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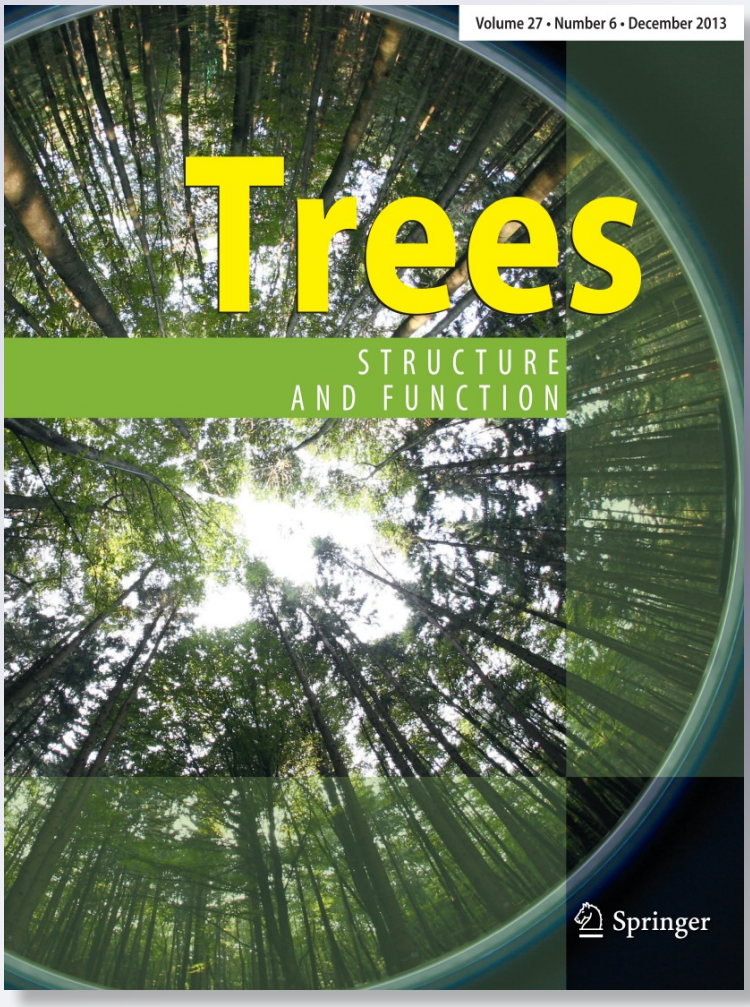

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ORIGINAL PAPER

Effects of soil calcium and aluminum on the physiology of balsam fir and red spruce saplings in northern New England

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Abstract We examined the influence of calcium (Ca) and aluminum (Al) nutrition on the foliar physiology of red spruce (*Picea rubens* Sarg.) and balsam fir [*Abies balsamea* (L.) Mill.] in northern New England, USA. At the Hubbard Brook Experimental Forest (NH, USA), spruce and fir saplings were sampled from control, Al-, and Ca-supplemented plots at a long-established nutrient perturbation (NuPert) study in fall 2008. Measurements included cation concentrations (roots and foliage), dark-adapted chlorophyll fluorescence (F_v/F_m), soluble sugar concentrations, and ascorbate peroxidase (APX) and glutathione reductase (GR) activity in current-year foliage. Additional untreated saplings were sampled from base-rich Sleepers River (VT) and base-poor Jeffers Brook (NH) for F_v/F_m and foliar nutrient concentrations. At NuPert, there were significantly greater Ca concentrations and Ca:Al ratios in roots from the Ca end vs. the Al end of the Al-control-Ca addition gradient. There were also trends toward greater foliar Ca and Ca:Al ratios and lower Al concentrations across the treatment gradient at NuPert and for foliage at Sleepers River vs. Jeffers Brook. At NuPert, F_v/F_m and APX activity increased across the treatment gradient, and red spruce was higher in these measures than balsam fir. These patterns were also observed when Jeffers Brook and Sleepers River were compared. Increased Ca availability appeared to enhance the ability of red spruce and balsam fir to repair oxidative stress damage, including photooxidation. Our findings support work indicating a greater contemporary level of stress for balsam fir relative to red spruce, which is surprising considering the well-documented regional decline of spruce.

