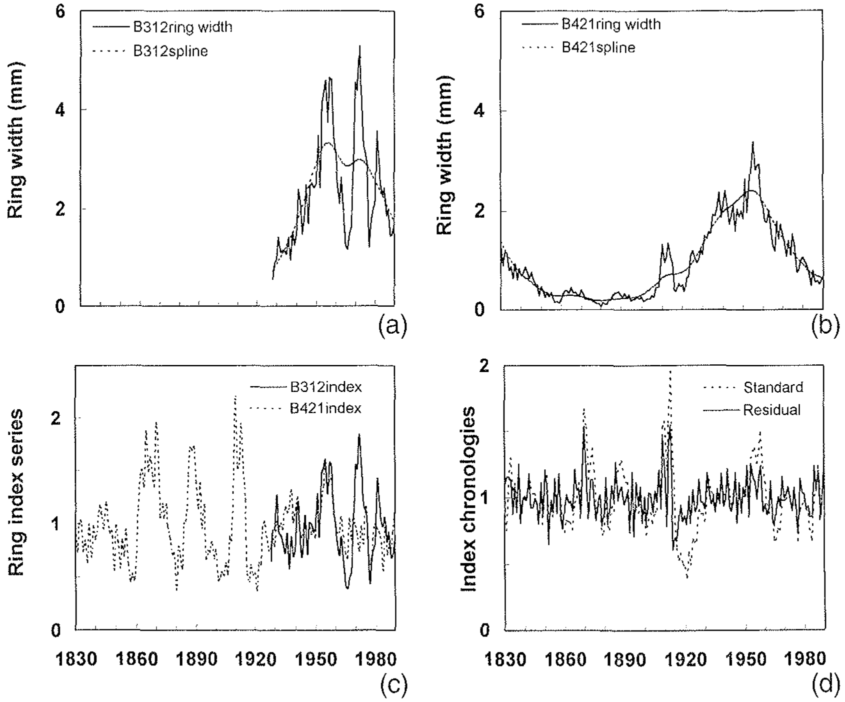

Figure 7.4. Tree ring data from red spruce sampled at Kossuth, Maine. (a,b) Splines representing individual growth trends were fitted to ring width series. (c) For dendroclimatic analysis, dimensionless index series were calculated by dividing the ring width by the trend for each year. (d) Index series were averaged to produce a standard chronology for each location. Each index series was also fitted with an autoregressive model and the residuals averaged to produce a residual chronology.

resulted in a standard index chronology for each location (Fig. 7.4d). One property of ring width series, index chronologies, and most naturally occurring time series is autocorrelation. Autocorrelation is the property that each value is dependent in part on the value for the preceding year or years. Given the physiological carryover of tree energy reserves, foliage production and retention (in the case of conifers), etc. autocorrelation may be expected. Autocorrelation violates the underlying assumption of independence for regression analysis. Additionally, as index chronologies may be more autocorrelated than climatic series, autocorrelation in tree rings may obscure sensitivity to climate. To reduce this effect, each index series was fitted with an autoregressive model. The mean of the residuals produced from autoregressive modeling was used to produce a residual chronology for each forest location. Although similar through most of the time period, the residual chronologies did diverge from the standard chronologies (Fig. 7.4d).

The principle of limiting factors states that although growth is integrative, growth is restricted by the essential factor or condition that is most limiting in supply. Ring width may be limited by damage to shoots caused by winter injury in the previous year or a shortened growing season due to adverse weather conditions. This principle is analogous to the "rate-limiting step" of chemical reactions, that the overall rate of a multi-part reaction is determined by the rate of the slowest component step. Limiting factors in common throughout a stand can result in the formation of "pointer years," an unusually narrow ring or series of rings that aids in the synchronization or crossdating of tree-ring series.

To determine the overall growth trend, tree-ring series were partitioned by location and age class. Bootstrap confidence intervals were calculated for mean ring widths to indicate variation about the mean (Cook, 1990). Trends in certain combinations of tree species and forest locations produce ring series with identifiable characteristics. For example, ring series of red spruce from Maine frequently contain growth suppression about 1920 (Fig. 7.5, plots for Howland, Kossuth, and Bear Brook). This suppression was likely due to defoliation by the spruce budworm followed by decreased availability of carbohydrate for growth. As this form of defoliation primarily takes place in the bud, and spruce typically retains functional needles for several years, the period of growth suppression can persist beyond the actual outbreak period. The general pattern for all age classes of red spruce sampled across the northeastern United States includes a peak in ring width about 1960 followed by a decline in radial growth (see Fig. 7.5).

The principle of range sensitivity states that trees sampled at or near the edge of their range are likely to contain a stronger common signal, especially of climate. Range in this case may be determined by latitude, elevation, edaphic, or climatic characteristics. This concept is related to

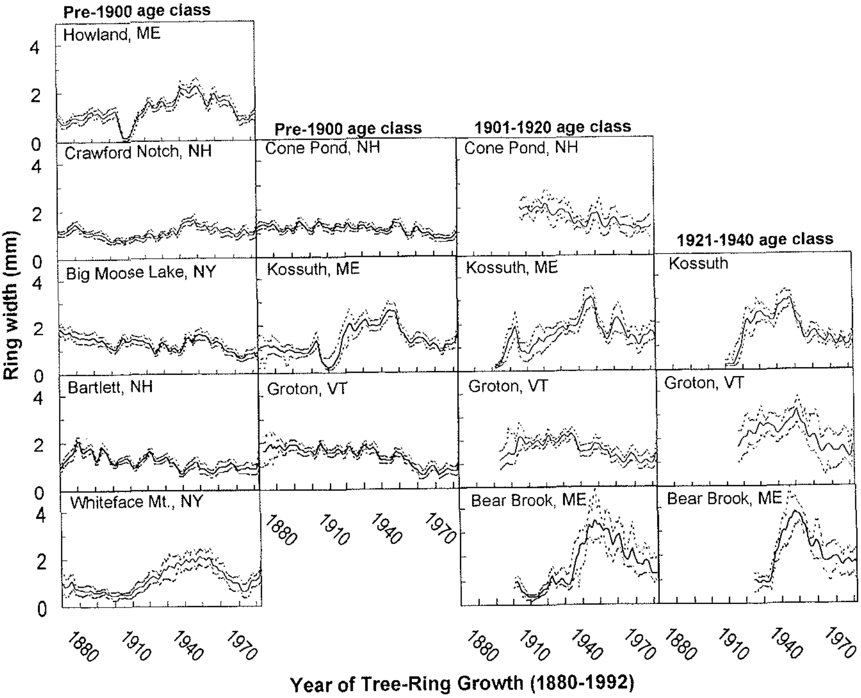

Figure 7.5. Mean ring width and 95% confidence intervals for red spruce at nine locations and various age classes. For each plot, the *x*-axis represents the years of growth from 1880–1993. The *y*-axis represents tree-ring width.

but not identical with ecological amplitude. Ecological amplitude is the capacity of a tree species to grow across a range of environmental conditions (Fritts, 1976). Ecological amplitude varies among species, from narrow, as in the case of coastal redwood, to broad, as in the case of red maple. Despite the breadth of the ecological amplitude, ring series from trees at the edge of the range are likely to contain a stronger common signal of environmental events.

The principle of site selection states that sampling sites can be identified as being likely to produce tree-ring series sensitive to the environmental variable being investigated. Investigations of tree health and forest decline may sample stands at various distances from the center of disturbance and decline (Bartholomay et al., 1997; McLaughlin et al., 1987).

The principle of crossdating is the process of matching patterns among several to many ring series. By including series with a known sampling date, the exact year of formation may be assigned to a particular ring. Crossdating is the rigorous process that moved tree-ring analysis from "ring counting" to dendrochronology. Crossdating is aided by the presence of a recurring environmental factor, such as drought or extreme cold, that periodically suppresses growth throughout the stand or region. In this manner, crossdating relies on common patterns of high frequency variation in ring width. Linkage of overlapping series contained in living trees, wooden structures, and subfossil remnants permits the construction of long tree-ring chronologies (Stokes and Smiley, 1968). Unfortunately, even long and perfectly constructed chronologies do not provide information on trends longer than the length of individual segments (the "segment length curse") (Cook et al., 1995).

Crossdating wooden timbers contained in old churches to existing chronologies allowed for the estimation of the timing of spruce budworm outbreaks for the 1700s and 1800s in Quebec (Krause, 1997). Krause (1997) constructed floating index chronologies (tree-ring chronologies not fixed to calendar dates) from structural members of buildings constructed of white spruce, a host species for the budworm, white cedar, a nonhost species, and other tree species. Crossdating with a local, recent spruce chronology allowed the assignment of calendar dates to both the white spruce and white cedar chronologies. Inspection of the crossdated spruce and cedar chronologies indicated periods of acute growth suppression in the spruce host chronologies that were not present in the cedar nonhost chronology. These periods of suppression were interpreted as indicators of episodic spruce budworm infestation (Krause, 1997).

Use of tree rings as a proxy record of climate or environmental disturbance depends on uniformity of linkage between external conditions and tree biology. This concept is an extension of the uniformitarian principle developed by 18th century naturalist James Hutton to describe geological processes. Briefly stated, "the present is the key to the past," those currently observable natural processes are the same processes that occurred in the past. Although this may seem to be self-evident, dendrochronology in a changing environment provides a cautionary note to the uncritical application of geological uniformitarianism to growth patterns of trees.

The decline of red spruce is still controversial with respect to causes and implications, although the dendrochronology of spruce decline has been well studied and reviewed (Cook and Zedaker, 1992; Johnson et al., 1995). The linkage at some locations between red spruce growth and climate markedly changed at about 1960 (Van Deusen, 1990). We tested this linkage at nine of our study locations. For all nine locations, significant regression models (nominal $P < 0.95$) of residual ring index chronologies were calibrated to climate variables for the 1896 to 1940. Although the true probability value was likely to have been less than the nominal value due to the multiple regression process, the probability values for the correlation coefficients determined for the verification periods could not have been similarly inflated. The portion of the index chronology for 1910 to 1929 was not included in the calibration of the Howland model due to previously discussed growth suppression. Each model was verified for two periods, 1941 to 1960 and 1971 to 1990. The endpoints for the verification period were chosen to test periods prior to and following the 1960s, the time of most rapid increases in environmental perturbation attributed to acidic atmospheric deposition. This procedure allowed model verification using independent data not used in the construction of the model. Significant models that related growth to temperature were used to predict ring indices for the verification periods. Total monthly precipitation had no significant effect on any of the constructed models. The predicted indices derived from the regression models were significantly correlated ($P \leq 0.05$) to actual indices for the 1941 to 1960 verification period at five locations (Fig. 7.6a–e). Actual and predicted indices were not significantly correlated for the 1941 to 1960 verification period at four locations (not shown). The lack of correlation for these four sites indicated that either there was no predictive relationship between index chronologies and mean monthly temperatures or that the nature of the predictive relationship differed between the calibration and the verification periods. For the 1971 to 1990 verification period, predicted indices were not correlated to the actual indices at any location. This suggests that at least five locations, the relationship of growth to temperature for the 1941 to 1960 verification period was similar to the 1896 to 1940 calibration period. However, growth in the 1971 to 1990 verification period was no longer responding to temperature as growth responded to temperature in the 1896 to 1940 calibration period.

