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Calcium fertilization increases the concentration of calcium in sapwood and calcium oxalate in foliage of red spruce

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ABSTRACT

Calcium cycling plays a key role in the health and productivity of red spruce forests in the northeastern US. A portion of the flowpath of calcium within forests includes translocation as Ca^{2+} in sapwood and accumulation as crystals of calcium oxalate in foliage. Concentrations of Ca in these tree tissues have been used as markers of environmental change due to acidic deposition or forest management practices. We compared the effects of Ca fertilization treatment on Ca concentration in wood and Ca and oxalate (Ox) concentration in foliage at two locations with different initial concentrations of Ca in the soil. We found greater amounts of Ca in wood from the high-Ca location than from the low-Ca location. Ca concentration was greater in wood formed in the 1970s than for wood formed in the 1980s, the outermost decadal band in these samples. The Ca-treatment was detected as an increased concentration of Ca in the 1970s and 1980s decadal bands. We also found that variation in Ca and Ox in foliage was essentially stoichiometric. The appearance and response to chemical tests of crystals in foliage were consistent with identification as calcium oxalate. The increased Ca in wood after Ca-treatment of the soil supports the use of dendrochemistry of base cations to investigate environmental change. However, differences in Ca concentration between the two outermost decadal bands of wood illustrate that internal processes of translocation and storage also affect Ca concentration. Calcium oxalate production in foliage diverts carbon from ordinary biosynthesis and energy-yielding processes. This sequestration, shedding, and decomposition of foliage may represent a significant and under-recognized contribution to carbon and Ca cycling.

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1. Introduction

Calcium is a crucial element in plant nutrition (Pilbeam and Morley, 2007). The health and productivity of red spruce (*Picea rubens* Sarg.) forests (Eagar and Adams, 1992) in the northeastern US has been linked to the biogeochemical cycling of Ca (Shortle and Smith, 1988; Borer et al., 2004; Lawrence et al., 1997). Competing ions such as Al are made more available by acidic deposition and can cause tree stress (Cronan and Grigal, 1995; Minocha et al., 1997) and reduce Ca availability through competition for ionic storage and exchange sites in the soil (Lawrence et al., 1995) and at the root tips of red spruce (Smith et al., 1995). Forest soils sensitive to disturbances in Ca biogeochemistry are low in base saturation and are naturally acidic, increasing Ca mobility and leaching.

Within trees, most Ca is in the form of the divalent cation Ca^{2+} that crosslinks cell wall materials, maintains the selective permeability of cell membranes, and acts as an enzyme cofactor and messaging molecule (McLaughlin and Wimmer, 1999). However, Ca is also found within trees in relatively insoluble crystalline form as both Ca oxalate and Ca carbonate (Katayama et al., 2008; Horner and Wagner, 1995). Under conditions of low availability of Ca, red spruce grow more slowly due to reduced potential for the crosslinking of cell walls and increased sensitivity to frost injury due to destabilization of cell membranes (DeHayes et al., 1999). In contrast, the metabolic challenge under conditions of calcium sufficiency is to regulate the comparatively large quantities of Ca in the apoplastic xylem sap (Smith and Shortle, 2001) while keeping low and finely controlled concentrations on cell membranes (Borer et al., 2004) and in the cytoplasm (Pilbeam and Morley, 2007).

When present in amounts greater than needed for metabolism, Ca^{2+} accumulates in plant foliage as salts of organic acids, particularly as crystals of calcium oxalate (CaOx). These crystals are minimally soluble in water and occur in a great variety of shapes and patterns of distribution in the foliage of a wide range of plant families (Lersten and Horner, 2008; Webb, 1999). Formation of CaOx plays a critical role in the regulation of Ca concentration and plant physiology (Franceschi and Nakata, 2005). The Ox anion is likely

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formed from ascorbic acid (Kostman et al., 2007; Nakata, 2003) and represents a diversion from normal energy-yielding or biosynthetic pathways (Debolt et al., 2007). Although some amount of CaOx formation appears to be constitutive, CaOx production can be enhanced by elevated amounts of Ca (Borer et al., 2004). This investment in CaOx has long been interpreted as providing protection from browsing herbivores (Büsgen and Münch, 1929) and also reduces the threat posed by the direct toxicity of Ca or of interference with metabolic processes that requires small, highly controlled amounts of Ca (Hirschi, 2004; Franceschi and Nakata, 2005).