Keywords *Picea rubens* · *Abies balsamea* · Chlorophyll fluorescence · Foliar cations · Soluble carbohydrates · Antioxidant enzyme activity

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Introduction

The northeastern US has received considerable inputs of acidic deposition in the past 50–100 years (Driscoll et al. 2001; McNulty et al. 2007). On poorly-buffered soils, this has led to the loss of significant amounts of calcium (Ca) and an increased availability of aluminum (Al) (Likens et al. 1998; DeHayes et al. 1999; Driscoll et al. 2001), which can be phytotoxic and competes with Ca for plant uptake (Marschner 2012). Calcium is an essential plant nutrient, and Ca depletion has been linked to declines in crown vigor and xylem growth and increases in mortality for multiple tree species in the northeastern US (Johnson and Andersen 1994; Johnson et al. 1994; Lawrence et al. 1997; DeHayes et al. 1999; Yanai et al. 2005; Schaberg et al. 2006; Bigelow and Canham 2007; Halman et al. 2008; Schaberg et al. 2011). Research conducted at the Hubbard Brook Experimental Forest (HBEF) in Thornton,

NH, has shown that at least some tree species have responded positively to a watershed-wide addition of Ca that was imposed in 1999 (Kobe et al. 2002; Fiorentino et al. 2003; Groffman et al. 2006; Hawley et al. 2006; Juice et al. 2006; Boyce 2007). Because HBEF contains a series of experimental watersheds, all located next to each other with similar vegetation and soils, it is an ideal location for this type of study. However, it is uncertain how results at HBEF reflect physiological responses to the broader range of Ca and Al nutrition that exists elsewhere in the Northeast.

Past work on the influence of Ca and Al on tree health and productivity has focused on two species that have experienced regional decline associated with acidic deposition in the northeast: red spruce (*Picea rubens* Sarg.) and sugar maple (*Acer saccharum* Marsh.) (e.g., DeHayes et al. 1999; Juice et al. 2006; Huggett et al. 2007; Halman et al. 2008). Foliar elemental levels and ratios have often been shown to be sensitive indicators of tree health and productivity for these species (e.g., McNulty et al. 1991, 1996; Aber et al. 1995; Kobe et al. 2002; Borer et al. 2004; St. Clair and Lynch 2004; St. Clair et al. 2005; Kulmaltiski et al. 2007). However, few studies have been conducted that evaluate the influence of cation nutrition on sympatric species experiencing both decline and no broad history of decline. In one of the few studies that compares the influence of Ca addition on the foliar physiology of a species in documented decline (red spruce) and one with no comparative decline [balsam fir; *Abies balsamea* (L). Carr.], Boyce (2007) found that balsam fir foliage showed greater evidence of stress than red spruce foliage–an unexpected result. That study found that Ca fertilization increased chlorophyll dark-adapted fluorescence (F_v/F_m) in both red spruce and balsam fir; however, spruce F_v/F_m was higher than that of fir (Boyce 2007), indicating that fir was more stressed than spruce. Currently, it is unknown if (1) apparent greater stress in balsam fir relative to red spruce is a widespread phenomenon, and (2) how Ca and Al nutrition may influence relative stress levels between these sympatric species.

A number of studies have shown that Ca plays an important role in plant stress response, photosystem function and carbohydrate metabolism (Bowler and Chua 1994; McLaughlin and Wimmer 1999; Snedden and Fromm 2001). Furthermore, in at least some species and locations, Ca addition can bolster these processes, thereby alleviating symptoms of decline (Hawley et al. 2006; Huggett et al. 2007; Halman et al. 2008; Schaberg et al. 2011). In particular, foliar sugar concentrations and the activities of certain antioxidant enzymes can provide protective benefits to foliage, but they are vulnerable to alteration following perturbations of Ca and Al nutrition (Halman et al. 2008). Soluble sugar buildups can increase cold tolerance and provide protection from winter freezing injury (Schaberg et al. 2000b). In addition, antioxidant systems break down reactive oxygen species (ROS) that are often created by environmental stresses such as photooxidative damage at low temperatures (Wise and Naylor 1987; Polle and Rennenberg 1994). Excess ROS that are not detoxified can result in severe cellular dysfunction (Foyer et al. 1994). The signal transduction pathways for many antioxidant enzymes require Ca (Bose et al. 2011), and Ca increases the activity of enzymes such as ascorbate peroxidase (APX) and glutathione reductase (GR) that help scavenge ROS (Jiang and Huang 2001; Jiang and Zhang 2003). In conifer species, APX activity is critical for tree health, especially, in the winter when temperatures frequently drop below freezing (Anderson et al. 1992).

