Anthropogenic Calcium Depletion: A Unique Threat to Forest Ecosystem Health?

Paul G. Schaberg,* Donald H. DeHayes,† and Gary J. Hawley†
*United States Department of Agriculture, Forest Service, Northeastern Research Station, S. Burlington, Vermont; †The University of Vermont, School of Natural Resources, Burlington, Vermont

ABSTRACT

Numerous anthropogenic factors can deplete calcium (Ca) from forest ecosystems. Because an adequate supply of Ca is needed to support fundamental biological functions, including cell membrane stability and stress response, the potential for Ca deficiency following the individual, cumulative, or potentially synergistic, influences of anthropogenic factors raises important questions concerning organism and ecosystem health. Past work has shown that one Ca-depleting factor (foliar acid mist exposure) reduces concentrations of biologically important membrane-associated Ca (mCa) from red spruce foliar cells, destabilizes these cells, and results in their increased susceptibility to the freezing injury responsible for red spruce decline in northeastern U.S. montane ecosystems. Data presented here indicate that these same disruptions can occur for other tree species and that soil-based Ca manipulation can also alter critical mCa pools. Considering the unique role Ca plays in the physiological response of cells to environmental change and stress, we hypothesize that depletion of biologically available Ca (e.g., mCa) could result in a scenario similar to recognized immune deficiency syndromes in animals. A hypothetical pathway through which anthropogenically induced Ca deficiencies could predispose plants, and possibly animals, to exaggerated injury following exposure to environmental stress is presented, and the potential implications of this scenario to ecosystem health are discussed.

SOIL CALCIUM DEPLETION

Calcium (Ca) is an abundant and critically important nutrient in plants and animals and a major cation in soil and surface waters. In pristine environments, Ca availability and cycling is governed by the balance of numerous natural processes, including atmospheric additions, mineral weathering, soil formation, plant uptake and growth, forest stand dynamics, and leaching losses (Likens *et al.* 1998; McLaughlin & Wimmer 1999). However, there is growing evidence that various anthropogenic factors may now be disrupting natural cycles and depleting Ca from forest soils. High acid loading that promotes Ca leaching is an important and consistent explanation for soil Ca depletion in north temperate forests (Kirschner & Lydersen 1995; Likens *et al.* 1996; Likens *et al.* 1998; Hyman *et al.* 1998). Other potential contributing factors include declines in atmospheric base cation deposition (Hedin *et al.* 1994), soil aluminum mobilization (Shortle & Smith 1988; Lawrence *et al.* 1995; Federer *et al.* 1989), nitrogen saturation (Aber *et al.* 1989; Aber *et al.* 1995; Schaberg *et al.* 1997), and changing climatic conditions (Tomlinson 1993). In addition, it is well recognized that Ca is one of the elements most susceptible to depletion following timber harvesting because of the high concentration of Ca in tree wood and bark (Mann *et al.* 1988; Federer *et al.* 1989; Dutch 1994; Fichter *et al.* 1998; Adams 1999). Whole tree harvesting in which branches and foliage are removed from sites along with tree boles, multiple

Address correspondence to: Paul G. Schaberg, USDA Forest Service, Northeastern Research Station, 705 Spear Street, S. Burlington, VT 05401, USA; E-mail pschaberg@fs.fed.us.

cuttings, or short rotation times increase the intensity of Ca loss (Hornbeck 1991, Adams 1999). Altered site conditions, including lower vegetative Ca uptake and increased soil acidification, also accelerate soil Ca leaching and loss following harvest (Hornbeck 1991, Adams 1999).

In addition to harvest-associated differences, the extent and rate of soil Ca depletion is clearly expected to vary with chemical weathering reactions and base cation availability (Hyman *et al.* 1998). However, reports of soil calcium losses are prevalent and appear to transcend both soil type and forest species associations (Mann *et al.* 1988). For example, European studies have documented soil Ca losses across a range of watersheds (Kirchner & Lydersen 1995) and have led to hypotheses relating cation imbalances caused by Ca losses to the decline of spruce (*Picea*) forests (Schulze 1989). In the United States, soil Ca losses have been documented in southern pine (*Pinus*) forests (e.g., Richter *et al.* 1994; Johnson *et al.* 1995) and numerous other conifer and hardwood forests throughout the northwest, northeast, and southeast (Federer *et al.* 1989; Wilson & Grigal 1995; Dhamala & Mitchell 1996; Huntington *et al.* 2000). In fact, soil Ca losses were evident at each of 11 forest stands examined throughout the United States, independent of harvesting history, harvesting practices, and the dominant species in each forest association (Mann *et al.* 1988). Furthermore, Ca losses were unique relative to other ions in that hydrologic losses were often as great as those resulting from harvest (Mann *et al.* 1988). Although it is often difficult to quantify the extent of soil Ca depletion over time, recent reports indicate that the magnitude of Ca losses may be substantial. For example, based on long-term chronological records and detailed biogeochemical studies, Likens *et al.* (1996) have estimated that the pool of Ca in the soil complex at the Hubbard Brook Experimental Forest (New Hampshire, USA) may have shrunk by more than 50% during the past 45 years. Soil losses of Ca were attributed to leaching caused by acid rain inputs, decreasing Ca deposition, and changing amounts of net Ca storage in plant biomass. If harvest-associated losses are added to those from other sources, the vulnerability of forests to Ca depletion becomes even more evident. Although Ca losses are substantial at times, remaining pools of weatherable or exchangeable Ca may be fully adequate for ecosystem function. The dilemma is determining how much Ca loss an ecosystem can sustain relative to existing pools and Ca inputs before depleting available stores below critical biological thresholds.

It is well recognized that Ca plays a vital role in supporting plant cell function (Bangerth 1979; Hanson 1984; Hepler & Wayne 1985; Roberts & Harmon 1992; Bush 1995; Sanders *et al.* 1999; Roos 2000). However, the physiological and ecological implications of anthropogenic perturbations to biological or soil Ca pools are only beginning to be unraveled for whole plant systems (McLaughlin & Wimmer 1999). Recent studies have implicated Ca disruption or changes in ion ratios (e.g., Ca/Al, Cronan & Grigal 1995) to reduced growth and vigor of woody angiosperms (Ellsworth & Liu 1994; Fyles *et al.* 1994; Wilmot *et al.* 1995) and coniferous tree species (DeHayes *et al.* 1999; Schaberg *et al.* 2000; Schulze 1989; Lawrence *et al.* 1995; McLaughlin *et al.* 1991). Despite this initial evidence, the overall threat that Ca depletion poses to forest health is largely unknown.

CALCIUM PARTITIONING AND PHYSIOLOGY

Plant productivity and health are fundamentally important to ecosystem sustenance and stability. Plants fulfill unique ecological roles and are keystone organisms that carry out essential ecosystem processes including solar energy capture, primary food production, nutrient and water cycling, gas exchange, oxygen release, soil enrichment, and erosion control. In recognition of the vital functions plants perform, any assessment of the biological risks of environmental Ca depletion must consider plant Ca dynamics and the unique distribution, form, and function of Ca in plants.

In all organisms, Ca is highly compartmentalized within cells and tissues, and this partitioning is a defining component of its physiological function. Because Ca has a high affinity for binding, thereby reducing the availability of biologically essential orthophosphate and phosphorylated organic compounds, cells have evolved mechanisms (including the pumped removal of Ca) to maintain very low cytoplasmic Ca concentrations (Roos 2000). As a result, estimated concentrations of Ca in plants varies greatly within cells, ranging from a low of 100-200 nM in the cytoplasm to a high of 1-10 mM in cell walls and vacuoles (Knight *et al.* 1996; Trewavas & Malho 1998). Localized concentrations of Ca support at least two important functions: (1) they add to the structural stability of cell

walls and membranes; and (2) labile Ca is a key constituent in the pathway that allows cells to sense and respond to environmental stimuli and change (Pandey *et al.* 2000; Roos 2000).