In an earlier investigation of spruce decline, concentrating especially on montane trees, Johnson et al. (1988) found a similarly timed change in climate dependence. They hypothesized that this change may have been due to a decade of unusually cold winters that killed foliage and buds and shifted carbon allocation away from wood production and into producing new shoots and foliage (Johnson et al., 1988). In contrast with Johnson et al. (1988), monthly climate variables with a significant effect on growth were specific to each sampled location (see Fig. 7.3). This may reflect that trees in our investigation were further removed from the edge of their elevation range and may have been affected differently by the factors causing the change in climate dependence. Other changes in climate dependence dated to about 1960 have been reported for red pine and Norway spruce (Leblanc et al., 1987) and shortleaf pine (Grissino-Mayer and Butler, 1993).

As a matter of faith, uniformitarianism is quite appealing. When all else is equal, conditions that limit growth in the present are likely to have limited growth in the past. However, environmental change and associated changes in tree energy allocation could change the growth response of trees to climate, as has been shown in white pine exposed to atmospheric ozone (Bartholomay et al., 1997). This is a cautionary note for dendroclimatic reconstructions based on the premise that trees had

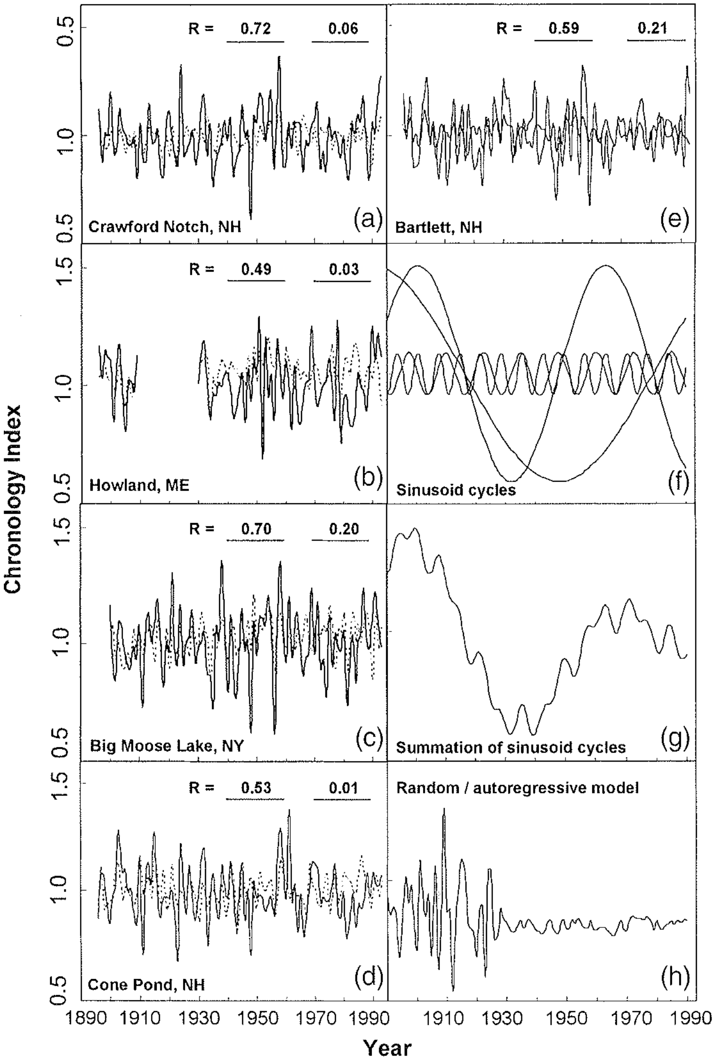

Figure 7.6. Red spruce and model growth series. (a–e) Residual index chronologies were compared with chronologies estimated by dendroclimatic models. Correlation coefficients (*R*) for the observed and estimated chronologies for the 1941 to 1960 and 1971 to 1990 verification periods are listed. Correlations were significant for all comparisons in the 1941 to 1960 period ($P \geq 0.95$). No significant correlations were observed for comparisons in the 1971 to 1990 period. Sinusoid waves (f) and their sum (g) approximate the appearance of tree ring data (after Reams and Huso, 1993). (h) An autoregressive process acting on random values assigned to 1890 to 1930 produces a smooth periodic series resembling series derived from tree rings.

the same response to climate in the past as trees in the present (Smith et al., 1999). Fig. 7.6a–e argues against the acceptance of simple uniformitarianism.

The principle of signal and noise arises from the integrative growth of tree rings and the variety of research questions to which the tree-ring record is applied. The principle is sometimes stated as the replication principal, that multiple observations within and among trees will remove individual growth responses and better represent growth of the stand or region. However, even with adequate replication, the maximization of the desired information (signal) still needs to be separated from the information not believed to be useful (noise) in answering the research question.

An individual tree-ring series may contain trends, for example, of low frequency due to tree age and canopy closure, medium frequency due to local gap formation and recovery, and high frequency due to weather. Which of these trends is signal and which is noise is entirely dependent on the research question. Year-to-year variation can be maximized by detrending (Fig. 7.4a–d) or minimized through averaging by tree age class (see Fig. 7.5).

An individual tree-ring series typically contains cyclic as well as unique peaks and valleys. Model cyclic process can be represented by sinusoid waves of specific frequency and amplitude (Fig. 7.6f). The summation of these cyclic processes can yield a pattern suggestive of ring series (Reams et al., 1993) (Fig. 7.6g). Numerical techniques such as the Kalman filter (Van Deusen, 1990) may be able to increase our ability to precisely identify the timing of the change in climate dependence. However, purely mathematical approaches are not likely to identify the biological basis for growth cycles. Robust interpretation of ring patterns will require coming to grips with the mechanisms of the underlying cyclicity. Any suggested linking mechanism is likely to still leave doubt. Successive random events, entering autoperiodic processes such as ring formation, can themselves result in cycles or regular periodicity (Slutzky, 1937). In Fig. 7.6h, y-values for the 1890 to 1930 period were randomly drawn from a normal distribution. The y-values for 1931 to 1990 were calculated as a first-order autoregressive process seeded from the 1890 to 1930 period. The modeled values (1931 to 1990) quickly dampened the wide variation of the earlier, random portion of the series and appear to contain some pattern. Consequently, random series acted on by an autoregressive process can form the basis of an apparently periodic series.

Dendrochronology provides a record of tree growth and current and past environmental change. Interpreting that record requires a clearly specified hypothesis that may not be obvious from the record itself. The dendrochronology of red spruce indicates that across the northeastern U.S., growth has declined since the 1960s (Cook and Zedaker, 1992). The growth decline is apparent at all elevations and for all age classes. The change in climate dependence of red spruce and other species following 1960 may be of greater significance than the growth decline itself. These results are consistent with a widespread, fundamental change in the response of trees to the environment. The potential relationship of this change to perturbations in soil chemistry, land use patterns, and overall stress loading is unclear. Defining that relationship of biology and environment is made more difficult through the presence of natural cyclic processes in growth and climate. Identifying the cyclic processes is itself hindered through the natural integration of cycles of various frequencies and amplitudes and the generation of cyclic processes through the autoregressive process itself.

The body of principles for dendrochronology will continue to evolve. As more researchers apply the concepts to diverse problems, both the limitations and strengths of the concepts will become more apparent. This emphasis on the conceptual structure of the process should enhance the power of the tree-ring record to identify and explain environmental change.

Dendrochemistry: Change in Cation Mobility

Dendrochemistry is the interpretation of radial trends in the chemistry of dated wood. Dendrochemistry is based on the chemical relationship between the environment external to the tree and to the wood active in water conduction. For dendrochemistry, increment cores or stem sections are dated and samples of wood formed at different time periods are researched and analyzed. The application of dendrochemistry to environmental change requires distinguishing between the chemical characteristics of internal processes of maturation, defense, and infection from external environmental conditions (Smith and Shortle, 1996).

Process models predicted a mobilization of essential base cations in response to acidic deposition (Shortle and Smith, 1988; Lawrence et al., 1995). A signal of the mobilization of the essential bases calcium (Ca) and magnesium (Mg) appears in the dendrochemical record of red spruce (Shortle et al., 1997). Interpreting this signal of potential mobilization requires an understanding of transport processes of Ca and Mg. The binding of divalent Ca and Mg to ion exchange sites in the wood cell wall system follows the characteristics of a Donnan equilibrium (Momoshima and Bondietti, 1990). In brief, the amount of Ca and Mg in wood is a function of pH, the peak concentration of Ca and Mg in the surrounding sap, and the number of binding sites available for exchange. Under uniform soil and sap conditions, Ca and Mg concentrations tend to decrease with increasing radial distance from the pith, due to decreasing numbers of available binding sites (Momoshima and Bondietti, 1990).