In wood, most Ca^{2+} is bound to ion exchange sites, primarily to the anionic carboxylic acid groups of pectin and hemicellulose (Momoshima and Bondietti, 1990; McLaughlin and Wimmer, 1999). Patterns of Ca concentration in dated tree rings have been used as a record to infer previous periods of Ca mobilization in the soils of red spruce forests (Shortle et al., 1997).

Because of the sensitivity of red spruce forests to Ca availability and interest in the applicability of the dendrochemical record to assess changes in availability of Ca in forest soil, we tested: (1) the effect of Ca fertilization on sapwood Ca concentrations and (2) the effect of Ca fertilization on Ca and Ox concentrations in foliage at a high- and a low-Ca location.

2. Materials and methods

2.1. Study areas

Calcium concentration in wood and calcium oxalate (CaOx) concentration in foliage were determined for red spruce at two locations that differed in initial concentrations of soil calcium. The concentration of exchangeable Ca in the Oa soil horizon at the low-Ca location (Big Moose Lake, in the Adirondack Mountains of New York, 43° 49′N, 74° 53′W, elev. 570 m), was 6.4 $cmol_c\ kg^{-1}$ and at the high-Ca location (Groton State Forest, Vermont, 46° 16′N, 72°17′W, elev. 510 m) was 13.7 $cmol_c\ kg^{-1}$ (David and Lawrence, 1996). As part of a larger regional investigation of the biogeochemistry of red spruce forests, subplots at each location were fertilized. Details from the larger study on the fertilization treatments and the effects of fertilization on forest productivity have been published (Kulmatiski et al., 2007).

Three replicate 30 m × 30 m plots at each of the two locations were assigned to calcium and control treatments. Ca-treatment consisted of the application of 160 kg $year^{-1}$ of Ca as equimolar amounts of $CaCl_2$ and $CaSO_4$. For this study, the annual Ca-treatment was applied in three equal increments during the four growing seasons of 1992–1995.

2.2. Wood chemistry

Before fertilization in 1992, two increment cores were collected from each of two canopy dominant or codominant red spruce in the three Ca-treated and three untreated control plots at each location. The 48 increment cores collected in 1992 were air-dried, mounted, and surfaced using standard dendrochronological techniques (Stokes and Smiley, 1968). Ring widths were measured, crossdated, and the year of formation assigned. The decade of wood formation was marked along the length of each core. In 1995, after four growing seasons, two additional increment cores were collected from each of the trees sampled in 1992 and similarly processed. The resulting 96 cores were screened for the absence of visible microbial infection and the presence of sufficient wood to support chemical sampling. Pairs of cores from five trees for each combination of location and treatment (40 cores from 20 trees) were selected for calcium analysis. One core of each pair was collected before and the other core was collected after fertilization.

The calcium concentration was determined separately for the outer two decadal bands of wood using the method of Minocha and Shortle (1993). In brief, wood shavings for chemical extraction were removed from one or two holes bored in the center of the decadal bands formed in the 1970s and 1980s (Shortle et al., 1997). In all cases, the 1980s decadal band contained sapwood only, identified on the basis of its relative translucency and water-soaked appearance at the time of collection. The 1970s band was variable in type and could contain sapwood, heartwood, or the transitional zone between them. Sample shavings (30 mg) from the 1970s and 1980s decadal bands were extracted in 6 mL of 10 mM HCl and three freeze–thaw cycles (Minocha and Shortle, 1993). Ca concentration in extracts from cores collected in 1992 was determined by direct-current plasma–atomic emission spectroscopy (Beckman Spectroscan V ARL, Fullerton, CA) using the US Environmental Protection Agency's method 66-AES0029 (1986). Ca concentration in extracts from cores collected in 1995 was determined by inductively-coupled plasma–atomic emission spectroscopy (Varian Vista CCD, Palo Alto, CA). Comparative tests of the two methods of Ca analysis showed good agreement (data not shown).