In its structural role, Ca is a key constituent of the middle lamella of cell walls where it helps to bind adjacent cells together and strengthen overall construction (Marschner 1995). Ca also influences membrane structure and function, stabilizing membranes and influencing permeability by bridging phosphate and carboxylate groups of membrane phospholipids and proteins (Palta & Li 1978; Legge *et al.* 1982; Davies & Monk-Talbot 1990). The plasma membrane plays a critical role in plant cell function and, by influencing membrane architecture, Ca influences numerous critical physiological processes, such as solution movement across membranes and the ability of cells to resist dehydration and freezing (Pomeroy & Andrews 1985; Steponkus 1990; Arora & Palta 1986; Arora & Palta 1988; Guy 1990; DeHayes *et al.* 1999; Schaberg & DeHayes 2000; Sutinen *et al.* 2001). Extracellular Ca also exists within and between cell walls as a component of crystalline deposits (Fink 1991). These deposits have low physiological availability and may only function as a depository for excess Ca.

Ca also serves as an important second messenger in the perception and transduction of environmental and stress signals (Figure 1) (Hepler & Wayne 1985; Roberts & Harmon 1992; Bush 1995; Sanders *et al.* 1999; Pandey *et al.* 2000; Roos 2000). Because extremely little free Ca exists in the cytoplasm of cells, environmental stimuli that temporarily alter the permeability of the plasma membrane to Ca, allow labile extracellular Ca to flow into cells along a steep concentration gradient (Sanders *et al.* 1999). Data suggest that Ca concentrated within cellular organelles (notably the vacuole, endoplasmic reticulum, and mitochondria) can also serve as a source of messenger Ca (Roos 2000). Whether originating from extra- or intracellular sources, once in the cytoplasm, Ca quickly binds to Ca-specific proteins such as calmodulin. Activated Ca-protein complexes then initiate a chain of physiological modifications (e.g., changes in enzyme activity, gene transcription, etc.) that help cells adjust to the environmental conditions that triggered the response cascade. This entry of Ca into the cytoplasm acts as a "messenger" of environmental information into cells and appears to be an essential first step in triggering a wide range of physiological responses needed by plants to successfully adjust to environmental change or defend against pests and pathogens. Indeed, a series of independently conducted studies have implicated a critical message perception and transduction role for Ca in response to an array of environmental stresses, including salinity (Lynch *et al.* 1987), low temperature (Arora & Palta 1988; Dhindsa *et al.* 1993; Monroy *et al.* 1993; DeHayes *et al.* 1997; DeHayes *et al.* 1999), drought (Sheen 1996), reduced light (Sheen 1996), fungal infections (Salzer *et al.* 1996; Hebe *et al.* 1999), and insect infestations (McLaughlin & Wimmer 1999). For example, Ca influx to the cytoplasm leads to the closure of stomata that helps limit plant water loss and enhances plant survival during droughts (Ng *et al.* 2001). Similarly, Ca signal transduction is involved in cell recognition of fungal infection and initiates the hypersensitive response that contributes to pathogen resistance (Xu & Heath 1998). Specificity of the Ca signal to relate the nature and extent of environmental stimuli likely results from the amplitude, duration, frequency, and location of Ca influx, as well as interactions of the Ca signal with other cellular components and signal pathways (Sanders *et al.* 1999; Pandey *et al.* 2000).

Conifer needles provide an excellent example of the biological fractionization and function of Ca. Here, the majority of Ca exists as insoluble Ca oxalate and pectate crystals in cell walls and extracellular spaces (Fink 1991). In contrast to these large reserves, ions in equilibrium within the plasma membrane region (including some free and displaced apoplastic Ca from the cell wall) are biologically available and of major physiological importance. This pool of membrane-associated Ca (mCa), although a relatively small fraction of total foliar ion pools, is the portion that influences membrane stability and the response of cells to changing environmental conditions and multiple stress signals (Atkinson *et al.* 1990; DeHayes *et al.* 1997). Recent studies have demonstrated the dynamic and independent nature of the mCa pool as a structurally and functionally distinct compartment of the total foliar Ca pool in red spruce (*Picea rubens*) leaf tissue (DeHayes *et al.* 1997) as well as its unique sensitivity to direct manipulation from acidic deposition (DeHayes *et al.* 1999).

DIRECT IMPACTS OF ACID RAIN ON FOLIAR CALCIUM

Environmental Ca depletion is most commonly associated with the loss of soil Ca. However, re-

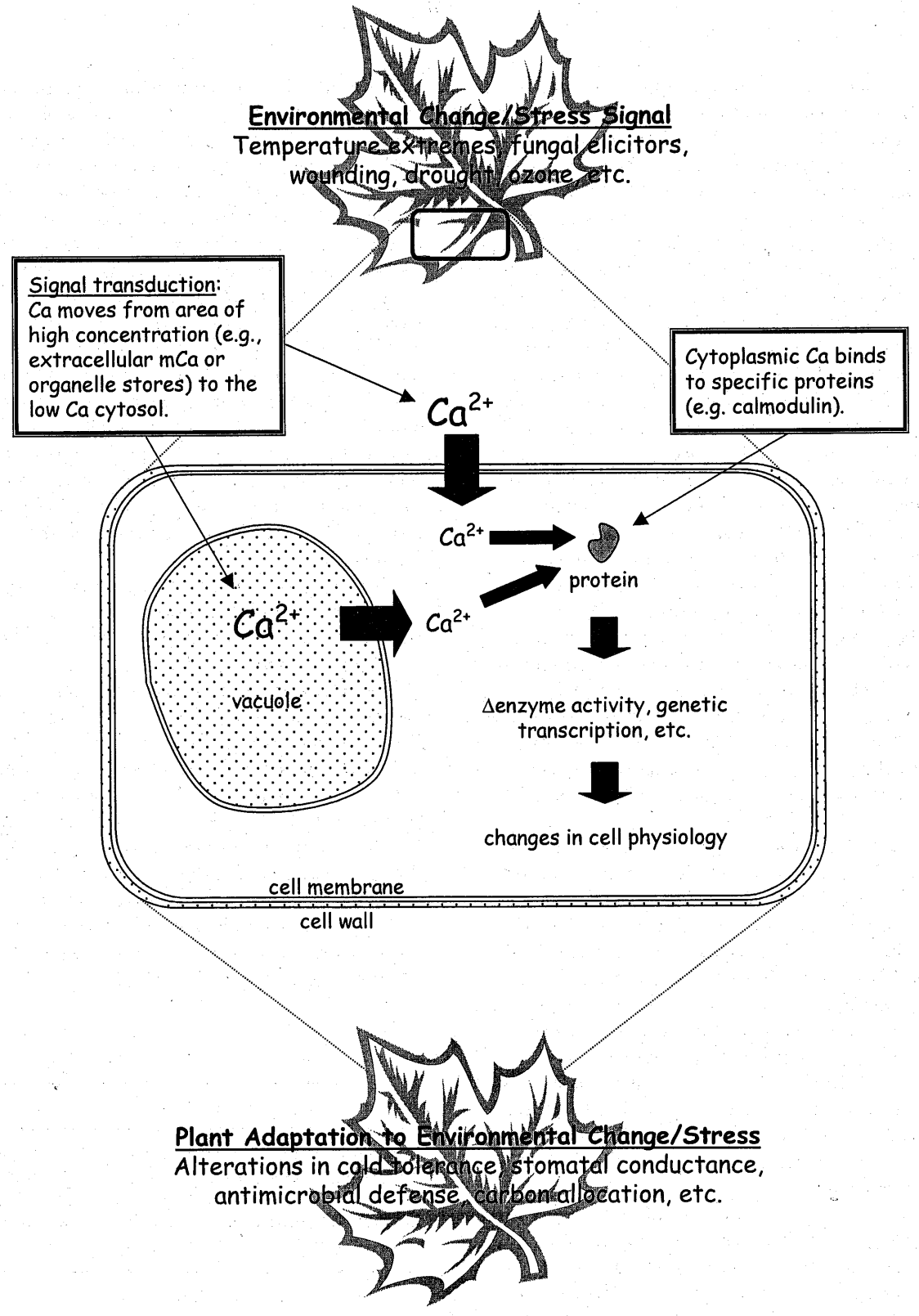

FIGURE 1. Schematic representation depicting the transduction of environmental stimuli (e.g., a stress event) into chemical signals (calcium (Ca) influx to the cytoplasm) that trigger alterations in cell physiology and promote plant adaptation and survival. Signal-induced alterations in membrane permeability allow Ca to enter the cytoplasm along a steep concentration gradient. Incoming Ca can bind to and activate Ca-specific protein complexes that interact with other cell components (existing enzymes, DNA, etc.) to modify cell physiology in response to the instigating environmental cue. We propose that depletion of biologically labile Ca (e.g., membrane-associated Ca (mCa)) could perturb Ca signal transduction and diminish the ability of plants to sense and respond to enviromental change/stress.