The objective of the current study was to examine how a range in Ca and Al nutrition influences the physiology of red spruce and balsam fir in northern New England. Physiological assessments included chlorophyll fluorescence, soluble sugar concentrations, and the activities of APX and GR in current-year foliage, processes known to be Ca-sensitive. A broad range of Ca and Al nutrition was targeted by examining spruce and fir at two types of plots: (1) A replicated Ca- and Al-addition study at HBEF, and (2) two sites elsewhere in the region with naturally occurring differences in base cation nutrition.

Methods

Site characteristics and sampling

Chlorophyll fluorescence, cation and soluble sugar concentrations, and antioxidant activities of APX and GR were obtained from foliar samples collected in late October 2008 from 12 balsam fir and 12 red spruce saplings (<0.5 m tall) growing in four forest plot replicates of three treatments: (1) Al addition, (2) control (no treatment), and (3) Ca addition in the NuPert (Nutrient Perturbation) study at HBEF, which is 730–760 m in elevation and located west of Watershed 6 at 43° 57′, N 71° 45′ W (Berger et al. 2001; Phillips and Yanai 2004; Huggett et al. 2007). In 1995, the 12 forested plots (each 45 × 45 m) in NuPert were equally and randomly divided among the three treatments. Control plots have experienced a long-term reduction in soil Ca (Likens et al. 1998), whereas Ca addition was conducted to increase soil Ca above ambient levels, and Al addition was conducted to reduce Ca availability through competitive inhibition (Huggett et al. 2007). Treatment applications of $AlCl_3$ or $CaCl_2$ occurred in fall or spring during leafless periods. Annual additions of $CaCl_2$ were halted and replaced with a one-time application of wollastonite ($CaSiO_3$) pellets in 1999. Details of treatment applications are given in Huggett et al. (2007). At the time of sampling,

NuPert plots had received a total of 8.1 g Al m^{-2} or 48 g Ca m^{-2}. Treatments at NuPert were designed to produce a gradient in Ca and Al nutrition, ranging from the Ca-addition plots (highest Ca and lowest Al) to the Al-addition plots (lowest Ca and highest Al), with the control plots being intermediate in the availability of these cations. At this time of year, temperatures usually rose to >10 °C during the day but often fell below 0 °C at night, and leaves from the primarily deciduous canopy had fallen. All measurements were taken on the same samples. Due to the small size of NuPert foliar samples, shoots were bulked before analysis, resulting in a total of three samples per species per treatment plot (a total of 12 samples/species per treatment).

Additional data were collected in early November 2008 from Sleepers River in northeastern Vermont at 44°29′ N, 72°9′ W (Shanley et al. 2004; Mitchell et al. 2008). Elevation in the sampled catchment ranges from 519 to 686 m, and the site is underlain by a sulfidic calcareous granulite interbedded with micaceous phyllites and biotite schists, leading to base-rich, high-pH, bicarbonate sulfate waters. Thus, this site was expected to be much like the Ca-addition NuPert plots. Much of the forest cover is northern hardwoods (*Acer saccharum*, *Fagus grandifolia* Ehrh., and *Betula alleghaniensis* Britton), with balsam fir and red spruce making up <5 % of total cover (Mitchell et al. 2008). A total of 12 balsam fir and 12 red spruce saplings were sampled from an area dominated by these two species.

Data were also collected from an additional 12 red spruce and 12 balsam fir in November 2008 from Jeffers Brook, NH, on the west side of Mt. Clough, at 44°2′ N 71° 53.5′ W and an elevation of ~680–730 m. While considered more fertile than sites at HBEF (Bae et al. 2011), this site is less fertile than Sleepers River and therefore represents an intermediate nutritional measure. Saplings at both Sleepers River and Jeffers Brook were taller than at NuPert, usually 1.5–3.0 m tall.