Tests of skewness and kurtosis showed no marked deviation from normality of distribution among treatments or within each treatment combination. Variation in Ca concentration was analyzed using a repeated measures ANOVA with three main treatment factors (each with two levels): location (high-Ca, low-Ca soil), treatment (Ca-treated, untreated control), and wood position (1970s, 1980s decadal bands) and one repeated measures factor of timing (sample collected before or after fertilization treatment). Significance of the treatment factors and interactions were tested at $p < 0.05$. For significant treatments and treatment interactions, means were separated using the Bonferroni correction for multiple comparisons. All statistical analysis was performed using Systat version 10.2 software (Systat Software, Inc. Chicago, IL).

2.3. Foliar chemistry

To test the stoichiometric relationship of foliar calcium and oxalate, two canopy dominant or codominant red spruce were selected and sampled from the Ca-treated and untreated control plots at each location. Two sun-lit branches from trees in each combination of Ca-treatment and location were collected with a pole pruner. Needles formed during the current year and those formed during the previous two years were removed and kept separate. A portion of the needle collections was reserved for microscopy. Needles were pooled from both branches, oven-dried at 75 °C for 36 h, and ground to a fine powder. For Ca analysis, ground needles were wet-digested as described for the analysis of ground wood in Minocha and Shortle (1993). Ca concentration in needle digests were determined as for wood extracts, above.

Oxalate concentrations were determined by a modification of the procedure of Huang and Tanudjaja (1992). Ground needles were extracted with 1 M H_2SO_4 for 24 h in the dark. Extracts were diluted with water 20:1 (v/v), passed through a 0.2 mm filter, and analyzed by a high-performance liquid chromatograph (Hewlett-Packard 1050 with DAD detector, Palo Alto, CA) with a polystyrene divinylbenzene column (Bio-Rad Aminex HPX-87H, Hercules, CA) and a 12.5 mM H_2SO_4 mobile phase. Oxalate peak areas were integrated and compared to standard curves prepared for each analytical run.

Three statistical outliers (out of a total of 172 analyses of samples collected from 22 trees) with a studentized residual >3.5 were omitted from further analysis. The relationship of Ca and Ox concentration was tested by regression analysis of the means of the various treatment combinations. Variation in concentration of the constituent ions of calcium oxalate were analyzed by ANOVA with the four main treatment factors of ion (calcium, oxalate), location (high-Ca, low-Ca soil), treatment (Ca-treated, untreated control),

Table 1
Mean concentration (and standard error, $n = 5$) of calcium (mmol kg^{-1}) in the two outer decadal bands of red spruce wood in Ca-treated and untreated control plots at two forest locations that differ in initial concentration of soil Ca.

Timing	Wood position[a]	Big Moose Lake, NY (low-Ca soil)				Groton, VT (high-Ca soil)			
		Control		Ca-treated		Control		Ca-treated	
Before treatment	1970s	17.2	(1.1)	16.9	(0.3)	18.6	(1.2)	16.8	(0.9)
	1980s	14.7	(0.5)	13.7	(0.5)	16.6	(0.9)	15.5	(0.2)
After treatment	1970s	16.4	(0.9)	19.2	(1.5)	18.4	(1.2)	19.2	(0.7)
	1980s	16.0	(0.5)	15.0	(0.8)	15.9	(0.6)	17.9	(0.6)

[a] Position refers to decade of wood formation.

and needle age (current year, previous years) and their interactions. Significance of the treatment factors and interactions were tested at $p < 0.05$. For significant treatments and treatment interactions, means were separated using the Bonferroni correction for multiple comparisons.