cent evidence has demonstrated that the leaching of Ca from foliage can also be substantial. Direct acid-induced foliar Ca leaching has been demonstrated in many north temperate forest tree species, including red spruce (Joslin *et al.* 1988; DeHayes *et al.* 1999; Schaberg *et al.* 2000), white spruce (*Picea glauca*, Scherbatskoy & Klein 1983), sugar maple (*Acer saccharum*, Lovett & Hubbell 1991), red maple (*Acer rubrum*, Potter 1991), yellow birch (*Betula alleghaniensis*, Scherbatskoy & Klein 1983), and eastern white pine (*Pinus strobus*, Lovett & Hubbell 1991). Furthermore, acidic deposition-mediated Ca disruption has been directly implicated in the widespread and well-documented decline of montane red spruce forests. We have shown that acidic deposition on red spruce foliage preferentially displaces Ca ions specifically associated with plasma membranes of mesophyll cells. This loss of mCa destabilizes membranes, may disrupt the messenger function of Ca, and significantly reduces foliar cold tolerance (DeHayes *et al.* 1999; Schaberg *et al.* 2000). This sequence of physiological perturbations leads to the commonly observed secondary symptoms of freezing injury in northern regions and could be associated with elevated tissue respiration (McLaughlin *et al.* 1993) and an enhanced susceptibility to other stresses that compromise overall forest health (DeHayes *et al.* 1999).

Importantly, there is reason to believe that acid-induced mCa disruption is not unique to red spruce, although the implications may be exacerbated in this species because of its unique sensitivity to subfreezing temperatures. In fact, recent evidence from our laboratory indicates that the same fundamental physiological responses documented for red spruce also occur in other northern coniferous species. We conducted a series of *in vivo* experiments that used proven protocols (DeHayes *et al.* 1999; Schaberg *et al.* 2000; Schaberg & DeHayes 2000) to treat seedlings of four species (eastern white pine, eastern hemlock (*Tsuga canadensis*), balsam fir (*Abies balsamea*), and red spruce for comparison) with simulated acid mists (ionic composition patterned after regional cloud chemistry with pH levels adjusted to 3.0 or 5.0 using H_2SO_4) for one growing season (July through October). Following treatment, foliar samples were analyzed to test the influence of aerial acid mist application on: (1) concentrations of mCa on the plasma membranes of mesophyll cells; (2) plasma membrane stability; and (3) midwinter (January) freezing tolerance. Membrane-associated Ca was measured using the fluorescent probe chlorotetracycline (CTC) with computer image processing to quantify the intensity of mCa-specific fluorescent emissions (Borer *et al.* 1997; DeHayes *et al.* 1997). Freezing tolerance was assessed by measuring electrolyte leakage (determined by conductivity measurements) from the current-year foliage from each tree following a series of controlled freezing tests (DeHayes & Williams 1989; Schaberg & DeHayes 2000). Relative electrolyte loss from non-frozen controls used in cold tolerance assessments was used to evaluate baseline (before freezing stress) levels of membrane integrity (Schaberg *et al.* 2000). Limitations in the quantity of white pine, hemlock, and balsam fir foliage prevented the evaluation of multiple measurement parameters for these species. However, red spruce foliage was abundant enough to provide a consistent and standardized comparison for all response assays.

The results of these experiments showed consistent and parallel physiological responses for red spruce and the other species examined. Overall, the reductions in mCa concentration (Table 1), decreases in foliar membrane stability (Table 2), and declines in foliar cold tolerance (Table 3) experienced by red spruce seedlings treated with pH 3.0 compared with pH 5.0 mists were fully consistent with our past results (DeHayes *et al.* 1999; Schaberg *et al.* 2000; Schaberg & DeHayes 2000). For the first time, however, this data indicated that the same pattern of response occurred with other tree species. Similar to red spruce, eastern hemlock seedlings treated with pH 3.0 mist had lower mCa concentrations than hemlock exposed to pH 5.0 mist (Table 1), and balsam fir treated with pH

TABLE 1

Relative concentration of membrane-associated calcium (mCa) on mesophyll cell membranes in current-year foliage of eastern hemlock and red spruce seedlings following application of pH 5.0 or 3.0 simulated cloud water treatments

	Relative mCa				
Species	pH 5.0	pH 3.0	P	n	ΔmCa (%)
Eastern hemlock	0.32	0.29	0.02	20	−10
Red spruce	0.18	0.12	0.05	40	−22

TABLE 2

Relative stability of cell membranes in current-year foliage of balsam fir and red spruce seedlings following application of pH 5.0 or 3.0 simulated cloud water treatments

Species	Membrane stability (relative electrolyte leakage) pH 5.0	pH 3.0	P	n	Δmembrane leakage (%)
Balsam fir	0.24	0.32	0.03	40	+33
Red spruce	0.24	0.29	0.05	40	+21

3.0 mist exhibited greater membrane destabilization than fir exposed to pH 5.0 mist (Table 2). Acid mist exposure even reduced the cold tolerance of white pine seedlings (Table 3). This reduction in white pine cold tolerance does not have the same significance to plant health and survival as similar reductions have for red spruce because white pine are approximately 20°C more cold tolerant in midwinter than red spruce prior to acid-induced reductions (DeHayes *et al.* 2000). Nonetheless, the fundamental similarity in response underscores the consistency in mechanistic response among all species tested. Indeed, these data raise the possibility that acid-induced foliar Ca disruption may be a more general phenomenon than the highly visible freezing injury syndrome evident in red spruce.

TABLE 3

Cold tolerance of current-year eastern white pine and red spruce foliage following application of pH 5.0 or 3.0 simulated cloud water treatments

Species	Cold tolerance (°C) pH 5.0	pH 3.0	P	n	Δcold tolerance (%)
White pine	−54.2	−51.8	0.004	30	−4.4
Red spruce	−49.0	−44.9	0.008	40	−8.4

SOIL CALCIUM LIMITATIONS

Although the above experiments and our past work highlight the potential for aerial acid exposure to alter plant physiology and health, they do not specifically resolve the potential risks of soil Ca depletion. Numerous anthropogenic factors have been implicated in the loss of soil stores that are the source of plant Ca. In past experiments, we found that soil Ca additions had no discernable impact on biologically important mCa concentrations or related physiology (DeHayes *et al.* 1999; Schaberg *et al.* 2000). However, these experiments could not resolve whether further soil Ca depletion (below experimental starting points) could result in plant Ca deficiencies and physiological dysfunction. Therefore, we recently conducted a controlled experiment to specifically examine if soil-based Ca limitations would restrict the accrual of biologically meaningful mCa levels on foliar cell membranes. Here red spruce seed was germinated and grown in a low Ca perlite potting media ($<1 \mu g \cdot g^{-1}$ Ca based on inductively coupled plasma emission spectroscopy) and supplied with nutritionally complete simulated soil solutions that differed in their Ca concentration (supplied as $CaCl_2$, Ingestad 1959). Forty plants were randomly divided between two experimental groups: 20 seedlings to a 5 μg/l Ca treatment and 20 seedlings to a 0 μg/l Ca treatment. Following eight months of daily watering with soil solutions in a climate-moderated greenhouse (temperature range 20-25°C, ambient light), four needles from each seedling were removed and their mCa concentrations measured using established methods (Borer *et al.* 1997; DeHayes *et al.* 1997; DeHayes *et al.* 1999; Schaberg *et*

TABLE 4

Relative concentration of membrane-associated calcium (mCa) on mesophyll cell membranes in current-year foliage of red spruce seedlings treated with soil solution Ca additions (0 or 5 μM Ca)

Relative mCa				
Soil solution Ca				
0 μM	5 μM	P	n	ΔmCa (%)
0.10	0.16	0.005	40	+60

al. 2000). Results from this experiment reflect an experimentally induced "worst case scenario" (severe short-term Ca restrictions). Nonetheless, they verify that soil-based Ca limitations can impede the accrual of biologically available Ca on foliar membranes (Table 4).