Foliar and root cations

Following chlorophyll fluorescence measurements (see below) at all sites, current-year shoots were excised, sealed in plastic bags, and transported to the laboratory. As another measure of plant nutrition at NuPert, fine roots (<2 mm) of five red spruce and five balsam fir per treatment plot were carefully collected by hand in August 2009 and analyzed for cations. Roots were followed from the base of each sapling to ensure proper identification of individuals and species. Once exhumed, roots were wrapped in moist towels and placed in plastic bags for transport to the laboratory in a cooler. In preparation for cation analysis, roots were washed in distilled-deionized water three times to remove all soil and debris. Foliage and roots were dried for 2 weeks at 65 °C, ground to pass a 2-mm sieve, and digested by heating with nitric acid and hydrogen peroxide in a block digester (adapted from Jones and Case 1990). Digested foliar and root tissues were analyzed for total cations (Al, Ca, Fe, K, Mg and Mn) by inductively coupled plasma atomic emission spectroscopy (ICP-AES, PlasmaSpec 2.5, Leeman Labs, Lowell, MA, USA). Eastern white pine (*Pinus strobus* L.) needles from the National Bureau of Standards and Technology (SRM 1575) and sample duplicates and blanks were analyzed for procedural verification. Tissue standards were within 5 % of certified values.

Chlorophyll fluorescence

Dark-adapted chlorophyll fluorescence (F_v/F_m) was measured with an OS5-FL modulated chlorophyll fluorometer (Opti-Sciences, Hudson, NH, USA) on current-year foliage, following the procedure outlined in Boyce (2007). One shoot from each sapling was sampled by dark-adapting for 15 min before measurement.

Soluble sugar analysis

Foliar samples to be analyzed for foliar soluble sugar concentration (NuPert only) were stored on ice after collection in October 2008 for transport to the laboratory, freeze–dried, ground and stored at −80 °C until further analysis. Cuticular waxes were removed using hexane, and sugars were extracted in 80 % ethanol (Hinesley et al. 1992). Concentrations of fructose, glucose, raffinose, stachyose, sucrose, and xylose were determined as described by Schaberg et al. (2002) with a Waters HPLC equipped with an Alliance 2695 separations module, a 2414 differential refractometer, and a Waters Sugar-Pak column (Waters, Milford, MA, USA). Data were analyzed for individual and total sugar concentrations with Waters Empower Pro software and expressed as mg g^{-1} dry mass.

Ascorbate peroxidase and glutathione reductase activity

Foliar antioxidant data were obtained only from NuPert plots. Current-year foliage was excised in the field in October 2008, transported on ice to the laboratory and stored at −80 °C prior to enzyme analyses. The methods of Nakano and Asada (1981) were used to measure APX activity (μmol ascorbate min^{-1} mg^{-1}), based on spectrophotometric measurements (DU800 UV/VIS spectrophotometer, Beckman-Coulter, Inc., Fullerton, CA, USA) of the linear decrease in absorbance at 290 nm (extinction coefficient 2.8 mM^{-1} cm^{-1}) using a 1 mL reaction mixture of 50 mM potassium phosphate (pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, 0.15 mM H_2O_2, and 10 μL of

sample extract. Final activity was corrected by subtraction of ascorbate activity and non-enzymatic ascorbate breakdown. The methods of Smith et al. (1988) and Pell et al. (1999) were used to quantify GR activity (μmol TNB min^{-1} mg^{-1}) in a 1 mL reaction mixture consisting of 50 mM potassium phosphate (pH 7.8), 0.1 mM EDTA, 0.2 mM nicotinamide adenine dinucleotide phosphate (NADPH), 0.5 mM 5,5′-dithiobis 2-nitrobenzoic acid (DTNB), 0.2 mM glutathione oxidoreductase (GSSG), and 10 μL of sample extract. Linear increases in absorbance of DTNB reduced to GSH were measured at 412 nm (extinction coefficient 14.15 mM^{-1} cm^{-1}) for 120 s.

Statistical analysis

All statistics were performed using R (R Development Core Team 2012). Due to differences in sapling sizes and sampling dates, data from NuPert were analyzed separately from Jeffers Brook and Sleepers River. A split-plot analysis of variance (ANOVA) design was used for within-site comparisons at NuPert, where treatment (Al, control or Ca) was the whole-plot factor, and species (fir or spruce) was the split-plot factor. For comparisons between Jeffers Brook and Sleepers River, a two-way completely randomized ANOVA was used, where the two factors were site and species. In addition, linear correlations of balsam fir and red spruce combined were performed on F_v/F_m and the Al:Ca foliar ratio at all three sites; foliar APX activity was also compared to these two factors at NuPert. Because foliage was aggregated for cation and APX measurements at NuPert, data were aggregated by plot before correlation was performed.