In a dendrochemical analysis of red spruce across the northeastern U.S. (Shortle et al., 1997), wood formed in the 1960s was more frequently enriched in Ca (Fig. 7.7a) and Mg (Fig. 7.7b) than at any other time over the past eight decades ($P < 0.05$). The enrichment frequency of 28% for Ca and 52% for Mg in wood formed from 1961 to 1970 is remarkable because

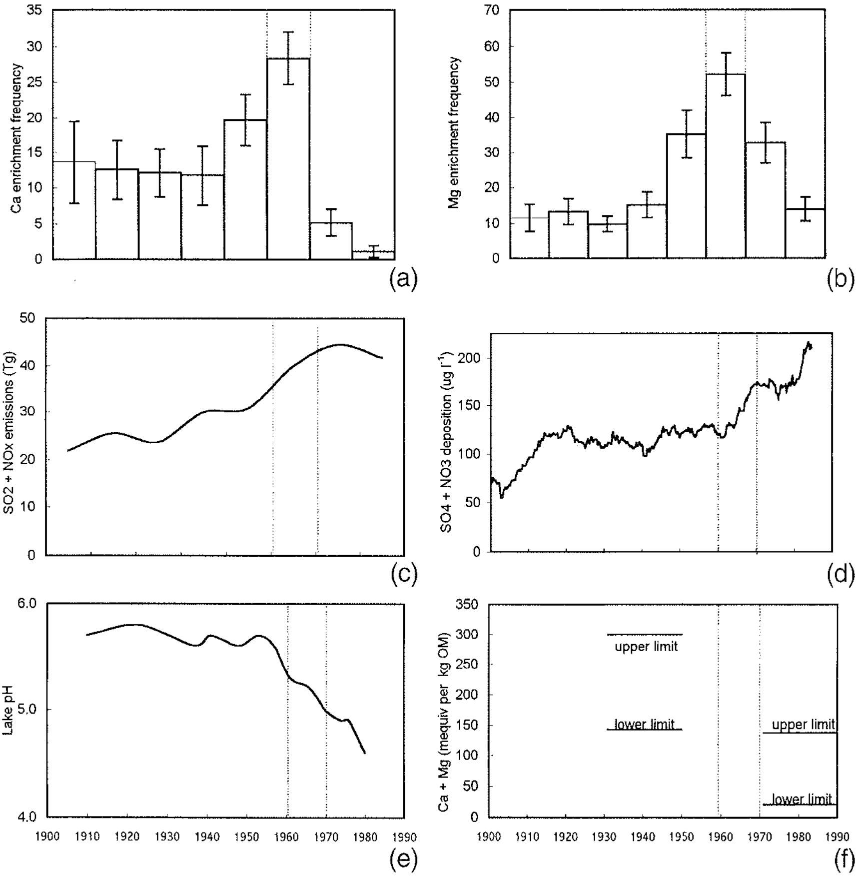

Figure 7.7. Dendrochemical markers of cation mobilization and coincident environmental changes. The vertical, dashed lines mark the years 1960 and 1970. (a) Percent frequency of Ca enrichment by decade. For example, the 1961 to 1970 bar indicates that the mean frequency of Ca enrichment relative to the preceeding decade was 28% across all sample locations (±SE). (b) Enrichment frequency for Mg was calculated as for Ca in (a). (c) Combined emissions of SO_2 and NO_x for the United States (NAPAP, 1993). (d) Combined deposition of nonmarine SO_4 and NO_3 in ice core 20D collected from south central Greenland (Mayewski et al., 1986). (e) Water pH at Big Moose Lake in the southwest Adirondacks of NY. Acidity was estimated through stratigraphy of diatoms in sediments (Charles, 1984). (f) Historical range of concentration of Ca and Mg in the forest floor of the northeastern U.S. according to available reports (Shortle and Bondietti, 1992).

the enrichment indicates a perturbation in the equilibrium between soil chemistry, sap chemistry, and cation binding sites in stemwood. The observed Ca and Mg enrichment is also remarkable because of its regional nature.

What internal or external factors could cause the enrichment in Ca and Mg for wood formed in the 1960s? Internal infection processes and the tree response to injury and infection can greatly alter Mg and Ca concentration in the wood of living trees. However, the careful screening of both candidate trees and cores argue against that explanation. In some tree species, especially those (unlike red spruce) that contain a highly colored heartwood, heartwood formation can affect wood chemistry. Simply because of tree age and the maturation process of red spruce, 31 of the 40 (78%) marked sapwood–heartwood boundaries occurred in wood formed in 1961 to 1970 (Shortle et al., 1997). However, the sapwood–heartwood boundary coincided with peak Ca enrichment in only 22 of the 40 cores (55%). We suggest that if Ca enrichment was a constitutive feature of heartwood formation in red spruce, essentially all, rather than the observed 55%, of the peak Ca enrichment would coincide with the sapwood–heartwood boundary.

The pattern of stemwood enrichment in Ca and Mg is consistent with the hypothesis that Ca and Mg were mobilized in the soil as acidic deposition increased, and transported in unusually high concentrations to stemwood via root uptake and sap transport (Shortle et al., 1997). This temporary increase in binding of Ca and Mg in wood formed in 1961 to 1970 coincided with (1) increased sulfur dioxide (SO_2) and oxides of nitrogen (NO_x) emissions (NAPAP, 1993; Fig. 7.7c), (2) increased deposition of sulfate (SO_4) and nitrate (NO_3) in the Greenland ice sheet (Mayewski et al., 1986; Fig. 7.7d), (3) decreased pH of lake water in the Adirondacks (Charles, 1984; Fig. 7.7e), and (4) reduced concentrations of Ca and Mg in the forest floor (Shortle and Bondietti, 1992; Fig. 7.7f).

If further reductions in the concentration of Ca and Mg from the forest floor were due to exchange of hydrogen (H) for Ca, either from plant growth (Johnson and Anderson, 1994) or from acidic deposition, then the concentration of exchangeable Ca and Mg ($[\mathrm{Ca + Mg}]_{\mathrm{ex}}$) would be negatively correlated with the concentration of exchangeable H ($[\mathrm{H}]_{\mathrm{ex}}$). Across our 12 study locations (Shortle et al., 1997), soil analysis indicated that $[\mathrm{Ca + Mg}]_{\mathrm{ex}}$ was not significantly related to $[\mathrm{H}]_{\mathrm{ex}}$ for untransformed or $\log_e$ transformed data from the forest floor ($P = 0.12$) or mineral soil ($P = 0.70$) (Fig. 7.8a). However, $\log_e [\mathrm{Ca + Mg}]_{\mathrm{ex}}$ was significantly and negatively correlated to $\log_e [\mathrm{Al}]_{\mathrm{ex}}$ in the forest floor, the principle rooting zone of red spruce (Fig. 7.8b, $P < 0.001$). Lawrence et al. (1995) have attributed this negative correlation between exchangeable Ca and exchangeable aluminum (Al) in the forest floor to increased translocation of Al from the mineral soil, where acidic deposition has increased Al dissolution. Acid-extractable Al in the forest floor increased over the past

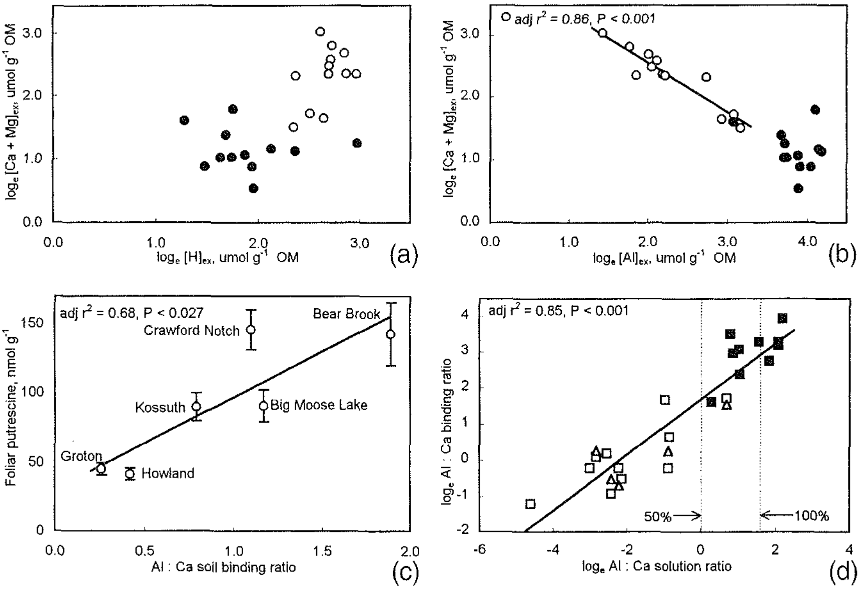

Figure 7.8. Relationships among major cations in forest soils and soil solutions (David and Lawrence, 1996; Lawrence et al., 1995) and the stress marker, putrescine. (a) Regression analysis indicates no significant relationship between log$_e$ [Ca + Mg]$_{ex}$ to log$_e$ [H]$_{ex}$ either in mineral soil (closed circles, $P < 0.70$) or forest floor (open circles, $P < 0.12$). (b) Regression analysis indicates a significant relationship between log$_e$ [Ca + Mg]$_{ex}$ and log$_e$ [Al]$_{ex}$ in the forest floor (open circles, $P < 0.001$) but no significant relationship in mineral soil (closed circles, $P < 0.36$). (c) Regression analysis indicates that concentrations of putrescine in red spruce foliage are significantly related to the Al/Ca binding ratio (molar charge ratio of exchangeable Al to exchangeable Ca) of the forest floor (open circles, $P < 0.027$). (d) Regression analysis indicates a significant relationship between log$_e$ Al/Ca ratio in the forest floor soil solution to the log$_e$ Al/Ca binding ratio in the forest floor (open squares) and fine root tips (triangles), and between log$_e$ Al/Ca ratio in the mineral soil solution to the log$_e$ Al/Ca binding ratio in mineral soil (closed squares, $P < 0.001$). Vertical, dotted lines indicate the suggested 50 and 100% risk level of forest damage due to adverse Al/Ca ratios.

two decades at the Hubbard Brook Experimental Forest, and ratios of Al to Ca in mineral soil solutions (but not forest floor solutions) were strongly correlated with exchangeable Al content in the forest floor. Mineral dissolution of Al in the forest floor was ruled out as a cause for this relation because mineral Al concentrations were unrelated to exchangeable Al concentrations (Lawrence et al., 1995). Leaching of Ca by SO_4^{2-} has directly contributed to depletion of forest floor Ca, but mobilization of Al in the mineral soil has also indirectly contributed to this process by (1) reducing uptake of Ca from the mineral soil (2) providing a supply of reactive Al that exchanges with Ca in the forest floor, enabling the leaching of Ca from the forest floor, and (3) increasing Al saturation so that the number of exchange sites available for adsorbing added Ca is reduced.

The foliar concentration of the stress marker putrescine in putatively healthy red spruce trees was significantly correlated ($P < 0.02$) to the Al/Ca binding ratio of the forest floor (Fig. 7.8c). This indicates that even healthy-appearing trees are stressed due to adverse Al/Ca ratios. Regression analysis indicates that $\log_e$ Al/Ca binding ratio was strongly related with the $\log_e$ Al/Ca ratio in the soil solution of both the forest floor and the mineral soil (Fig. 7.8d, $P < 0.001$). Nonwoody absorbing root tips in the forest floor appear to be binding Al in the same manner as the surrounding soil, at the expense of Ca and Mg (Fig. 7.8d; Shortle and Bondietti, 1992; Smith et al., 1995). The Al/Ca ratio in the soil solution (expressed as the reciprocal Ca/Al ratio) was described as an ecological indicator of approximate thresholds beyond which the risk of forest damage increased due to Al stress and nutrient imbalances (Cronan and Grigal, 1995). The risk of forest damage was assessed at 50% when the soil solution Al/Ca ratio was 2 and at 100% when the ratio was 5. At all locations in the regional red spruce study, Al/Ca ratios of mineral soil solutions exceeded the 50% risk level and half of the locations were above the 100% risk level (Shortle et al., 1997). Most forest floor solution ratios were below the 50% risk level and trees appeared generally healthy. The forest floor solution ratio was above the 50% risk level at only one location, Mt. Abraham, Vermont, where half of the canopy spruce were dead or dying. Because we sampled the most healthy portions of the red spruce forest at each location, the area we investigated at Whiteface Mountain, New York, did not have a particularly high Al/Ca binding ratio (0.8) in the forest floor. This particular area contained neither evidence of unusual mortality or current tree decline.