Further indication of the potential for soil-based disruption of foliar Ca nutrition was recently provided by a study of the influence of long-term nitrogen (N) fertilization on the mineral nutrition and physiology of mature red spruce trees on Mount Ascutney, Vermont (Schaberg *et al.* in press). Of the ten experimental plots at this site, two plots have each received either 0, 15.7, 19.8, 25.6, or 31.4 $kg \cdot ha^{-1} \cdot yr^{-1}$ of N (added as NH_4Cl and/or $NaNO_3$) since 1988. Past work at this site showed that protracted N additions resulted in reduced total foliar Ca incorporation (including biologically unavailable Ca crystals) and led to higher rates of foliar respiration (Schaberg *et al.* 1997). Our new work has confirmed that soil treatments have specifically reduced foliar mCa levels and simultaneously reduced membrane stability and cold tolerance levels (Schaberg *et al.* in press). Together, greenhouse (Table 4) and field (Schaberg *et al.* in press) experiments indicate that soil-based Ca perturbations can result in nutritional and physiological disruptions comparable with those resulting from aerial acid mist exposure.

POTENTIAL IMPACTS ON ECOSYSTEM HEALTH

Our results suggest that physiologically meaningful mCa disruptions may be more pervasive than the direct acid-induced foliar alterations already noted for red spruce. In particular, it appears that the physiological implications of foliar Ca leaching are applicable to many tree species and that soil-based Ca limitations can result in foliar mCa deficiencies similar to those produced by leaching. But what do these findings tell us about the possible influence of Ca depletion on forest function and health?

If limitations in soil Ca develop in forest ecosystems, then associated plants could be prevented from accumulating physiologically adequate supplies of Ca. In addition, soil-based Ca limitations could be exacerbated by foliar leaching losses of Ca after uptake. Soil- and/or foliar leaching-driven limitations in biologically available Ca would have definite physiological implications, and some of the resulting deficiency symptoms (such as likely reductions in growth, Marschner 1995) might become evident through established monitoring efforts, including long-term forest inventory data. However, many factors affect growth including variations in water availability, temperature, competition, biotic stresses, age, land use history, and stand development. The influence of these and other factors, alone or in combination, could overshadow any influence of Ca depletion on forest growth. Nonetheless, reports of increases in tree growth within declining stands following Ca additions and/or liming may indicate that Ca limitations are already suppressing growth in at least some forests (Wilmot *et al.* 1994; Long *et al.* 1997; Moore *et al.* 2000). Still, it is interesting to consider that impaired Ca availability might not manifest itself in immediate physiological dysfunction.

Given the fundamental role Ca plays in plant stress response systems (Figure 1), biological Ca depletion could create a scenario analogous to the suppression of animal immune systems. For example, there are numerous circumstances (e.g., HIV infection, chemotherapy treatment, etc.) that diminish the normal function of human immune systems. An immunocompromised person may well appear, feel, and ostensibly function as if they were healthy. Nonetheless, when exposed to a disease agent they can experience declines in health that are exaggerated relative to a person with a fully functioning immune system. In this same way, it is possible that depletions of biologically available Ca (e.g., mCa) could suppress the ability of plants to adequately sense and respond to changes in their surroundings, and thereby, predispose them to disproportionate decline following exposure to perhaps even "normal" levels of stress that otherwise would pose no catastrophic threat to plants with fully functional biological response systems in place. Importantly, under this scenario, plants might appear to be normal and healthy even though their biological response systems were compromised. This "hidden" threat could be particularly pertinent when Ca deprivation was not severe (as in the early stages of soil Ca depletion) and the visible symptoms of acute plant Ca deficiencies were not yet evident.

We hypothesize that anthropogenic disruptions of biologically available Ca may impair the ability of plants to recognize and respond to environmental stress, and thus, predispose forests to decline. Specifically, we propose that depletion of biologically labile Ca would diminish the transduction of environmental/stress signals into cells

and limit full activation of physiological response systems (changes in enzyme activity, gene expression, etc.). Without a complete and appropriate cellular response, physiological accommodation and defense mechanisms could prove inadequate, especially if plants were confronted with numerous, interactive, or prolonged stresses. Reduced stress response would predispose plants to amplified injury following natural or anthropogenic stress events and increase the probability of injury and decline of individual plants, populations, and communities.

This hypothesis is not centered on any one species or decline syndrome, and several real world examples consistent with it have now been documented. Certainly, the acid-induced depletion of mCa that predisposes red spruce to freezing injury, foliar loss, and decline could result in part from disruptions of stress response systems as well as a structural weakening of cell membranes. In addition, it is known that acid rain exposure significantly increases the susceptibility of flowering dogwood (*Cornus florida*) trees to injury by the fungal pathogen dogwood anthracnose (*Discula destructiva*) (Anderson *et al.* 1993; Britton *et al.* 1996). Controlled studies have implicated acid-induced nutrient deficiencies in the altered disease susceptibility of dogwoods (Britton *et al.* 1996). Enhanced disease susceptibility has also been associated with environmental conditions (e.g., shade, low temperatures, wet cool summers, etc.) that result in low transpiration rates, and thus, reduced Ca accumulation in plants (McLaughlin & Wimmer 1999). The association of Ca deficiency and anthracnose susceptibility is also supported by an increased resistance to this disease following lime application (USDA 1991). Based on this and other evidence, McLaughlin and Wimmer (1999) speculated that Ca deficiencies driven by low soil Ca concentrations, low transpiration rates, and/or accelerated foliar leaching reduce the natural resistance of dogwood to anthracnose infection.

The well-documented decline of sugar maple in north temperate forests of the United States also appears consistent with the hypothesis that depleted biological Ca may predispose trees to inadequate stress response and decline. Several environmental factors, notably drought and insect defoliations (Allen *et al.* 1992; Payette *et al.* 1996), freezing injury (Gregory *et al.* 1986), and nutritional deficiencies (especially Ca, Ellsworth & Liu 1994; Wilmot *et al.* 1995; Long *et al.* 1997) have been associated with maple decline. And, experimental additions of Ca and/or lime have been shown to reduce decline symptoms (Wilmot *et al.* 1995; Long *et al.* 1997; Moore *et al.* 2000). The possible synergy of Ca depletion and stress imposition was highlighted by a study examining the factors associated with the decline of sugar maples on the Allegheny Plateau in Pennsylvania (Horsley *et al.* 2000). Here, maple stands on unglaciated summits generally had soils low in base cations, leaves with lower Ca and magnesium (Mg), and higher manganese (Mn) concentrations, and experienced significantly higher levels of tree mortality after repeated insect defoliations (Horsley *et al.* 2000). Although the specific predisposing influence of Ca depletion on sugar maple decline has not been experimentally tested, it is noteworthy that plant physiological response systems associated with all the stresses specifically connected to maple decline (drought, insect defoliation, and freezing injury) involve activation by Ca signal transduction.

Regardless of the specific species involved, we propose that diminished stress response may be particularly important now because numerous human activities (e.g., pollution production, ozone depletion that elevates UV exposure, climate change, the spread of exotic pests and pathogens, etc.,) are simultaneously subjecting forest ecosystems to an unprecedented level and diversity of stresses. The combined influence of elevated stress exposure and reduced stress response/tolerance may represent a unique emerging burden on the health and sustainability of forest ecosystems.

In addition to alterations in plant systems, environmental Ca depletions can lead to Ca deficiencies in animal populations, negatively altering their physiology and reproductive success. This phenomenon has been well documented in Europe, where soils exposed to protracted inputs of acid deposition can become depleted of Ca and incapable of supporting normal snail population levels (e.g., Graveland *et al.* 1994; Graveland & van der Wal 1996). Declines in snail populations reverberate through the food chain, diminish the Ca intake for birds dependent on this source of Ca, and result in elevated rates of eggshell defects, clutch desertions, and empty nests (Graveland *et al.* 1994; Graveland & van der Wal 1996). Even here, the threat of Ca depletion appears to be that of a predisposing agent: low Ca incorporation makes eggs more susceptible to final injury through desiccation or breakage (Graveland *et al.* 1994; Graveland & van der Wal 1996).