Results

Foliar and root cations

There were significant differences in foliar cation concentrations at NuPert between balsam fir and red spruce for all elements tested except Cu and Fe (Table 1). Fir had lower Ca:Al ratios but higher Ca:Mn and Mg:Mn ratios. Across the treatment gradient (Al-control-Ca addition), i.e., from Al addition to control to Ca addition plots, only Mn showed a significant treatment-associated difference (a decline).

Table 1 Foliar cation concentrations and cation ratios (mass balance) of current-year balsam fir and red spruce foliage by treatment at NuPert, with standard deviations given in parentheses ($n = 12$)

Cation	Species	Al	Control	Ca	*P*-values	
					Species	Treatment
Mean concentration (mg/kg) per treatment						
Al	Fir	256.3 (13.1)	229.4 (49.1)	237.3 (41.6)	**<0.0001**	0.3629
	Spruce	56.5 (7.1)	48.6 (16.1)	45.4 (15.2)		
Ca	Fir	2,646.2 (59.4)	2,792.5 (212.1)	3,104.1 (671.2)	**<0.0001**	0.2689
	Spruce	1,066.3 (68.5)	943.6 (64.9)	1,069.6 (75.0)		
Cu	Fir	3.8 (0.7)	3.3 (0.1)	3.7 (0.2)	0.9999	0.8483
	Spruce	3.5 (0.4)	3.8 (0.8)	3.4 (0.3)		
Fe	Fir	36.8 (5.4)	43.8 (7.0)	39.4 (3.3)	**0.0018***	0.0750
	Spruce	28.2 (3.3)	36.7 (7.6)	40.2 (5.9)		
K	Fir	7,784.6 (398.5)	8,187.1 (701.9)	8,328.0 (738.2)	**0.0251**	0.1280
	Spruce	7,875.8 (841.7)	9,396.9 (2,163.2)	8,871.3 (412.8)		
Mg	Fir	982.7 (50.3)	941.0 (44.2)	1,002.7 (75.9)	**<0.0001**	0.1139
	Spruce	806.0 (25.8)	844.4 (76.5)	896.7 (21.6)		
Mn	Fir	593.4 (38.6)	535.9 (80.0)	406.2 (38.4)	**<0.0001**	**0.0019**
	Spruce	843.5 (118.1)	818.2 (107.8)	688.2 (39.7)		
Mean cation ratio (mass balance) per treatment						
Ca:Al	Fir	10.5 (0.6)	12.9 (2.9)	14.7 (4.8)	**<0.0001**	0.0938
	Spruce	21.5 (4.5)	27.0 (12.1)	33.2 (8.2)		
Ca:Mn	Fir	4.5 (0.3)	5.5 (1.4)	7.6 (0.9)	**<0.0001***	**0.0022**
	Spruce	1.3 (0.1)	1.2 (0.2)	1.6 (0.2)		
Mg:Mn	Fir	1.7 (0.1)	1.8 (0.3)	2.6 (0.3)	**<0.0001***	**0.0001**
	Spruce	1.0 (0.2)	1.1 (0.1)	1.3 (0.1)		

P-values for differences between species and treatments are shown in boldface for significant values; *asterisks* after species *P*-values indicate a significant species × treatment effect

Foliar Ca:Al increased across the treatment gradient, but statistical significance was only moderate ($P = 0.0938$, Table 1). Foliar Ca:Mn and Mg:Mn both increased significantly across the Al-control-Ca addition gradient; the significant species × treatment effects indicated that the amount of increase differed between species.

In contrast to foliar samples, root samples did not require bulking, thereby resulting in a slightly larger sample size (five per species and plot) for cation analysis. There were no differences between spruce and fir for Al, Ca, Mg, and the Ca:Al ratio; while Mn concentrations were similar in the Al-treated sites, they decreased in fir and increased in spruce in the control and Ca-treated sites (Table 2). Root Ca concentrations and Ca:Al ratios significantly increased in both species across the Al-control-Ca addition gradient. Interestingly, although changes in Ca:Mn and Mg:Mn ratios of spruce and fir across the Al-control-Ca addition gradient were not different, the trends were in opposite directions—fir concentration ratios increased while those of spruce decreased.