These results support and expand upon the model that Robert Hartig proposed in 1897 to relate sulfate deposition to damage ("smoke injury") of spruce–fir forests (Hartig, 1897). Hartig stated that essential base cations would be lost from the root zone of trees subjected to high inputs of sulfate. We suggest that mobilization of Al has accelerated the loss of essential bases. We make this suggestion because of the evidence of a general mobilization of base cations in the mid-20th century and the dynamics of Al behavior as described above. As Hartig described, the loss of essential bases would suppress root growth. Roots not able to grow into new areas of the forest soil would eventually starve and die, making the belowground parts of trees susceptible to attack by facultative pathogens. Aboveground parts would respond to the loss of roots by shedding needles. Most trees would survive but grow poorly, be more frequently infected by facultative pathogens and insects, and be less tolerant of abiotic stressors such as drought.

In conclusion, the patterns of the dendrochemical marker of Ca and Mg enrichment in red spruce stemwood is inconsistent with a stable chemical environment in forest soil for the 20th century in the northeastern United States. The enrichment in wood formed in the 1960s is consistent with mobilization of base cations in the mid-20th century. This hypothetical mobilization coincides with increases in the atmospheric emission and deposition of nitrates and sulfates. The biochemical marker, foliar putrescine concentration, indicated that even apparently healthy trees are under stress due to adverse Al/Ca ratios in the forest floor. The hypothetical mobilization of Al, which would result in greater Al/Ca ratios, would be enhanced by acidic deposition (Cronan and Goldstein, 1989; Driscoll et al., 1984; Lawrence and Fernandez, 1991). Spruce stands most vulnerable to multiple stressors, such as those at high elevations, have already been damaged. We anticipate that red spruce and other tree species growing under less harsh conditions will become stressed and more vulnerable with continued depletion of essential base cations and mobilization of Al.

Biochemical Markers: Indicators of Changing Stress

Trees, by their perennial nature, are constantly exposed to a variety of biological and environmental stresses. Plants, including forest trees, respond to these stresses with physiological and developmental changes that lead to either an avoidance or a tolerance of the stress factors. Depending upon the duration of exposure, plants employ either short-term responses that overcome stress or long-term adaptation to that stress by long-lived epigenetic changes. Some species have developed genetic mechanisms to adapt to harsh and variable environments. The complex response to stress begins with the perception of stress which, through a series of signal transduction pathways, leads to changes at the cellular, physiological, and developmental levels (Vernon et al., 1993). While some physiological responses may be specific to the stressor, other responses are generic. Also, whereas some of these responses may be localized to the tissue or organ that was stressed, others may be systemic responses of the whole tree. In all cases, stress-induced genes are activated, some of which lead to metabolic changes that can be used as physiological and biochemical markers of stress.

Whereas some of these metabolic changes are quantitative, for example, an increase or decrease in cell size or the cellular content of one or more metabolites, others are qualitative, for example, the presence or absence of certain metabolites or proteins. The first step in alleviating stress in plants is to detect and characterize the stress response at an early stage. Certain forms of stress (e.g., drought) occur intermittently and the stress periods are followed by recovery periods. Other forms of stress are more constant and require different adaptive mechanisms. For example, plants grown in high salinity develop mechanisms to exclude sodium (Na^+) or accumulate potassium (K^+) ions. On the other hand, plants exposed intermittently to salt may accumulate organic solutes, such as proline and polyamines, to stabilize cell membranes and achieve osmotic balance. In most cases these changes persist for some time even after the stress is temporarily removed. Thus, if these changes could be detected early, they could be useful indicators or markers of stress in visually nonsymptomatic trees.

The need to assess a number of early physiological and biochemical markers of stress in trees is obvious. With slow and long-term changes in the soil and environmental factors due to human activity, forest trees have been affected in many ways, leading to loss of productivity in the forest. Unfortunately, in most cases, symptoms of stress appear too late to manage or treat trees to reverse the effects of stress. Thus, the availability of markers that can assess the current status of stress in apparently healthy trees in a forest is crucial for planning a potential treatment or a management practice for either alleviating the deleterious effects of the stress or removing the cause of stress. To be useful, a physiological or biochemical marker should be a part of an adaptive mechanism and not a short-term response to applied stress. A useful marker is easily detected, quantified, and able to reliably estimate the health status of a stand.

The process to identify such markers involves (1) a comparison across a gradient of environmental change (e.g., acid precipitation, urban pollution, increased fertilization, etc.) and (2) experiments to study the effects of specific stressors on plant metabolism. While a few studies of the former type have been reported with forest trees, most of the experimental work has been done with either crop plants or young seedlings and tissue cultures of tree species.

Osmotic stress, salt stress, mineral deficiency, and increased solubilization of Al are some of the soil factors that have changed in recent years and have affected forest productivity. Among the important atmospheric factors are increased O_3, NO_x, sulfur oxides (SO_x), and particulate deposition. All of these factors can contribute to a decline in tree health and forest productivity. Metabolic changes associated with soil and environmental factors are changes in photosynthetic rates, foliage fluorescence characteristics, organic metabolites or inorganic ions, phytochelatins, activities of antioxidative enzymes, and the presence of certain stress-induced proteins. The following discussion examines these changes in plants, including trees, followed by a summary of our work on biochemical indicators of Al stress in woody plants.

Many stress factors, such as ozone, low temperature, and drought, induce the production of reactive oxygen species, such as oxygen radicals and hydrogen peroxide (H_2O_2). These oxidants damage cellular metabolism, particularly photosynthesis. To prevent oxidative damage, plant cells utilize a scavenging system consisting of low molecular weight antioxidants (e.g., ascorbic acid, glutathione, and vitamin E) and a set of inducible protective enzymes, including superoxide dismutase (SOD), glutathione reductase (GTR), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and ascorbate peroxidase (APX). Thus, the constant presence of elevated levels of these oxygen-scavenging compounds can be indicative of one or more forms of stress, such as O_3 stress in birch and drought stress in oak and pine (Schwanz et al., 1996). Spruce showing symptoms of decline disease at high elevation have higher concentrations of antioxidants (Nageswara et al., 1991; Bermadinger-Stabentheiner, 1996). Molecular genetic techniques to affect stress tolerance through the modulation of cellular levels of these antioxidants and antioxygenic enzymes are being investigated in several species, including poplar (Foyer et al., 1995).

Polyamines (putrescine, spermidine, and spermine) are a group of open-chained polycations of low molecular weight that are found in all organisms and exist in both free and bound forms. They play an important role in the growth and development of all organisms. The biological function of the polyamines could be partly attributed to their cationic nature and their electrostatic interactions with polyanionic nucleic acids and negatively charged functional groups of membranes, enzymes, or structural proteins in the cell (Houman et al., 1991). Recently, considerable attention has been paid to the study of changes in polyamine metabolism in plants subjected to various kinds of environmental stress. External stress can result in an increase or a decrease in cellular polyamines, depending upon the type of stress, the plant species, and the time of stress application (Zhou et al., 1995). Abiotic stress conditions that affect polyamine concentration include low pH, SO_2, high salinity, osmotic shock, nutrient stress (e.g., K or Ca deficiency), low temperature, and ozone (Flores, 1991 and references therein), and Al (Minocha et al., 1992, 1996a,b, 1997). Polyamines generally show a reverse proportionality to ions such as Ca, Mg, manganese (Mn), and K in response to Al treatment (Minocha et al., 1992, 1997; Zhou et al., 1995). Houman et al. (1991) observed a 25-fold increase in putrescine in the leaves and an 80-fold increase in roots of *Populus maximowiczii* stem cuttings grown in low K. Spermidine and spermine content decreased under low K conditions. The increased levels of putrescine were sustained over several weeks, thus acting as useful markers of K deficiency.

In addition to polyamines that may stabilize cellular membranes, nucleic acids, and some proteins, another mechanism often used by plants to protect themselves under prolonged stress conditions is the accumulation of compatible solutes. These solutes are a group of compounds that are not highly charged, but are polar and highly soluble. They include proline, glycerol, glycine, betaine, and so forth. Besides osmoregulation, they perform the important function of osmoprotection by helping to preserve the native conformation of proteins (Rudolph et al., 1986;

Carpenter and Crow, 1989). While higher concentrations of inorganic ions may be inhibitory to enzyme activities, compatible solutes at higher concentrations are not inhibitory to most enzyme activities (Pavlicek and Yopp, 1982; Richter and Kirst, 1987). At present, the literature on the role of compatible solutes in osmoprotection in trees is scarce. An increase in foliar proline content has been shown in several species of oak in response to drought stress (Kim and Kim, 1994), and in spruce and *Eucalyptus* in response to elicitor-induced stress and insect herbivory, respectively (Lange et al., 1995; Marsh and Adams, 1995). Factors that affect fine root structure and function, which would in turn affect the water and nutrient status of the plant, could affect the levels of compatible solutes in the foliage.

Plants growing in soils rich in heavy metals are known to accumulate high concentrations of metal-binding peptides called phytochelatins (Grill et al., 1987). These peptides, which have the general structure (γ-Glu-Cys)$_n$-Gly ($n = 2$ to 10), are synthesized from glutathione by enzymatic peptide synthases and not by direct translation of messenger ribonucleic acid (mRNA). Phytochelatins have also been referred to occasionally and erroneously as metallothioneins. True metallothioneins are a group of small metal-binding proteins found mostly in bacteria and animals (Hamer, 1986). Metallothioneins are direct products of the genes synthesized through mRNA translation. More recently, metallothioneins have been located in plants. The correlation of metallothein to phytochelatin concentrations remain unknown. Phytochelatins bind to heavy metals, such as cadmium (Cd), lead (Pb), and chromium (Cr), and effectively reduce the cellular concentrations of free metals. The biosynthesis of phytochelatins is induced only by heavy metals and thus their presence in high quantities is a reliable and specific indicator of heavy metal stress (Grill et al., 1988). Gawel et al. (1996) have proposed that heavy metals may be the likely contributing factor in the decline of forests in the northeastern U.S. Their data showed that the phytochelatin concentrations were higher in red spruce, a species in decline in northeastern U.S., as compared with balsam fir, a species that is not. Phytochelatin concentrations increased with increasing altitude and had a positive correlation with decline. They also increased across the region in forest stands with increasing tree damage.