The transfer of Ca deficiencies from soils to supported plants and animals has also been re-

ported for North American forest systems. Mahony *et al.* (1997) examined the mineral nutrition of healthy and declining hardwood forests in Ontario, Canada. They found that declining forests had lower Ca and Mg concentrations and calcium: aluminum (Ca:Al) ratios in their soils than healthy forests. The Ca, Mg, and phosphorus concentrations and the Ca:Al ratios in the foliage of sugar maple trees were also lower in declining sites, possibly indicating that extensive acid leaching from foliage and soils had altered mineral nutrition and tree health (Mahony *et al.* 1997). Importantly, alterations in foliar nutrition also resulted in reductions in Ca and Mg in caterpillars (*Geometridae*) feeding within tree canopies. Although mineral deficiencies were not detected in the birds feeding on Ca-depleted caterpillars (presumably because their diets were supplemented by Ca from other sources), this study verified that Ca depletion could be conveyed up forest trophic levels (Mahony *et al.* 1997).

Combining evidence from geochemical, plant, and animal studies, we have developed a hypothetical pathway through which anthropogenic Ca depletion could lead to declines in forest ecosystem health (Figure 2). This pathway highlights the possibility that both soil- and canopy-based Ca loss may result in plant Ca deficiencies that could instigate broad-based alterations of ecosystem function. Specifically, we propose that multiple anthropogenic drivers could work to reduce Ca concentrations in plants through the reduced uptake and/or enhanced foliar leaching loss of Ca. Inadequate accretion/excessive loss of Ca in plants could deplete biologically available Ca, alter cellular membrane stability and function, and diminish the physiological capacity of plants to recognize and successfully respond to natural and human-induced environmental change. Without an adequate capacity for stress response, plants with deficient biological Ca supplies would be more vulnerable to damage following inevitable exposures to environmental stresses. Probabilities of plant injury and decline would be enhanced if anthropogenic stresses increase in number and/or severity, and damage would also be expected to increase through repeated and/or interactive stress impositions. If extensive, the resulting decline of individuals would radiate through plant communities and alter the competition and survival of populations and perhaps even species, including animals at higher levels of forest food chains. Plant Ca deficiencies could be readily transferred to dependent herbivores, altering their Ca nutrition and associated physiology. These alterations would likely include declines in the reproductive success of some bird species, but could conceivably also result in Ca deficiencies among a broader spectrum of animals. For example, Ca deficiencies might result in reduced Ca incorporation and a weakening of insect exoskeletons and vertebrate bones, or even lead to reductions in the quantity or quality of mammalian milk production. Just as thinned eggshells predispose bird populations to reproductive failure when combined with desiccation or physical stress, Ca deficiencies in other animals might be most pertinent when combined with other stress factors (i.e., predation, freezing temperatures, extended migrations, etc.) to alter animal health and survival.

Through its influence on the nutrition and physiology of constituent plants and animals, and in conjunction with the presence of secondary environmental stresses, anthropogenic Ca depletion could perturb the structure, function, and health of forested ecosystems, and jeopardize the ecosystem goods and services (e.g., primary production, nutrient cycling, water filtration, oxygen generation, etc.) forests provide. Among other possible manifestations, by acting as a predisposing agent to decline, Ca depletion could promote the development of Ecosystem Distress Syndrome (Rapport *et al.* 1985). This syndrome is characterized by reduced biodiversity, altered primary and secondary productivity, increased disease prevalence, reduced efficiency of nutrient cycling, increased prominence of exotic species, and enhanced dominance of short-lived opportunistic species within impacted ecosystems (Yazvenko & Rapport 1996; Rapport & Whitford 1999). In addition to the fundamental threat posed to biological and ecological health, the scenario depicted in Figure 2 would have direct economic implications as timber production and other forest commodities were impacted. While more difficult to quantify, associated disruptions of other ecosystem services would also have economic repercussions (Costanza *et al.* 1997).

Although the fundamental connections between components of the proposed pathway have been documented, many details remain uncertain and are currently being evaluated. For example, the specific biochemistry of Ca-dependent plant stress response systems is currently a topic of great scientific interest and activity. Despite some uncertainty, by synthesizing information from disparate scientific disciplines, this pathway provides a uniquely comprehensive overview of the poten-

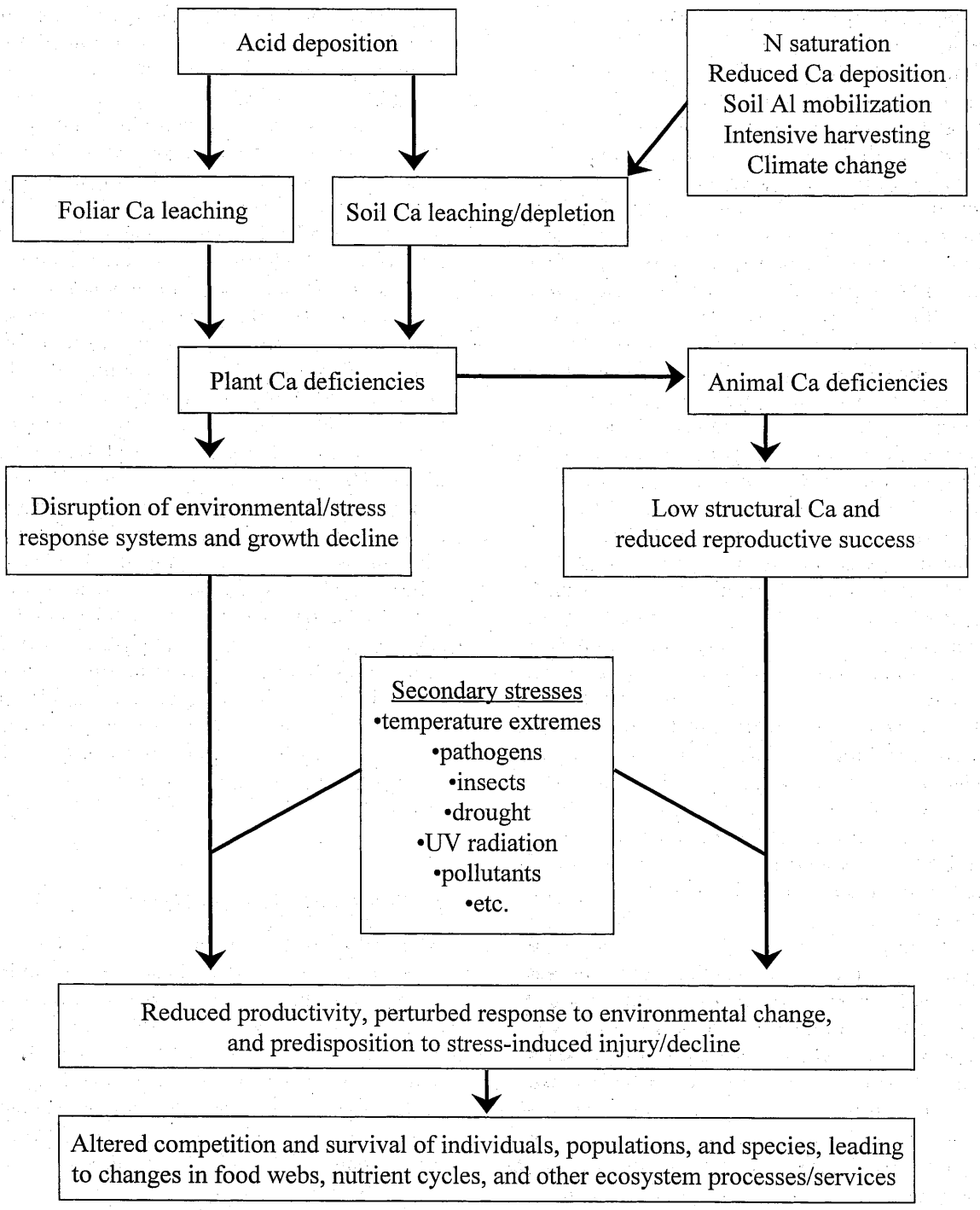

FIGURE 2. Hypothetical pathway through which anthropogenic calcium (Ca) depletion could lead to declines in forest ecosystem health. This pathway highlights the possibility that the inadequate uptake/excessive loss of Ca in plants could induce biological Ca deficiencies that diminish the physiological capacity of plants to recognize and successfully respond to environmental change/stress. Suppressed stress response in plants along with possible Ca deficiencies in animals could predispose biotic populations to exaggerated injury following inevitable exposures to natural and anthropogenic stresses, and thereby, threaten the health, function, and stability of forest ecosystems. This scenario may have an enhanced relevance if human activities continue to subject forest ecosystems to an elevated level and diversity of stresses.

tial ecological threat posed by anthropogenic Ca depletion.