2.4. Microscopy

Needles reserved for scanning electron microscopy (SEM) were air-dried, gold-coated, and viewed by SEM (Amray AMR-1000A, Bedford, MA) at 5 kV. Needles reserved for transmission electron microscopy (TEM) and light microscopy (LM) were placed in formalin:acetic acid:alcohol fixative at the time of collection (Berlyn and Miksche, 1976). Needles for TEM were post-fixed in 0.2% buffered glutaraldehyde and osmium tetroxide (Bozzola and Russell, 1992), embedded in resin, sectioned with an ultramicrotome, and viewed by TEM (Philips CM-10, Amsterdam, Netherlands). Free-hand sections of fixed needles were viewed by LM using bright-field and phase-contrast illumination in tests for the presence of calcium carbonate.

2.5. Crystal identification

Crystals in needle sections were identified as CaOx using several positive and negative tests. The alternative identification of the crystals as a carbonate salt was tested through LM observation of needle sections exposed to HCl. Bubble formation was presumed to indicate the presence of carbonate salts in the tissue. Ca salts were detected by exposing needle sections to EDTA (pH 7.2) or H_2SO_4 (V.R. Franceschi, personal communication). Disappearance of crystals through the formation of a chemical complex with EDTA and crystal dissolution followed by the formation of needle-shaped crystals of $CaSO_4$ were presumed to indicate the presence of calcium salts in the tissue. Oxalate in needles was detected using the oxalate oxidase spectrophotometric assay, measured at 590 mm (Horner et al., 2005).

3. Results

3.1. Wood chemistry

In the two outer decadal bands of red spruce wood, mean Ca concentration varied from 14–19 mmol kg^{-1} for all combinations of factors of forest location, sapwood position, Ca-treatment, and timing of sample collection (Table 1). The coefficient of variation ranged from 2% to 15% across all combinations of factors.

ANOVA of Ca concentration indicated significant effects of location (high-Ca soil > low-Ca soil, $p = 0.026$) and wood position (1970s > 1980s decadal band, $p < 0.001$) (Table 2). No interaction of treatment factors was statistically significant between subjects. The variation within subjects was significantly related to the repeated measure of the timing of sample collection (after Ca-treatment > before Ca-treatment) and the interaction of timing with Ca-treatment. Ca concentration was similar in sapwood from both

Table 2
Analysis of variance of calcium concentration in the outermost two decadal bands of red spruce wood from Ca-treated and untreated control plots at two forest locations that differ in initial concentration of soil Ca.

Source of variation	SS	df	MS	*F*	*P*
Between subjects					
Location	29.695	1	29.695	5.5	0.026
Treatment	0.042	1	0.042	<0.1	0.930
Wood position	93.442	1	93.442	17.2	<0.001
Location × treatment	0.088	1	0.088	<0.1	0.899
Location × wood position	2.606	1	2.606	0.5	0.494
Treatment × wood position	2.251	1	2.251	0.4	0.524
Location × treatment × wood position	12.324	1	12.324	2.3	0.142
Error	173.842	32	5.433		
Within subjects					
Timing	20.12	1	20.12	11.2	0.002
Timing × location	0.003	1	0.003	<0.1	0.967
Timing × treatment	24.376	1	24.376	13.6	0.001
Timing × wood position	0.102	1	0.102	<0.1	0.813
Timing × wood position × treatment	1.953	1	1.953	1.1	0.305
Timing × location × wood position	0.942	1	0.942	0.5	0.474
Timing × treatment × wood position	2.185	1	2.185	1.2	0.278
Timing × location × treatment × wood position	4.032	1	4.032	2.2	0.144
Error	57.427	32	1.795		

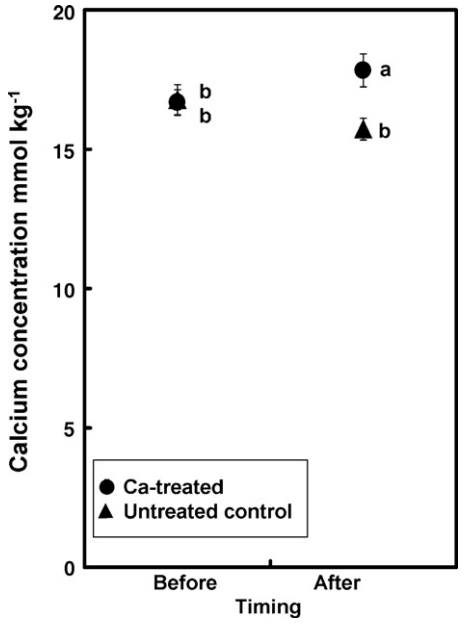

Fig. 1. Significant interactions of calcium treatment and the timing of sample collection identified by ANOVA of Ca concentration in red spruce wood (Table 2). Within each Ca-treatment, means marked by a different lower-case letter are significantly different ($p < 0.05$).