There are many classes of stress-inducible proteins, for example, heat-shock proteins (hsp), metallothionein-like proteins, late embryogenic abundant (lea) proteins, calmodulin, and several proline-rich cell wall proteins that have also been reported to be synthesized in stressed cells of several plant species. In most cases, specific functions of these proteins are not known. Likewise, increased cellular levels of salicylic acid, pathogen-related proteins (PRPs) and changes in foliage fluorescence have been suggested as potential markers of stress.

Biochemical Indicators of Aluminum Stress

The adverse effects of acidic deposition on soil productivity, due to the solubilization of Al and leaching of bases, are of major concern to forest land managers because such processes may impact growth over large areas. Monomeric Al is an important toxic species to plants, both in the rhizopshere and within the plant symplasm (Shortle and Smith, 1988; Cronan and Grigal, 1995; Kochian, 1995) Aluminum interferes with cation uptake and can cause damage to plant cells by interaction with sensitive macromolecules (Haug, 1984; Sucoff et al., 1990). Aluminum is known to affect the needle biomass, root growth, seedling height, and cellular inorganic ion content in plants (Schier et al., 1990). However, very little is known about the primary site(s) of action of Al and the chain of biochemical and molecular events associated with short- and long-term effects of Al. We have employed suspension tissue cultures of two woody plants, periwinkle (*Catharanthus roseus*) and red spruce as a model experimental system to study cellular responses to Al. We have also conducted field studies to correlate foliar polyamines and inorganic cations to soil Al/Ca ratios.

Tissue Culture

Cell cultures are a reliable tool for gaining insights into the effects of certain elements on plant cells under controlled conditions. This is a valuable approach as it is extremely difficult and expensive to create a controlled environment for mature trees. Model systems using cell cultures can identify biochemical and molecular processes that are early indicators of a particular stress, for example, Al exposure. Methods developed using the model system may then be applied to foliage or other tissues of mature trees collected across a natural or manipulated stress gradient to evaluate the current and future health status of forest stands.

Rapid methods for the extraction of free polyamines and exchangeable inorganic ions and for the quantitation of polyamines using high pressure liquid chromatography (HPLC) were developed in our laboratory using several herbaceous and woody plant tissues (Minocha et al., 1990, 1994; Minocha and Shortle, 1993). Preliminary work on Al stress was done using a woody plant model culture system of periwinkle until a suspension culture model system of red spruce became available in our laboratory.

Addition of 0.2, 0.5 or 1.0 mM aluminum chlorate ($AlCl_3$) to 3-day-old periwinkle cells showed a small but significant increase in cellular levels of putrescine at 4 hours followed by a sharp decline by 16 hours (Minocha et al., 1991). There was no further decline in putrescine during the next 32 hours. Spermidine levels did not change appreciably as compared with the control cultures. However, spermine levels increased by 2- to 3-fold at 24 and 48 hours. Whereas all concentrations of Al caused a slight decrease in total cell number, cell viability was affected only by 1.0 mM Al. There was a decrease in the cellular levels of Ca, Mg, Na, K, Mn, P, and iron (Fe) in the cells treated with Al at 4 hours but a significant increase by 16 and 24 hours. The results suggest that both the absolute amounts of Al and the length of exposure to it are important for cell toxicity (Minocha et al., 1992).

Similar to the results with periwinkle, treatment of 3-day-old red spruce cultures with 0.2, 0.5, and 1.0 mM $AlCl_3$ caused a dose-dependent inhibition of fresh weight production. These levels of Al also caused a significant increase in cellular putrescine concentrations. This increase was dose-dependent up to 2 days and could be observed as early as 4 hours (Fig. 7.9). Spermidine levels were either not affected or showed a slight increase, which was not always dose-dependent. Al generally caused a dose-dependent increase in spermine and a decrease in K, Ca, Mg, and Mn (Minocha et al., 1996a).

Aluminum tolerance varies considerably among species and even among genotypes within species (Delhaize and Ryan, 1995). The different responses observed for putrescine content during long-term incubation with Al for periwinkle (Minocha et al., 1992) and red spruce suspensions are probably due to their different sensitivities to Al. Periwinkle showed an increase in putrescine content in response to Al treatment during the first 4 hours, but an inhibition thereafter in response to Al treatment. In contrast, this stress response (increase in putrescine levels) lasted for several days in red spruce. Differences in the growth rates of cell cultures (i.e., periwinkle is faster growing than red spruce) may be partly responsible for this observed difference (Minocha et al., 1996a).

Under conditions of increased oxidative stress, such as low temperature and/or nutrient deficiencies, adjustments of enzymatic activities and antioxidant levels have been observed in herbaceous plants as well as spruce needles (Hendry and Broklebank, 1985; Price and Hendry, 1991; Baker, 1994). Aluminum has been reported to cause Ca deficiency in spruce. Thus Al may also induce cells to respond to nutrient deficiency by changing their antioxidant levels.

There is no available information in the literature on the effects of Al on antioxidants and their biosynthetic enzymes in plants. However, research done using rats, animal cell culture systems, and in vitro assays has shown that aluminum was responsible for altered GTR and SOD activities (Zaman et al., 1990; Shainkin-Kestenbaum et al., 1989). Our work with red spruce suspension cultures treated with Al showed a dose-dependent decrease in GTR, MDHAR, and APX. Thse changes in GTR were not consistent from experiment to experiment.

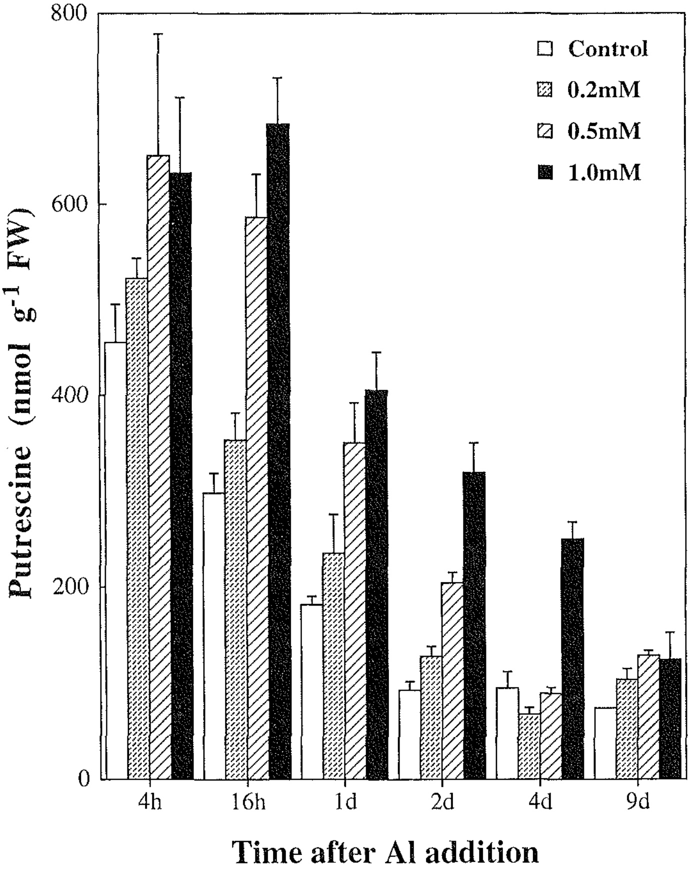

Figure 7.9. Effects of $AlCl_3$ on cellular putrescine. Al was added to 3-day-old red spruce cell suspension cultures of red spruce and subsequently analyzed. Values are mean ± SE of three replicates.

Recently, several researchers have postulated a protective role for organic acids (e.g., malate or citrate) in alleviating Al stress in herbaceous plants (Delhaize and Ryan, 1995 and references therein). The amount of malate released by an Al-tolerant genotype of wheat was 5- to 10-fold greater than the amount released from a near-isogeneic, but Al-sensitive genotype (Ryan et al., 1995). Following these suggestions we undertook a study (1) to determine which organic acids, if any, are accumulated or secreted by red spruce cultures in response to Al treatment and (2) to determine if exogenous addition of any of these organic acids along with Al would reverse the effects of Al on red spruce cultures. Our data show that addition of 0.5 and 1.0 mM $AlCl_3$ to 3-day-old suspension cultures caused a significant increase in the cellular content of succinate with a concomitant decrease in the concentration of oxalate at 48 hours.

However, both succinate and oxalate were present in significantly higher amounts in the spent media of Al-treated cell cultures. The cellular amounts of malate, ascorbate, and citrate were not affected by Al and they were not present in detectable quantities in the spent media. Exogenous addition of succinate alone or along with Al had no effect on cellular polyamine concentrations or the cell mass. Addition of either oxalate, ascorbate, or citrate alone to the cultures caused an increase in polyamine concentrations without any effects on cell mass. However, while the addition of malate by itself had no effect on cellular putrescine and cell mass, its addition along with Al caused a complete reversal of Al effects on putrescine metabolism and the growth rate of cells. The data support the postulated role for malate in alleviating Al stress.

Field Studies

The next step in the study was to validate observable changes in polyamine metabolism to Al exposure under field conditions. If valid, we could then use polyamine chemistry as an early biochemical marker of stress in apparently healthy trees (Minocha et al., 1996b, 1997). Six red spruce stands from the northeastern U.S. were selected for collection of soil and foliage samples. These stands all had soil solution pH values below 4.0 in the Oa horizon but varied in their soil Al concentrations. Some of these sites were apparently under some form of environmental stress as indicated by a large number of dead and dying red spruce trees. Samples of soil and needles (from apparently healthy red spruce trees) were collected from these sites four times during a 2-year period. The data showed a strong positive correlation between Ca and Mg content in the needles and that of the Oa horizon of the soil, the area of greatest occurrence of fine absorbing roots. Needles from trees growing on relatively Ca-rich soils with a low exchangeable Al concentration and a low Al/Ca soil solution ratio had significantly lower concentrations of putrescine and spermidine than those growing on Ca-poor soils with a high exchangeable Al concentration and a high Al/Ca soil solution in the Oa horizon. The magnitude of this change was several-fold higher for putrescine concentrations than for spermidine concentrations. Neither putrescine nor spermidine was correlated with soil solution chemistry (Ca, Mg, and Al concentrations) in the B horizon. The putrescine concentrations of the needles always correlated significantly with exchangeable Al ($r^2 = 0.73$, $P \leq 0.05$) and soil solution Al/Ca ratios (Fig. 7.10) ($r^2 = 0.91$, $P \leq 0.01$) of the Oa horizon. This suggests that in conjunction with soil chemistry, putrescine and/or spermidine may potentially be used as early indicators of Al stress before the appearance of visual symptoms in red spruce trees.