Importantly, this pathway also emphasizes several points that have received little attention regarding the possible consequences of Ca depletion. One is that the biological impacts of Ca deficiency on stress tolerance and health could easily remain a latent and unnoticed threat when overall environmental stress levels were low. The ominous potential for inadequate physiological response and exaggerated injury/decline would only occur following exposure to natural or anthropogenic stress. Even when stress levels increased, resulting damage could well be attributed solely to stress exposure and the contributing influence of Ca deficiency may not be recognized. Indeed, traditional assays of forest health that rely heavily on visible indicators of decline (e.g., growth reductions, leaf loss, etc.) and causality (signs of insect damage, fungal infections, etc.) would likely overlook biological Ca deficiency as a possible predisposing factor in observed health declines. Furthermore, the influence of Ca depletion could remain hidden despite focused attempts to examine it if only traditional measurements of plant Ca status were made. Common measurements of total tissue Ca are dominated by unavailable (crystalline and structural) forms of Ca, which can be poorly associated with biological pools, such as mCa (DeHayes *et al.* 1997; DeHayes *et al.* 1999; Schaberg *et al.* 2000). If only total Ca concentrations were monitored, the biological Ca deficiencies fundamental to the decline scenario could remain undetected.

The compound nature of the threat depicted in Figure 2 (requiring both Ca deficiency and environmental stress), also greatly complicates the process of predicting future forest health conditions. Because the dynamics of Ca physiology within and among species and sites would have to be integrated with information on the presence, degree, distribution, and potential interaction of environmental stresses, accurate predictions of future damage could be very difficult to make. Details of localized Ca cycling and availability, species differences in Ca uptake and utilization, as well as the dynamics of stress exposure and response would all likely influence the biological and ecological threat posed by Ca depletion. Regardless of predictions for any one location, the unsettling prospect exists that overall damage to forest systems could increase greatly over time as a key component of stress response systems (biological Ca signaling) was itself diminished as part of the decline scenario.

Environmental Ca depletion has now been documented for forest ecosystems around the world including portions of North America (Likens *et al.* 1996; Huntington 2000), Europe (Kirchner & Lydersen 1995; Wesselink *et al.* 1995; Thimonier *et al.* 2000), and Asia (Nykvist 2000). Indeed, in some locations where intensive harvesting and other Ca depleting factors coexist, recent reports indicate that sustainable forest harvests cannot occur without managed Ca fertilization (Sverdrup and Rosen 1998; Nykvist 2000). Still, the biological and ecological threat posed by such losses remains vague. How much Ca can be lost from a system (or how little need remain) before meaningful deficiencies develop? Which pools of Ca should be monitored to evaluate biologically important Ca loss? Which biological and ecological processes are likely to be most vulnerable to Ca depletion and should, therefore, be preferentially examined? Is it likely that the full impacts of biological Ca loss will only be evident following the imposition of other secondary stresses? The relationships outlined in Figure 2 provide a useful focus for answering questions like these, help guide the development of biologically meaningful monitoring and risk assessment strategies, and supply a theoretical foundation for controlled experiments needed to determine if anthropogenic Ca depletion poses a unique threat to forest ecosystem health.

ACKNOWLEDGMENTS

We thank Paula F. Murakami, Dr. G. Richard Strimbeck, Amy S. Burfeind, and John D. Alexander for their help with treatment imposition, data collection, and analysis. We also thank C. Eagar, R. Costanza, T. Hutchinson, and D. Rapport for their helpful review comments. The research described in this paper was supported in part by a cooperative agreement with the U.S. Environmental Protection Agency and USDA CSREES McIntire-Stennis Forest Research Program funds.

REFERENCES

Aber, J.D., Magill, A., McNulty, S.G., Boone, R.D., Nadelhoffer, K.J., Downs, M., Halle, R. (1995) Forest biogeochemistry and primary production altered by nitrogen saturation. *Water, Air, and Soil Pollution* **85**, 1665–1670.

Aber, J.D., Nadelhoffer, K.J., Steudler, P., Melillo, J.M.

(1989) Nitrogen saturation in northern forest ecosystems. *BioScience* **39**, 378–386.

Adams, M.B. (1999) Acid deposition and sustainable forest management in the central Appalachians, USA. *Forest Ecology and Management* **122**, 17–28.

Allen, D.C., Barnett, C.J., Millers, I., Lachance, D. (1992) Temporal change (1988-1990) in sugar maple health, and factors associated with crown condition. *Canadian Journal of Forest Research* **22**, 1776–1784.

Anderson, R.L., Berrang, P., Knighten, J., Lawton, K.A., Britton, K.O. (1993) Pretreating dogwood seedlings with simulated acidic precipitation increases dogwood anthracnose symptoms in greenhouse-laboratory trials. *Canadian Journal of Forest Research* **23**, 55–58.

Arora, R. & Palta, J.P. (1986) Protoplasmic swelling as a symptom of freezing injury in onion bulb cells. *Plant Physiology* **82**, 625–629.

Arora, R. & Palta, J.P. (1988) *In vivo* perturbation of membrane-associated calcium by freeze-thaw stress in onion bulb cells. *Plant Physiology* **87**, 622–628.

Atkinson, M.M., Keppler, L.D., Orlandi, E.W., Baker, C.J., Mischke, C.F. (1990) Involvement of plasma membrane calcium influx in bacterial induction of the K^+/H^+ and hypersensitive responses in tobacco. *Plant Physiology* **92**, 215–221.

Bangerth, F. (1979) Calcium-related physiological disorders of plants. *Annual Review of Phytopathology* **17**, 97–122.

Borer, C.H., DeHayes, D.H., Schaberg, P.G., Cumming, J.R. (1997) Relative quantification of membrane-associated calcium in red spruce mesophyll cells. *Trees* **12**, 21–26.

Britton, K.O., Berrang, P., Mavity, E. (1996) Effects of pretreatment with simulated acid rain on the severity of dogwood anthracnose. *Plant Disease* **80**, 646–649.

Bush, D.S. (1995) Calcium regulation in plant cells and its role in signaling. *Annual Review of Plant Physiology and Plant Molecular Biology* **46**, 95–122.

Costanza, R., d'Agre, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Naeem, S., Limburg, K., Paruelo, J., O'Neill, R.V., Raskin, R., Sutton, P., van den Belt, M. (1997) The value of the world's ecosystem services and natural capital. *Nature* **387**, 253–260.

Cronan, C.S. & Grigal, D.F. (1995) Use of calcium/aluminum ratios as indicators of stress in forest ecosystems. *Journal of Environmental Quality* **24**, 209–226.

Davies, H.W. & Monk-Talbot, L.S. (1990) Permeability characteristics and membrane lipid composition of potato tuber cultivars in relation to Ca^{2+} deficiency. *Phytochemistry* **29**, 2833–2835.

DeHayes, D.H., Schaberg, P.G., Hawley, G.J., Borer, C.H., Cumming, J.R., Strimbeck, G.R. (1997) Physiological implications of seasonal variation in membrane-associated calcium in red spruce mesophyll cells. *Tree Physiology* **17**, 687–695.

DeHayes, D.H., Schaberg, P.G., Hawley, G.J., Strimbeck G.R. (1999) Acid rain impacts calcium nutrition and forest health. *BioScience* **49**, 789–800.

DeHayes, D.H., Schaberg, P.G., Strimbeck, G.R. (2000) Red spruce cold hardiness and freezing injury. In: Bigras, F.J. & Colombo, S.J. (eds.) *Conifer Cold Hardiness*. pp. 495–529. Kluwer Academic Publishers, Dordrecht, Netherlands.

DeHayes, D.H. & Williams, M.W. (1989) *Critical Temperature: A Quantitative Method of Assessing Cold Tolerance.* General Technical Report NE-134. U.S. Department of Agriculture Forest Service, Northeastern Forest Experiment Station, Broomall, PA.

Dhamala, B.R. & Mitchell, M.J. (1996) Soil disturbance and elemental dynamics in a northern hardwood forest soil, USA. *Water, Air, and Soil Pollution* **88**, 343–353.