Table 3
Mean concentration (and standard error, $n = 7–12$) of calcium and oxalate (mmol kg^{-1}) ions in needles of red spruce in Ca-treated and untreated control plots at two forest locations that differ in initial concentration of soil calcium.

Ion	Needles[a]	Big Moose Lake, NY (low-Ca soil)				Groton, VT (high-Ca soil)			
		Control		Ca-treated		Control		Ca-treated	
Ca	Current	45	(6)	76	(7)	80	(4)	115	(8)
	Previous	76	(10)	111	(10)	146	(8)	227	(13)
Oxalate	Current	67	(5)	101	(8)	121	(8)	166	(8)
	Previous	77	(11)	110	(11)	173	(12)	232	(9)

[a] Needles formed during the current year and during the previous two years were analyzed separately.

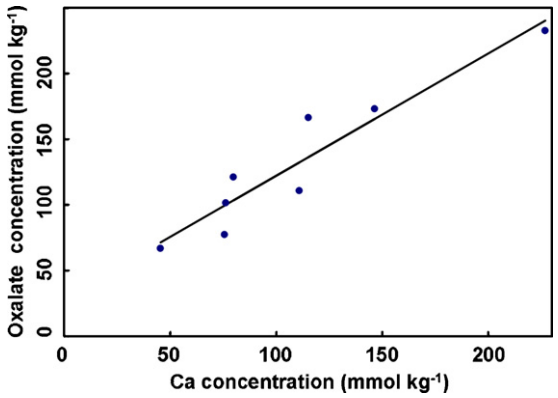

Fig. 2. Scatter diagram of concentration of calcium and oxalate in red spruce foliage. Each marker represents the mean of replicate observations for a combination of location, Ca-treatment, and needle age.

control and treated plots sampled before treatment and significantly increased in treated plots after treatment (Table 2 and Fig. 1).

3.2. Foliar chemistry

Concentration of the constituent ions of CaOx were determined for needles of red spruce grown in Ca-treated and untreated control plots (Table 3). The coefficient of variation ranged from 16% to 49% across all treatment combinations.

Regression analysis of the relationship between Ca and Ox concentration (Fig. 2) showed a strong linear relationship (adj. $R^2 = 0.88$, $p < 0.001$) described by the equation:

$$\hat{y} = 0.93x + 29.1$$

where $\hat{y}$ is the estimated Ox concentration and x is the measured Ca concentration.

Table 4
Analysis of variance of concentration for component ions of calcium oxalate in foliage of red spruce.

Source of variation	SS	df	MS	*F*	*P*
Ion	19023	1	19023	21.1	<0.001
Location	228626	1	228626	253.4	<0.001
Treatment	80731	1	80731	89.5	<0.001
Needle age	93224	1	93224	103.3	<0.001
Ion × location	3766	1	3766	4.2	0.043
Ion × treatment	53	1	53	0.1	0.809
Ion × needle age	7094	1	7094	7.9	0.006
Location × treatment	4791	1	4791	5.3	0.023
Location × needle age	28815	1	28815	31.9	<0.001
Treatment × needle age	2501	1	2501	2.8	0.098
Ion × location × treatment	108	1	108	0.1	0.730
Ion × location × needle age	142	1	142	0.2	0.693
Ion × treatment × needle age	853	1	853	0.9	0.332
Location × treatment × needle age	2017	1	2017	2.2	0.137
Ion × location × treatment × needle age	402	1	402	0.4	0.505
Error	138028	153	902		