We conclude that similar inferences on the effects of Al may be drawn from cell cultures and the foliage of mature red spruce in the field (Minocha et al., 1996a). This indicates that the cell culture studies are

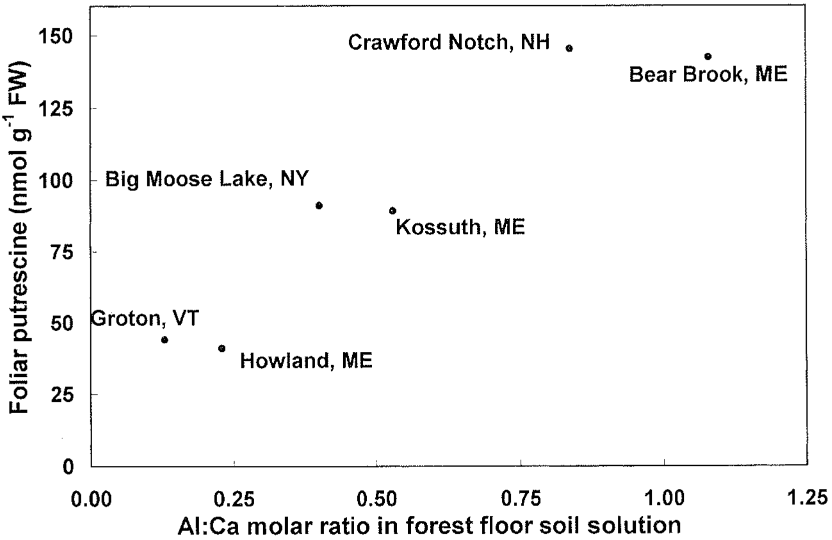

Figure 7.10. The relationship of foliar soluble putrescine to the Al/Ca molar ratio calculated for total monomeric Al and Ca in the soil solution of the forest floor. Foliar data are means of 40 replicate observations except for Kossuth, Maine, and Big Moose, New York, which are means of 20 replicate observations. Soil data are means of 12 replicate observations.

a valuable and reliable tool for investigating the effects of potential chemical stressors on trees. We have also demonstrated that an increase in foliar putrescine and/or spermidine concentrations in response to direct or indirect stress imposed on red spruce trees by Al exposure may possibly be used as an early warning tool for assessing and predicting tree health before the appearance of visual symptoms of damage.

Polyamines, in addition to a role in membrane stabilization, act as scavengers of free radicals, modulators of the metabolism of reduced N and modulators of free Ca in the cells (Slocum and Flores, 1991). Since their biosynthetic pathway competes with the biosynthesis of ethylene, a senescence hormone, they can also modulate ethylene metabolism. Thus, the elevated cellular polyamine content could help the plant in tolerating a variety of stresses that affect the above mentioned cellular functions.

Belowground Processes

Root Physiology and Pathology

Acidic deposition can deplete soil nutrients, promote soil acidification (Robarge and Johnson, 1992), and increase the concentration of anions.

Increased deposition of acidic anions increases the concentration of Al in the soil solution (Reuss, 1983). These changes adversely affect root viability and sustainability.

Root physiology and pathology are affected indirectly by acidic deposition primarily by two mechanisms. The first mechanism is a change in soil chemistry that modifies (1) root morphology, longevity, and turnover rates, (2) the mycorrhizal status of the root, and (3) root chemistry, metabolism, and ultimately the ability to take up nutrients. The second mechanism is a change in carbon allocation patterns to the roots caused by reductions in total tree carbon production due to reduced nutrient uptake, reduced photosynthetic area (foliage) related to dieback phenomena (e.g., winter injury in spruce), and increased root mortality. Change in carbon allocation reduces the available carbohydrate, required to maintain existing roots, replace dead and dying roots, and provide defense chemicals needed to protect roots against pathogenic fungi and other microorganisms (Bloomfield et al., 1996).

Ulrich (1983) proposed that the adverse effects of acidic deposition on forest ecosystems resulted from decreases in the base cations in soil solution, especially Ca levels, resulting in low Ca/Al ratios. Studies on fine root deterioration in conifer forests in Germany linked fine root death, decreases in growth, deteriorating crowns, and increased tree mortality to changes in the molar ratio of Al/Ca in the forest floor (Bauch and Schroeder, 1982; Bauch et al., 1985a,b; Stienen et al., 1984). These studies found that where decreased growth or tree mortality occurred, Al/Ca ratios in the cortex of fine roots were >1.

Higher levels of Al seem to decrease the uptake of Ca by tree roots eventually resulting in the loss of root integrity. Radioisotope studies showed that when equimolar concentrations of Al^{3+} and Ca^{2+} existed at pH 4, Ca uptake by the roots was reduced (Schroder et al., 1988). The Ca/Al molar ratio of the soil solution seems to be one of the prime mechanisms by which acidic deposition affects forest growth and it has been proposed as an important indicator of potential stress in forest ecosystems (Cronan and Grigal, 1995). This ratio is especially critical in the forest floor where the fine root systems of coniferous species are predominantly located and capture nutrients (Vogt et al., 1981).

Fine Root Distribution in the Forest Floor

The forest floor is an important source of nutrients for forest trees, especially for coniferous trees in cold temperate and boreal forests (Cole and Rapp, 1981; Vogt et al., 1986); the importance of this horizon as the dominant site for nutrient capture increases as the stand ages and develops. In the Pacific Northwest, Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) forests about 70 years old obtained almost 100% of their nutrient requirements from the forest floor (Johnson et al., 1982b). At this stage of stand development, trees have fully occupied the site, and foliar and root biomasses have stabilized (Vogt et al., 1987). There is also some evidence that suggests that as the stand ages, fine roots concentrate in the forest floor. Fine roots in a 23-year-old stand of *Abies amabilis* Forbes in the Pacific Northwest was predominantly located in the E horizon while in an older stand (~180-year-old) root biomass was mostly located in the forest floor (Vogt et al., 1980).

The few studies conducted in forests of the northeastern U.S. show that fine roots of conifers occupy predominantly the forest floor, which in these forests can be quite deep (5 to 15 cm) (Fernandez, 1992; Joslin et al., 1992). In a survey of hardwood and spruce–fir forests in the Adirondack Mountains of NY, fine roots of red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* L.) occupied the forest floor predominantly, while fine roots of yellow birch (*Betula alleghaniensis* Britt.), sugar maple (*Acer saccaharum* Marsh.), and beech (*Fagus grandifolia* Ehrh.) were distributed throughout the forest floor and mineral horizons (Hopkins, 1939). For example, about 95% of balsam fir roots and 100% of red spruce roots were found in the forest floor horizons designated as the F or fermentation layer (Oe) and H or humus layer (Oa). In contrast, yellow birch had about half (40 to 50%) of its fine roots in the forest floor, while beech (37%) and sugar maple (12%) had a third or less of their roots in the forest floor. More recent studies in the same area, but over a greater range of sites than used in the Hopkins study (Hopkins, 1939), showed that 70 to 80% of the conifer fine root biomass (<2-mm diameter) occurred in the forest floor, while more than 50% of the fine roots of hardwood species were in the mineral horizons.

This preponderance of fine roots in the surface organic horizons has also been observed in spruce–fir forests in the southern Appalachians, where fine roots were almost exclusively limited to the Oa horizon and surface of the underlying mineral horizon (Johnson and Fernandez, 1992; Kelly and Mays, 1989). Both of these reports indicated that fine roots (<0.5 cm) were found in the lower depths of the mineral horizon but they represented only a very small fraction of the total fine roots.

In these northern forests, the restriction of conifer roots primarily to the forest floor is in strong contrast to the distribution of hardwood roots. In the Adirondacks, fine root abundance of yellow birch in the mineral horizon ranged from 32 to 100% depending on associated species composition and site type (Hopkins, 1939). In the same sites, total abundance of sugar maple and beech roots in the mineral horizon ranged from 88 to 100% and 63 to 100%, respectively. The same trend has also been recorded for mixed mesophytic forests in eastern Kentucky. Root abundance was consistently and significantly higher in the mineral soil and represented 79%, 83% and 88% of the total root abundance for oak, transitional, and beech forest types, respectively (Kalisz et al., 1987).

Soil Chemistry Effects on Fine Roots

The effects of acidic deposition on the humus–root relationships in sensitive forest sites and its role in subsequent localized damage was first proposed in the late 1800s in Germany for conifer sites apparently suffering from sulfur emissions (Hartig, 1897). Sulfur emissions were postulated to affect the soil, which led to suppressed root growth and nutrient uptake, and, eventually, dieback, decline, and mortality. However, it was not until the late 1970s and early 1980s that significant large-scale tree mortality was reported in Europe and stimulated research on its cause(s), which to some extent corroborated Hartig's hypothesis (Ulrich et al., 1979). This led to specific studies that showed significant disruption of root functions and damage to root tissues due to the mobilization of Al and its uptake by trees (Godbold et al., 1988).

In general, spruce on acidic "mor" soils carry larger bio- and necromasses and have greater turnover rates than trees on more fertile soils (Kottke, 1987; Ulrich, 1987; Vogt et al., 1986). Thus, trees must allocate a larger percentage of their net primary production to root system growth to provide sufficient nutrients and water for growth (Rehfuess, 1989). Any significant reduction in root growth and turnover rates would have a significant adverse effect on above ground productivity.

The impact of acidic deposition on root growth and hence on overall productivity is mediated most likely through its effect in the rooting zone on the abundance of aluminum (Al), which interferes with and regulates processes at the soil–root interface (Cronan and Grigal, 1995). The effect may be manifested through two mechanisms. One mechanism is the competition by Al for nutrient uptake exchange sites, and subsequent physiological alterations to plant growth and resource acquisition. The other is the restriction of the rooting zone where roots can acquire resources. The first mechanism is related to the affinity of root exchange sites for Al at low pH and high Al/Ca ratios (Cronan, 1991; Dahlgren et al., 1991). This allows Al to significantly interfere with the uptake of other important cations (Ca and Mg), thus eventually causing nutrient imbalances within the whole plant, for example, the observed Mg deficiencies in Norway spruce in Germany (Schlegel and Hutterman, 1990). Also, increased uptake of Al by the fine roots directly and adversely affects their physiology, causing reduced water uptake and decreased fine-root biomass, branching, and terminal elongation (Cronan et al., 1989).