Dhindsa, R.S., Monroy, A., Wolfraim, L., Dong, G. (1993) Signal transduction and gene expression during cold acclimation in alfalfa. In: Li, P.H. & Christersson, L. (eds.) *Advances in Plant Cold Hardiness*. pp. 57–72. CRC Press, Boca Raton, FL.

Dutch, J. (1994) Intensive harvesting of forests: the nutritional aspects and sustainability implications. *Proceedings of the IEA/BE workshop.* May 16–22, 1993, Fredericton, NB. Information Report M-X-191E. pp. 41–57. Canadian Forest Service, Maritimes Region, Fredericton, NB.

Ellsworth, D.S. & Liu, X. (1994) Photosynthesis and canopy nutrition of four sugar maple forests on acid soils in northern Vermont. *Canadian Journal of Forest Research* **24**, 2118–2127.

Federer, C.A., Hornbeck, J.W., Tritton, L.M., Martin, C.W., Pierce, R.S., Smith, C.T. (1989) Long-term depletion of calcium and other nutrients in eastern US forests. *Environmental Management* **13**, 593–601.

Fichter, J., Dambrine, E., Turpault, M.-P., Ranger, J. (1998) The Strengbach catchment (Vosger Mountains, N-E France). *Water, Air, and Soil Pollution* **104**, 125–148.

Fink, S. (1991) The micromorphological distribution of bound calcium in needles of Norway spruce (*Picea abies* (L.) Karst.). *New Phytologist* **119**, 33–40.

Fyles, J.W., Cote, B., Courchesne, F., Hendershot, W.H., Savoie, S. (1994) Effects of base cation fertilization on soil and foliage nutrient concentrations, and litter-fall and throughfall nutrient fluxes in a sugar maple forest. *Canadian Journal of Forest Research* **24**, 542–549.

Graveland, J. & van der Wal, R. (1996) Decline in snail abundance due to soil acidification causes eggshell defects in forest passerines. *Oecologia* **105**, 351–360.

Graveland, J., van der Wal, R., van Balen, J.H., van Noordwijk, A.J. (1994) Poor reproduction in forest passerines from decline of snail abundance on acidified soils. *Nature* **368**, 446–448.

Gregory, R.A., Wiliams, M.W., Wong, B.L., Hawley, G.J. (1986) Proposed scenario for dieback and decline of *Acer saccharum* in northeastern U.S.A. and southeastern Canada. *IAWA Bulletin* **7**, 357–369.

Guy, C.L. (1990) Cold acclimation and freezing stress tolerance: role of protein metabolism. *Annual Re-*

view of Plant Physiology and Plant Molecular Biology **41**, 187–223.

Hanson, J.B. (1984) The functions of calcium in plant nutrition. Vol. I. In: Tinker, P.B. & Lauchli, A. (eds,) *Advances in Plant Nutrition.* pp. 149–208. Praeger Publishers, New York.

Hebe, G., Hager, A., Salzer, P. (1999) Initial signaling processes induced by elicitors of ectomycorrhiza-forming fungi in spruce cells can also be triggered by G-protein-activating mastoparan and protein phosphatase-inhibiting cantharidin. *Planta* **207**, 418–425.

Hedin, L.O., Granat, L., Likens, G.E., Buishand, T.A., Galloway, J.N., Butler, T.J., Rodhe, H. (1994) Steep declines in atmospheric base cations in regions of Europe and North America. *Nature* **367**, 351–354.

Hepler, P.K. & Wayne, R.O. (1985) Calcium and plant development. *Annual Review of Plant Physiology* **36**, 397– 439.

Hornbeck, J.W. (1991) Nutrient depletion: a problem for forests in New England and eastern Canada? *Proceedings of the conference on the impacts of intensive harvesting,* January 22, 1990. pp. 56–67. Fredericton, NB. Forestry Canada, Maritimes Region, Fredericton, NB.

Horsley, S.B., Long, R.P., Bailey, S.W., Hallet, R.A., Hall, T.J. (2000) Factors associated with the decline disease of sugar maple on the Allegheny Plateau. *Canadian Journal of Forest Research* **30**, 1365–1378.

Huntington, T.G. (2000) The potential for calcium depletion in forest ecosystems of southeastern United States: review and analysis. *Global Biogeochemical Cycles* **14**, 623–638.

Huntington, T.G., Hooper, R.P., Johnson, C.E., Aulenbach, B.T., Cappellato, R., Blum, A.E. (2000) Calcium depletion in a southeastern United States forest ecosystem. *Soil Science Society of America Journal* **64**, 1845–1858.

Hyman, M.E., Johnson, C.E., Bailey, S.W., April, R.H., Hornbeck, J.W. (1998) Chemical weathering and cation loss in a base-poor watershed. *GSA Bulletin* **110**, 85–95.

Ingestad, T. (1959) Studies on the nutrition of forest tree seedlings. II. Mineral nutrition of spruce. *Physiologia Plantarum* **12**, 568–593.

Johnson, D.W., Binkley, D., Conklin, P. (1995) Simulated effects of atmospheric deposition, harvesting, and species change on nutrient cycling in a loblolly pine forest. *Forest Ecology* **76**, 29–45.

Joslin, J.D., McDuffie, C., Brewer, P.F. (1988) Acidic cloud water and cation loss from red spruce foliage. *Water, Air, and Soil Pollution* **39**, 355–363.

Knight, H., Trewavas, A.J., Knight, M.R. (1996) Cold calcium signaling in arabidopsis involves two cellular pools and a change in calcium signature after acclimation. *Plant Cell* **8**, 489–503.

Kirchner, J.W. & Lydersen, E. (1995) Base cation depletion and potential long-term acidification of Norwegian catchments. *Environmental Science and Technology* **29**, 1953–1960.

Lawrence, G.B., David, M.B., Shortle, W.C. (1995) A new mechanism for calcium loss in forest-floor soils. *Nature* **378**, 162–165.

Legge, R.L., Thompson, E., Baker, J.E., Lieberman, M. (1982) The effect of calcium on the fluidity and phase properties of microsomal membranes isolated from postclimateric Golden Delicious apples. *Plant Cell Physiology* **23**, 161–169.

Likens, G.E., Driscoll, C.T., Buso, D.C. (1996) Long-term effects of acid rain: Response and recovery of a forest ecosystem. *Science* **272**, 244–246.

Likens, G.E., Driscoll, C.T., Buso, D.C., Siccama, T.G., Johnson, C.E., Lovett, G.M., Fahey, T.J., Reiners, W.A., Ryan, D.F., Martin, C.W., Bailey, S.W. (1998) The biogeochemistry of calcium at Hubbard Brook. *Biogeochemistry* **41**, 89–173.

Long, R.P., Horsley, S.B., Lilja, P.R. (1997) Impact of forest liming on growth and crown vigor of sugar maple and associated hardwoods. *Canadian Journal of Forest Research* **27**, 1560–1573.

Lovett, G.M. & Hubbell, J.G. (1991) Effect of ozone and acid mist on foliar leaching from eastern white pine and sugar maple. *Canadian Journal of Forest Resources* **21**, 794–802.

Lynch, J., Cramer, G.R., Lauchli, A. (1987) Salinity reduces membrane-associated calcium in corn root protoplasts. *Plant Physiology* **83**, 390–394.

Mahony, N., Nol, E., Hutchinson, T. (1997) Food-chain chemistry, reproductive success, and foraging behaviour of songbirds in acidified maple forests of central Ontario. *Canadian Journal of Zoology* **75**, 509–517.

Mann, L.K., Johnson, D.W., West, D.C., Cold, D.W., Hornbeck, J.W., Martin, C.W., Riekerk, H., Smith, C.T., Swank, W.T., Tritton, L.M., Van Lear, D.H. (1988) Effects of whole-tree and stem-only clearcutting on postharvest hydrologic losses, nutrient capital, and regrowth. *Forest Science* **34**, 412–428.

Marschner, H. (1995) *Mineral Nutrition of Higher Plants.* Academic Press, New York.

McLaughlin, S.B., Anderson, C.P., Hanson, P.J., Tjoelker, M.G., Roy, W.K. (1991) Increased dark respiration and calcium deficiency of red spruce in relation to acidic deposition at high elevation southern Appalachian Mountain sites. *Canadian Journal of Forest Research* **21**, 1234–1244.