Variation in the concentration of constituent ions of CaOx in red spruce needles was related to the main treatment factors of ion, location, treatment, and needle age (Table 4). Significant two-way interactions were also identified for four pairs of main treatments. The first two significant interactions depended on the identity of the ion. Ox concentration was significantly greater than Ca concentration at the high-Ca location but was not significantly different at the low-Ca location (Fig. 3A). Ox concentration was significantly greater than Ca in current needles, but not in needles formed in previous years (Fig. 3B). The last two significant interactions did not include the identity of the ion as a significant factor, so the concentration data for Ca and Ox were pooled. Ca-treatment increased the concentration of pooled Ca and Ox to a greater degree at the high-Ca location than at the low-Ca location (Fig. 3C). Older needles accumulated more pooled Ca and Ox at the high-Ca location than at

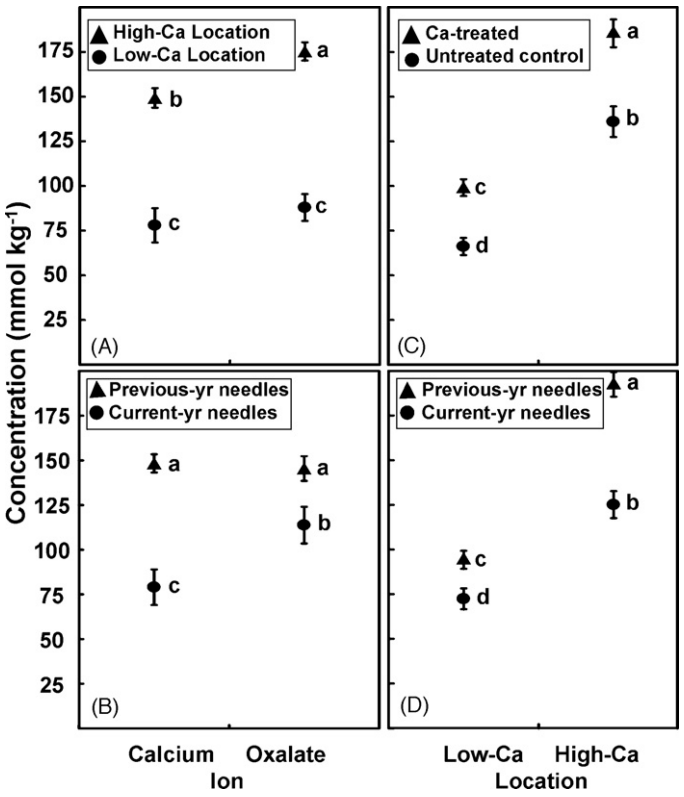

Fig. 3. Significant interactions identified by ANOVA in the concentration of component ions of calcium oxalate (Table 4). For each set of interactions, means marked by a different lower-case letter are significantly different ($p < 0.05$). (A) Ion × location (high-Ca marked by triangles, low-Ca marked by circles). (B) Ion × needle age (current-year needles marked by circles, previous-year needles marked by triangles). (C) Location × Ca-treatment (Ca-treated marked by triangles, untreated control marked by circles). (D) Location × needle age (current-year needles marked by circles, previous-year needles marked by triangles). Values in C and D are the pooled mean concentrations for calcium and oxalate.