The second mechanism is related to the potential toxicity of Al in the rooting medium (Dahlgren et al., 1991). Organic horizons typically have high cation exchange capacities and low Al/Ca ratios; B horizons, in contrast, have lower exchange capacities, higher relative amounts of exchangeable acidity, and high Al/Ca ratios (Cronan, 1994; Dahlgren et al., 1991). Thus, as concentrations of anions from acidic deposition increase in the mineral soil, the concentration of Al (primarily Al^{3+}) in the soil solution increases making the mineral horizon an inhospitable rooting medium for roots of Al-sensitive species. Root concentrations of Al tracked closely with the Al^{3+} concentrations but not total aqueous Al concentrations showing that the form of Al is important to toxicity (Dahlgren et al., 1991). In addition, Al concentrations in the forest floor increased with increased depth. This relationship was due both to changes in the chemical affinity for Al and to the progressively greater incorporation of mineral soil and/or mineral soil aluminum with increasing depth into the organic horizon (Rustad and Cronan, 1995). This increase in Al concentrations in the B mineral horizon and lower depths of the organic horizon will decrease the availability of hospitable rooting zones and further decrease the ability of tree roots to capture necessary quantities of base cations other than Al.

The effects of these chemical changes on roots and their relationships with aboveground deterioration have been documented for several forest systems. Prolonged suppression of cambial growth and crown dieback and mortality were linked with high molar Al/Ca ratios in fine roots of red spruce in stands in New England (Shortle and Smith, 1988). Shortle and Bondietti (1992) showed that in stands receiving high acidic deposition, Ca/Al ratios in the root tips were low (0.3 to 1.1), root density was sparse, and tree conditions were poor, based on crown condition and abundance of mortality. The Ca/Al ratio was higher (2.5 to 10) in root tips of red spruce in stands receiving less acidic deposition and where tree condition was judged good. Similar relationships with acidic deposition were observed in high-elevation red spruce stands in the southern Appalachians (Joslin and Wolfe, 1992). Restricted root growth was attributed to an unfavorable soil chemical environment characterized by high Al concentrations in the soil solution and a low Ca/Al molar ratio. Fine roots of red spruce have a high affinity for Al at low soil pHs and especially at low Ca/Al ratios; Al is taken up in preference to "nontoxic" cations. In the root itself, Al is co-precipitated with phosphate (PO_4) and oxalate thereby decreasing the availability of P to the trees. This co-precipitation of Al with PO_4 decreases with depth and thereby decreases the ability of roots to avoid Al toxicity by co-precipitating Al (Dahlgren et al., 1991). Smith et al. (1995) showed that this preferential uptake can occur differently depending on the location of roots in the organic horizon. Root tips in the upper portion of the organic horizon (Oa, F) had higher Ca and lower Al concentrations than root tips in the lower portion of the organic horizon (Oa, H). Smith et al. (1995) also observed that the Al/Ca ratios in the root tips were consistently higher at higher-elevation sites compared with lower-elevation sites. This differential concentration of Ca and Al in the fine root tips corresponds to the observation that the Al concentrations are higher in the lower portion (Oa) of the organic horizon in these stands (Rustad and Cronan, 1995).

Mycorrhizal Relationships

Not a great amount of work has been done in the northern forest ecosystems on the relationship of mycorrhizae and acidic deposition. One study in West Virginia compared ectomycorrhizal-basidiomycete communities in red spruce with those in northern hardwood forests (Bills et al., 1986). Twenty-seven species of putative mycorrhizal partners were identified by sporocarp presence in red spruce stands, and thirty-five were found on northern hardwood stands; eight of the species were common to both areas. The spruce sites were on ridge crests between 1200 and 1350 m but there was no comparison with stands at higher elevations that are potentially more polluted. In a limited study of root pathology on red spruce on Mt. Abraham, Vermont, fewer mycorrhizae were found on fine roots of declining (crown deterioration—dieback, needle loss) trees than on healthy trees in a high elevation stand (Wargo et al., 1993). In addition to lower percentages of root tips that were mycorrhizal, declining trees had fewer mycorrhizae morphotypes (Glenn et al., 1991; Wargo et al., 1993).

In a more extensive study of root pathology on red spruce conducted on trees in plots above and below cloud base and on windward and leeward aspects on Whiteface Mountain, NY, no consistent relationships between mycorrhizal measurements and tree health were detected on random samples of fine roots from healthy and declining trees (Wargo et al., 1993). In general, percentages of mycorrhizae on the Whiteface Mountain site (avg. 27%) were significantly lower than those reported for other red spruce sites in mesic and wetland sites in New Jersey (avg. 89%) (Glen et al., 1991) and at high-elevation sites on Mt. Mitchell in North Carolina (avg. 35%) (Bruck, 1984).

Data on abundance of mycorrhizae on red spruce from the Whiteface Mountain study and studies at Mt. Mitchell (Bruck, 1984) suggest that trees in high-elevation sites, regardless of health, have fewer mycorrhizae. This could be a function of soil type, climate, or nutrient status. In the Whiteface Mountain study, the percentage of root tips that were mycorrhizal on randomly selected fine roots was significantly lower than on fine roots chosen for their healthy appearance. Percentages of mycorrhizae on these healthy-appearing roots were 2 to 3 times higher than on the randomly chosen fine-root systems and were similar to those found on healthy red spruce at lower-elevation sites on Mt. Mitchell, North Carolina (Bruck, 1984). This suggests that the turnover rate of mycorrhizal roots is higher in those high-elevation sites.

Studies in Bavaria related changes in mycorrhizae on declining Norway spruce (*Picea abies* L.) to soil chemistry (Meyer et al., 1988). In these Bavarian stands, mycorrhizae numbers were highly and positively correlated with the molar Ca/Al ratio in the mineral soil. The low incidence of mycorrhizae on red spruce at Whiteface Mountain sites also could be related to the Al/Ca soil chemistry. For example, total Ca and

Mg concentrations in the forest floor on Whiteface Mountain were higher at the low-elevation spruce site than at the higher-elevation site, and were lower at the single site on Mt. Abraham (Shortle and Bondetti, 1992) where root damage was greater than at both elevations on Whiteface Mountain (Wargo et al., 1993). Also, exchangeable Ca and Mg were considerably lower on Mt. Abraham (Shortle and Bondietti, 1992). In addition to the low Ca and Mg, both sites had relatively high Al/Ca ratios; it was 0.9 on Whiteface Mountain and 3.1 on Mt. Abraham. The value for Whiteface Mountain is just slightly above the Ca/Al ratio at which Cronan and Griegel (1995) estimate that there is a 50:50 risk of impact on tree growth or nutrition. The Ca/Al ratio at Mt. Abraham is well below that estimated to have a 75% chance of causing or resulting in damage.

Other studies have shown negative relationships between Al and degree of mycorrihzal abundance on fine-roottips. The degree of mycorrhizal infection on Norway spruce in Germany was positively related to the Ca/Al ratio in the mineral soil and increased as the ratio increased (Schneider et al., 1989). Mycorrhizal formation on balsam fir seedlings growing in environmental chambers was inhibited by Al at 50 and 100 mg Al g^{-1} substrate and at pH 3 and 4 (Entry et al., 1987). Reduction in mycorrhizal colonization on pitch pine seedlings also was caused by Al at 50 mg l^{-1} concentration (McQuattie and Schier, 1992). In addition, the mantle hyphae were devoid of cytoplasm, and cortical cells of the fine roots contained dense material and were often disrupted. Also, the zone of actively dividing cells in Al-treated seedlings was significantly less than controls, thus significantly reducing the amount of overall root growth.

Similar relationships of colonization rates by mycorrhizal fungi and Ca and Al properties of the soil have also been observed in northern hardwoods. A survey of 18 mature sugar maples showed that the frequency of endomycorrhizal formation was positively related to soil pH but negatively related to the level of H + Al held on the soil exchange sites (Ouimet et al., 1995). In this same study, the frequency of mycorrhizal formation also was positively correlated with the foliar and fine root Ca content. In contrast, evaluation of fine roots in sugar maple stands that had been treated with a base cation mixture showed that fertilization had no effect on the incidence of endomycorrhizae (Cooke et al., 1992; Moutoglis and Widden, 1996). However, this lack of response to fertilizer may reflect the short duration (1 year) between treatment and evaluation of mycorrhizal colonization of fine roots.

Fine Nonwoody Root Pathology

The basic processes of root turnover and senescence in forest trees have been recently reviewed (Bloomfield et al., 1996). However, the role of fine root pathogens in contributing to root turnover and senescence is not well understood, nor are the biotic and abiotic factors that influence the importance of fine-root pathogens in forests.

Primary fungal pathogens of fine nonwoody roots of trees, though common on tree seedlings in nurseries, have been rarely observed in natural northern forests. Most of the known aggressive fine root pathogens have been documented in the lower latitudes where soils are generally warm (Sinclair et al., 1987). Even fine root pathogens that develop on tree seedlings growing in nurseries fail to spread to other seedlings or trees when infected seedlings are transplanted to forest soils (Sinclair et al., 1987). It has been suggested that for those fine root fungal pathogens that do develop in forest settings, some form of stress may be required to predispose roots to infection by these fungi (Manion, 1981).

The relationship between stress and fine root pathogens has not been thoroughly studied. Woody roots in both deciduous and coniferous trees are commonly predisposed by stressors (e.g., drought, defoliators, temperature extremes, pollutants, etc.) to less aggressive root pathogens such as *Armillaria* spp. (Carey et al., 1984; Hudak and Singh, 1970; Wargo, 1977, 1981; Wargo and Houston, 1974). The same phenomenon probably occurs for fine roots; however, since these relationships have not been studied extensively, the pathogens have not been identified.

One potential stress-induced pathogen of fine roots of conifers is *Mycelium radicis atrovirens* Melin. This name was originally given to a sterile gray fungus that was isolated from fine roots of *Pinus sylvestris* L. and *Picea abies* Karst. (Melin, 1922). It was designated a pseudomycorrhiza that was potentially harmful to tree roots. Richard and Fortin (1973) observed the same fungus on fine roots of black spruce (*Picea mariana* [Mill] B.S.P.) and *M. r. atrovirens* has been isolated from fine roots of declining Norway spruce (*P. abies*) in Bavaria (Livingston and Blaschke, 1984). Subsequent work has shown that many isolates of dark-pigmented fungi that were designated as *M. r. atrovirens* actually represent many different fungi (Kowalski, 1973). Two such fungi have been identified as *Phialocephala demorphospora* Kendrick (Kendrick, 1961) and *Phialocephala fortinii* Wang and Wilcox (Wang and Wilcox, 1985). Both form pseudomycorrhizal associations with conifer roots (Wang and Wilcox, 1985). *Phialocephala fortinii* is considered to be a pathogen of fine roots and may be triggered to cause mortality of fine roots on trees weakened by stress that accelerates senescence and reduces resistance in root tissue. Recent studies have shown that *P. fortinii* was pathogenic on red pine seedlings in greenhouse experiments (Harney, 1994; Wang and Wilcox, 1985).