McLaughlin, S.B., Tjoelker, M.G., Roy, W.K. (1993) Acid deposition alters red spruce physiology: Laboratory studies support field observations. *Canadian Journal of Forest Research* **23**, 380–386.

McLaughlin, S.B. & Wimmer, R. (1999) Tansley Review No. 104, Calcium physiology and terrestrial ecosystem processes. *New Phytologist* **142**, 373–417.

Monroy, A.F., Sarban, F., Dhinza, R.S. (1993) Cold-induced changes in freezing tolerance, protein phosphorylation and gene expression: evidence for a role of calcium. *Plant Physiology* **102**, 1227–1235.

Moore, J.D., Camiré, C., Ouimet, R. (2000) Effects of

liming on the nutrition, vigor, and growth of sugar maple at the Lake Clair Watershed, Québec, Canada. *Canadian Journal of Forest Research* **30**, 725–732.

Ng, C.K.-Y., Carr, K., McAinish, M.R., Powell, B., Hetherington, A.M. (2001) Drought-induced guard cell signal transduction involves sphingosine-1-phosphate. *Nature* **410**, 596–599.

Nykvist, N. (2000) Tropical forests can suffer from a serious deficiency of calcium after logging. *Ambio* **29**, 310–313.

Palta, J.P. & Li, P.H. (1978) Cell membrane properties in relation to freezing injury. In: Li, P.H. & Sakai, A. (eds) *Plant Cold Hardiness and Freezing Stress.* pp. 93–115. Academic Press, London.

Pandey, S., Tiwari, S.B., Upadhyaya, K.C., Sopory, S.K. (2000) Calcium signaling: linking environmental signals to cellular functions. *Critical Review in Plant Science* **19**, 291–318.

Payette, S., Fortin, M-J, Morneau, C. (1996) The recent sugar maple decline in southern Quebec: Probable causes deduced from tree rings. *Canadian Journal of Forest Research* **26**, 1069–1078.

Pomeroy, M.K. & Andrews, C.J. (1985) Effects of low temperature and calcium on survival and membrane properties of isolated winter wheat cells. *Plant Physiology* **78**, 484–488.

Potter, C.S. (1991) Nutrient leaching from *Acer rubrum* leaves by experimental acid rainfall. *Canadian Journal of Forest Research* **21**, 222–229.

Rapport, D.J., Regier, H.A., Hutchinson, T.C. (1985) Ecosystem behavior under stress. *American Naturalist* **125**, 617–640.

Rapport, D.J. & Whitford, W.G. (1999) How ecosystems respond to stress. *BioScience* **49**, 193–203.

Richter, D.D., Markewitz, D., Wells, C.G., Allen, H.L., April, R., Heine, P.R., Urrego, B. (1994) Soil chemical change during three decades in an old-field loblolly pine (*Pinus taeda* L.) ecosystem. *Ecology* **75**, 1463–1473.

Roberts, D.M. & Harmon, A.C. (1992) Calcium-modulated proteins: Targets of intracellular calcium signals in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**, 375–414.

Roos, W. (2000) Ion mapping in plant cells—methods and applications in signal transduction research. *Planta* **210**, 347–370.

Salzer, P., Hebe, G., Reith, A., Zitterell-Haid, B., Stransky, H., Gaschler, K., Hager, A. (1996) Rapid reactions of spruce cells to elicitors released from the ectomycorrhizal fungus *Hebeloma crustuliniforme*, and inactivation of these elicitors by extracellular spruce cell enzymes. *Planta* **198**, 118–126.

Sanders, D., Browniee, C., Harper, J.F. (1999) Communicating with calcium. *The Plant Cell* **11**, 691–706.

Schaberg, P.G. & DeHayes, D.H. (2000) Physiological and environmental causes of freezing injury in red spruce. In: Mickler, R., Birdsey, R., Hom, J.L. (eds) *Responses of Northern U.S. Forests to Environmental Change.* pp. 181–227. Springer-Verlag, New York.

Schaberg, P.G., DeHayes, D.H., Hawley, G.J., Strimbeck, G.R., Cumming, J.R., Murakami, P.F., Borer, C.H. (2000) Acid mist, soil Ca and Al alter the mineral nutrition and physiology of red spruce. *Tree Physiology* **20**, 73–85.

Schaberg, P.G., DeHayes, D.H., Hawley, G.J., Strimbeck, G.R., Murakami, P.F., McNulty, S.G. (In press) Effects of experimentally induced N saturation on foliar membranes, cold tolerance, and carbon storage in montane red spruce. *Canadian Journal of Forest Research.*

Schaberg, P.G., Perkins, T.D., McNulty, S.G. (1997) Effects of chronic low-level N additions on foliar elemental concentrations, morphology, and gas exchange of mature montane red spruce. *Canadian Journal of Forest Research* **27**, 1622–1629.

Scherbatskoy, T. & Klein, R.M. (1983) Response of spruce and birch foliage to leaching by acidic mists. *Journal of Environmental Quality* **12**, 189–195.

Schulze, E.D. (1989) Air pollution and forest decline in a spruce (*Picea abies*) forest. *Science* **244**, 8–15.

Sheen, J. (1996) Ca^{2+}-dependent protein kinases and stress signal transduction in plants. *Science* **274**, 1900–1902.

Shortle, W.C. & Smith, K.T. (1988) Aluminum-induced calcium deficiency syndrome in declining red spruce. *Science* **240**, 1017–1018.

Steponkus, P.L. (1990) Cold acclimation and freezing injury from a perspective of the plasma membrane. In: Katterman F. (ed) *Environmental Injury to Plants.* pp. 1–16. Academic Press, New York.

Sutinen, M.-L., Arora, R., Wisniewski, M., Ashworth, E., Strimbeck, R., Palta, J. (2001) Mechanism of frost survival and freeze-damage in nature. In: Bigras, F.J. & Colombo, S.J. (eds) *Conifer Cold Hardiness.* pp. 89–120. Kluwer Academic Publishers, Netherlands.

Sverdrup, H. & Rosen, K. (1998) Long-term cation mass balances for Swedish forests and the concept of sustainability. *Forest Ecology and Management* **110**, 221–236.

Thimonier, A., Dupouey, J.L., Le Tacon, F. (2000) Recent losses of base cations from soils of *Fagus sylvatica* L. stands in northeastern France. *Royal Swedish Academy of Sciences* **29**, 314–321.

Tomlinson, G.H. (1993) A possible mechanism relating increased soil temperature to forest decline. *Water, Air, and Soil Pollution* **66**, 365–380.

Trewavas, A.J. & Malho, R. (1998) Ca^{2+} signaling in plant cells: the big network! *Current Opinions in Plant Biology* **1**, 428–433.

USDA (United States Department of Agriculture) (1991) *Results of the 1990 Dogwood Anthracnose Impact Assessment and Pilot Test in the Southern United States.* Protection Report R8-PR20. USDA Forest Service, Southern Region, Asheville, NC.

Yazvenko, S.B. & Rapport, D.J. (1996) A framework for assessing forest ecosystem health. *Ecosystem Health* **2**, 40–51.

Wesselink, L.G., Meiwes, K.J., Matzner, E., Stein, A.

(1995) Long term changes in water and soil chemistry in spruce and beech forests, Solling, Germany. *Environmental Science and Technology* **29**, 51–58.

Wilmot, T.R., Ellsworth, D.S., Tyree, M.T. (1994) Base cation fertilization and liming effects on nutrition and growth of Vermont sugar maple stands. *Forest Ecology and Management* **84**, 123–134.

Wilmot, T.R., Ellsworth, D.S., Tyree, M.T. (1995) Relationships among crown condition, growth, and stand nutrition in seven northern Vermont sugarbushes. *Canadian Journal of Forest Research* **25**, 386–397.

Wilson, D.M. & Grigal, D.F. (1995) Effects of pine plantations and adjacent deciduous forests on soil calcium. *Soil Science Society of America Journal* **59**, 1755–1761.

Xu, H. & Heath, M.C. (1998) Role of calcium in signal transduction during the hypersensitive response caused by basidiospore-derived infection of the cowpea rust fungus. *The Plant Cell* **10**, 585–597.