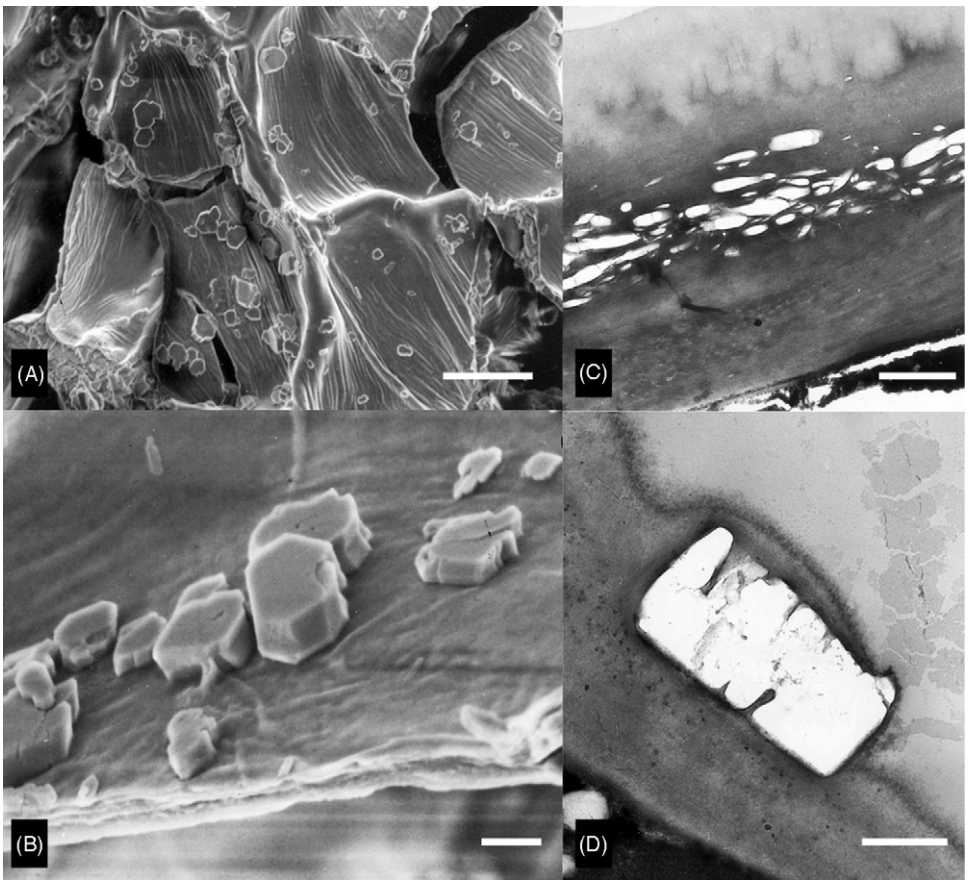

Fig. 4. Crystals inferred to be calcium oxalate in red spruce foliage. (A) SEM of crystals adhering to the outer walls of collapsed chlorenchyma cells (scale bar = 10 μm). (B) SEM of adhering crystals showing monoclinic structure (scale bar = 1.0 μm). (C) TEM of crystals embedded in the cuticle of the epidermal cell wall and beneath the epicuticular layer of wax (scale bar = 1.0 μm). (D) Crystal embedded in the wall of a chlorenchyma cell (scale bar = 0.25 μm).

the low-Ca location (Fig. 3D). None of the higher order interactions were significant (Table 2).

3.3. Microscopy and crystal identification

Microscopy of red spruce foliage indicated extensive deposition of crystals in the apoplast (Fig. 4A). The majority of crystals were monoclinic rectangular prisms (Fig. 4B). The inference that these crystals contained Ca was supported by dissolution with EDTA and with dissolution by H_2SO_4 followed by recrystalization of $CaSO_4$. Light microscopy showed no evidence of effervescence of crystals after application of HCl, indicating that the crystals were not calcium carbonate. Oxalate in needles was confirmed using the enzymatic test.

SEM showed crystals on the outside wall of needle chlorenchyma cells (Fig. 4A). TEM showed crystals within the cuticle and beneath the layer of epicuticular wax of epidermal cells (Fig. 4C). TEM also showed crystal deposits within the walls (Fig. 4D) and middle lamellae of chlorenchyma cells.

4. Discussion

Variation in initial concentration of soil Ca between the two locations was detected in the Ca concentration of the outer two decadal bands of wood. Variation in Ca concentration in healthy sapwood of red spruce depends on Ca concentration in xylem sap flowing through the wood, the presence of ion exchange sites that bind Ca, and hydrogen ion activity (expressed as pH) that affects the relative affinity of Ca ions to the exchange sites in the wood (Momoshima and Bondietti, 1990).