In studies on root vitality on declining red spruce, dematiaceous fungi were consistently isolated from putative dead, nonwoody roots from 10 sites in the states of New York, Vermont, New Hampshire, and Maine (Harney et al., 1995). Several of these isolates were identified by RFLP analysis of PCR amplified rDNA as being genetically related to *P. fortinii*

(Harney et al., 1995). These sites represented a putative west-to-east health and acid deposition gradient, and, consequently, a potential Al/Ca ratio gradient from a high deposition, poor health, central NY site, east to a low deposition, good health, ME site. There was a tendency for the sites with greater mortality or greater crown deterioration of red spruce to have greater levels of fine-root mortality but no correlation with these dematiaceous fungi have been verified as yet.

Tomato mosaic virus (ToMv) is another pathogen that has been found in fine roots of red spruce trees in areas potentially impacted by acid deposition. ToMv was initially detected in stream water exiting a deteriorating red spruce–balsam fir stand on Whiteface Mountain in New York (Jacobi and Castello, 1991). The virus was subsequently transmitted to herbaceous hosts. The virus also was detected in red spruce needles and subsequently transmitted to herbaceous hosts as well as red spruce, black spruce, and balsam fir seedlings (Jacobi et al., 1992). The virus was found in the roots of these infected seedlings, suggesting that the root system was the potential point of ToMv infection in infested soils in red spruce stands (Jacobi and Castello, 1992). Additional studies in windward and leeward red spruce stands, above and below cloud base (see Wargo et al., 1993), verified the presence of ToMv in fine roots of mature red spruce (Castello et al., 1995). The incidence and concentration of virus varied, but in general were greater in the healthier trees with little-to-moderate crown dieback than in declining trees with severe dieback. There was a higher percentage of virus-infected trees at the higher-elevation plots, that is, above cloud base on the windward and leeward aspects, but there was no aspect affect. Infected trees had smaller live crowns than uninfected trees, suggesting a long-term effect of virus infection on tree growth (Castello et al., 1995).

The role of this virus and other potential viruses in red spruce decline is unclear. The random pattern of virus occurrence in the fine nonwoody roots of red spruce (Castello et al., 1995) is coincident with a random pattern of fine root deterioration observed on these trees on Whiteface Mountain (Wargo et al., 1993). This coincidence suggests possible cause and effect. However, this random pattern of fine-root deterioration was in sharp contrast to apparent simultaneous and progressive deterioration that occurred on red spruce growing on Mt. Abraham in the Green Mountains of Vermont (Wargo et al., 1993). Root deterioration on trees on Mt. Abraham was related to low energy reserves (starch) in the woody root system and may indicate that these root systems were observed at a later stage of deterioration than those on trees on Whiteface Mountain.

The greater deterioration of fine-root systems on red spruce on Mt. Abraham also may be related to the chemical environment in which the roots were growing. The molar Al/Ca ratio of 3.1 in fine roots of red spruce in the humus layer on Mt. Abraham was significantly greater than the ratio of 0.9 measured in fine roots of red spruce on Whiteface Mountain (Shortle and Smith, 1988; Shortle and Bondietti, 1992). The soils on the two sites are also distinctly different, which helps explain the chemical differences in the fine roots. On Mt. Abraham plots, the forest floor rests directly on bedrock predominantly, while on Whiteface Mountain, plots had a well developed mineral soil (Wargo et al., 1993). Other studies on Whiteface Mountain on soil and foliar chemistry of red spruce have corroborated the lower Al to Ca ratio in soil, soil solution, and red spruce foliage on Whiteface Mountain (Johnson et al., 1994a,b).

Woody Root Pathology

No pathogens of the coarse woody root system have been consistently observed nor isolated from symptomatic trees and related to acidic deposition levels or putative gradients of acidic deposition in the northern forests. Armillaria root disease was reported on trees on Whiteface Mountain but the role of the fungus in the decline of red spruce was discounted because it was considered to be a secondary agent and not a direct cause of decline (Weidensaul et al., 1989). The secondary role of *Armillaria* seems to be exactly what its role in decline generally is, to attack trees after they have been weakened by stress agents (Wargo and Harrington, 1991). No information was given in the Weidensaul et al. report (1989) to determine if there was a relationship between *Armillaria* and decline severity or elevation. Lack of root pathogens as contributors to decline of red spruce and frazier fir also has been reported for the southern Appalachians (Bruck, 1989).

Where species of *Armillaria* have been observed and isolated from roots of declining red spruce, no consistent pattern or link between its presence and decline symptoms has been observed. In studies on red spruce on Mt. Abraham, Vermont and Whiteface Mountain, New York, *Armillaria* was found occasionally on some woody roots of trees classified to the severe decline category (Wargo et al., 1993). However, it was not on all severely declining trees and the fungus occurred only occasionally on trees with better crown conditions. In a survey to determine the incidence and severity of Armillaria root disease on red spruce at nine sites in the Northeast, the fungus was frequently found colonizing and killing trees at lower elevation sites (<800 m) (Carey et al., 1984). However, colonization by *Armillaria* was less severe and less frequent at elevations above 800 m. An aerial assessment of spruce mortality in New York, Vermont, New Hampshire, and the western mountains of Maine indicated that mortality was heaviest at elevations above 800 m (~2600 ft) (Miller-Weeks and Smoronk, 1993). The reasons for these differences by elevation were speculative. However, subsequent studies in seven sites (five in the Northeast and two in the southern Appalachians) on the distribution of rhizomorphs (the major infection and spreading structure of the fungus (Shaw and Kile, 1991) of *Armillaria* in soils in declining red spruce stands showed that both the frequency of occurrence and the abundance of rhizomorphs were significantly less in higher-elevation sites, thus decreasing the chances for root contact and infection (Wargo et al., 1987b). In this study on *Armillaria*, forest type or elevation, pH, and lead were significant variables that were related to rhizomorph density across all sites; all three variables were highly correlated among themselves. Lead concentration increases in the soil with elevation and has been related to atmospheric pollutant deposition (Johnson et al., 1982a). In addition, in vitro studies with *Armillaria* showed that concentrations of soluble lead as low as 10 ppm could significantly reduce thallus growth and inhibit rhizomorph formation and subsequent growth (Wargo et al., 1987a).

Species of *Armillaria* are not the only fungi that decrease in abundance with elevation in the northern forest ecosystems. Root and butt rot fungi in spruce–fir forests in New Hampshire decreased in importance as causes of gap-makers (trees that die and or snap-off and create forest gaps) as elevation increased (Worrall and Harrington, 1988). The relationship of acidic deposition or related factors with the reduction in fungi was not measured.

Conclusions

Few young trees survive to maturity. Tree survival depends on resisting the adverse effects of environmental stress. The stress to which an individual tree is subjected is the results of several to many stressors. Stressors include pests, pathogens, and adverse environmental conditions. Resistance to stress results from the application of metabolic energy within the genetic limits of the individual tree. Changes in landuse patterns, climate, soil nutrition, insect infestations, and the constant threat of disease all contribute to tree stress and all affect forest health and productivity.

Air pollution is an additional stressor to which trees have been constantly subjected throughout most of the 20th century. Air pollution comes in several forms, such as acids that alter the availability of essential elements in the soil, oxidants that disrupt leaf function, and heavy metals that block metabolism. Is there evidence that in the 20th century new stressors from human activity added to those already at work in the forest have had any impact on health and productivity?

We investigated the potential impact of acidic deposition on northern spruce forests. These forests naturally develop a thick raw or "mor" humus or organic layer in which the cation exchange capacity is highly sensitive to acid input. In this scenario, rapid increase in the atmospheric deposition of sulfate and nitrate in the middle of the 20th century mobilized essential Ca and Mg that were then available either for uptake by tree roots or for leaching from the rooting zone. This period of historical mobilization appears to be contained in the dendrochemical record of red spruce. Enhanced leaching may be responsible for the decreased storage of Ca and Mg in the soil as seen in the comparison with historical analyses. Acid deposition also mobilized Al from the mineral soil beneath the organic layer. Mobile Al ions displace Ca and Mg from the soil's organic layer and reduce the storage of Ca and Mg, which is essential for tree growth and development. In addition to displacing Ca and Mg from organic matter in the soil, Al binds to root cell walls, blocking the uptake of essential Ca and reducing root elongation. Roots unable to elongate and spread soon exhaust the local supply of essential elements and die. Dying roots are susceptible to attack by facultative root pathogens. Loss of roots leads to loss of crown and stem growth that results in reduced productivity and increased vulnerability to harmful abiotic and biotic stressors. Apparently healthy trees are stressed in relation to adverse Al:Ca ratios in the soil, as indicated by concentrations of putrescine, a biochemical marker in foliage.

These results taken together are consistent with widespread, fundamental change in the response of trees to the environment, which is due at least in part to acidic deposition. No one stressor, such as acid deposition, can be seperated from other perturbations of soil chemistry, landuse patterns, and overall stress loading, but the perturbation of cation cycling caused by acidic deposition can clearly be a significant contributing factor to the decline of productivity, and sometimes tree health, on base-poor forest sites.

In the 1980s Robert Hartig, the great German forest biologist, deduced that "smoke injury" (localized acidic deposition) reduced stem growth before the appearance of crown symptoms. Hartig hypothesized that loss of basic cations Ca and Mg from the root zone leads to root dysfunction, root death, and root infection. In the 1980s after modern technology had pushed acidic deposition to the regional level, Josef Bauch found that Al bound to the outer cortex of absorbing root tips reduced the uptake of Ca and Mg. The concept of aluminum-induced calcium deficiency syndrome was then developed to explain how acidic deposition can have an adverse effect on northern conifers growing on forest mor. Bauch further stated that the "bottom-up" disease described by Hartig coupled with "top-down" disease due to the direct action of air pollutants, such as ozone, on the crowns of trees could produce a "pincer effect." Either the bottom-up problem or the top-down problem alone caused growth suppression, but in combination caused mortality. Early detection, improved diagnosis, and management strategies designed to reduce levels of external stressors and enhance the effectiveness of trees to respond to stress will be needed to meet this challenge.

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