Red spruce showed significant environmental sensitivity with greater Ca concentration in wood from trees at the high-Ca location than from trees at the low-Ca location. We suggest that the differences in wood Ca concentration were due to the combined effects of higher Ca concentration and higher sap pH (Smith and Shortle, 2001) in trees at the high-Ca location relative to levels in the trees at the low-Ca location.

The outermost decadal band of wood (formed in the 1980s) contained less Ca than the adjacent decadal band (formed in the 1970s). This band, comprised exclusively of sapwood, contained a proportionately greater amount of living sapwood parenchyma than the adjacent band which contained a mixture of sapwood, heartwood, and the transitional layer between them. The more metabolically active outer band would likely participate to a greater degree in proton export into the xylem sap. This increase in hydrogen ion activity would decrease sap pH and reduce Ca binding.

The effect of Ca fertilization was recorded in the outer two bands of sapwood, supporting the concept that wood enrichment could be used as a record of changes in availability of soil Ca (Shortle et al., 1997). The timeline for this research was insufficient to test the stability of that record through wood maturity and heartwood formation (Meerts, 2002; Smith and Shortle, 2003).

The concentration of Ca in red spruce foliage was more highly variable than in the wood formed during the previous two decades. This may be due to variation in total hydraulic flux at the end of the transpiration stream (McLaughlin and Wimmer, 1999; Borer et al., 2004). The presence of dissociated ions such as Ca^{2+} may decrease hydraulic resistance (Zwieniecki et al., 2001) and increase hydraulic flux through the xylem and facilitate Ca accumulation in foliage.

The appearance and location of the crystals in the foliage were consistent with those described for CaOx by Fink (1991) in *Picea abies*. An especially rich area of crystal incorporation was the cuticular layer on the outer side of epidermal cells and beneath the fully differentiated, waxy cuticle. Also rich in crystals were the other walls of the chlorenchyma in the needle mesophyll. The presumptive chemical tests support the identification of the crystals as CaOx.

Foliar concentration of CaOx was greater in response to higher initial concentration of Ca in soil and to Ca-treatment. The near stoichiometric relationship of Ca and Ox in foliage above a minimum concentration of Ox indicated a dedicated diversion of plant metabolites, such as ascorbic acid, to sequester excess Ca. The *y*-intercept of about 29 mmol kg^{-1} of oxalate in the regression analysis with calcium concentration suggested that constitutive levels of soluble oxalate were maintained above levels needed for crystal formation (Franceschi and Nakata, 2005). Alternatively, the relationship between Ca and Ox may be nonlinear at concentrations lower than those observed here (Borer et al., 2004).

The flow of Ca within red spruce forests is complex and sensitive to changes in the chemical environment. The regulation of Ca flow crosses spatial scales from partitioning within individual cells, among tree tissues such as wood in various stages of maturity and decay, and in uptake from and return to forest soil. This cycling is tightly coupled to energy capture through protection from herbivory, allocation to Ca-intensive processes such as wood formation and membrane stabilization, and energy release during wood decomposition.

The formation of CaOx in foliage during conditions of Ca sufficiency protects cytoplasmic processes from Ca toxicity while providing a feedback mechanism that contributes to long-term availability of Ca in forest soil. As needles containing CaOx are shed, soil litter becomes enriched with a "slow release" form of Ca that is not immediately available for reuptake by red spruce. However, the Ca in CaOx is more likely to be retained in the forest floor for eventual uptake than other soluble forms of Ca that are more readily leached from the root environment (Graustein et al., 1977).

The CaOx contributed to the forest floor through the decomposition of foliage (Tait et al., 1999), ectomycorrhizal fungi (Cromack et al., 1979), and brownrot fungi during the wood decay process (Espejo and Agosin, 1991) also provides an energy source that supports the metabolism of soil-borne arthropods, bacteria, and actinomycetes (Daniel et al., 2007; Knutson et al., 1980). These soil-borne communities promote and stabilize the fertility of forest soils and enhance long-term carbon storage in the soil through the formation of carbonates (Verrecchia et al., 2006). Key biogeochemical processes in red spruce forests are linked through calcium and calcium ligands in wood and foliage.

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