Foliar cation concentrations of fir and spruce at Jeffers Brook and Sleepers River showed interspecific differences that were similar to those seen at NuPert, with all cation concentrations showing significant differences (Table 3). When collection sites were compared, foliar Al, Fe and Mn concentrations were highest at Jeffers Brook (all but Mn significantly), while Ca and Mg were highest at Sleepers River. The Ca:Al, Ca:Mn and Mg:Mn ratios were all higher at Sleepers River, and significant species × site interactions indicate that the amount of increase across sites varied between species. An additional analysis was used to compare control samples of NuPert with those of Jeffers Brook and Sleepers River and showed that NuPert foliar nutrient levels and ratios did not differ significantly from Jeffers Brook (data not shown).

Chlorophyll fluorescence

At NuPert, there were significant differences among treatments ($P = 0.0288$), with a significant linear increase in F_v/F_m across the Al-control-Ca addition gradient ($P = 0.0083$; Fig. 1a). Balsam fir F_v/F_m values were also significantly lower than red spruce ($P < 0.0001$). This same pattern was observed when fluorescence values from the Ca-rich Sleepers River sites were compared to the Ca-poor Jeffers Brook site (Fig. 1b), although here the significant site × species interaction ($P = 0.0449$) indicated that balsam fir F_v/F_m was much lower than red spruce at Jeffers Brook than at Sleepers River.

Soluble sugar analysis

At NuPert, foliar sugar concentrations were significantly different between species with the exception of xylose. Although there were no foliar sugar differences observed across treatments, fructose and stachyose concentrations

Table 2 Root cation concentrations and cation ratios (mass balance) of balsam fir and red spruce saplings by treatment at NuPert, with standard deviations given in parentheses ($n = 5$)

Cation	Species	Al	Control	Ca	*P*-values	
					Species	Treatment
Mean concentration (mg/kg) per treatment						
Al	Fir	1,533.9 (216.4)	1,234.0 (527.4)	899.1 (456.7)	0.9999	0.1412
	Spruce	1,451.1 (619.4)	828.6 (151.2)	1,411.5 (1,015.5)		
Ca	Fir	2,829.4 (481.0)	2,715.0 (837.3)	4,135.4 (224.5)	0.3118	**0.0035**
	Spruce	2,552.3 (306.9)	3,207.6 (282.6)	3,418.7 (453.7)		
Mg	Fir	1,032.0 (53.1)	936.8 (201.4)	1,114.4 (198.2)	0.9999	0.7042
	Spruce	1,045.8 (165.1)	1,045.2 (90.7)	1,025.2 (132.8)		
Mn	Fir	499.7 (118.7)	370.9 (72.7)	397.6 (59.4)	**<0.0001***	0.4469
	Spruce	518.9 (108.0)	637.4 (210.3)	765.1 (132.0)		
Mean cation ratio (mass balance) per treatment						
Ca:Al	Fir	2.6 (0.4)	3.9 (2.4)	8.5 (5.4)	0.1738	**0.0338**
	Spruce	2.1 (0.9)	9.3 (9.6)	14.1 (6.3)		
Ca:Mn	Fir	6.1 (1.1)	8.5 (2.8)	11.9 (1.9)	**<0.0001***	0.0611
	Spruce	5.6 (2.0)	6.0 (1.5)	4.7 (1.1)		
Mg:Mn	Fir	2.5 (0.8)	2.9 (0.9)	3.2 (0.5)	**<0.0001***	0.9881
	Spruce	2.4 (1.0)	2.0 (0.5)	1.4 (0.3)		

P-values for differences between species and treatments are shown in boldface for significant values; *asterisks* after species *P*-values indicate a significant species × treatment effect

Table 3 Foliar cation concentrations and cation ratios (mass balance) of current-year balsam fir and red spruce foliage at Jeffers Brook (NH) and Sleepers River (VT), with standard deviations given in parentheses ($n = 12$)

Cation	Species	Jeffers Brook	Sleepers River	*P*-values	
				Species	Site
Mean concentration (mg/kg) per site					
Al	Fir	277.3 (57.3)	164.0 (67.7)	**<0.0001***	**<0.0001**
	Spruce	26.8 (7.3)	17.1 (6.6)		
Ca	Fir	3,847.8 (1,111.9)	5,580.0 (1,079.7)	**<0.0001***	**<0.0001**
	Spruce	1,378.1 (321.0)	1,850.4 (606.3)		
Fe	Fir	55.7 (9.8)	29.3 (6.6)	**0.0129**	**<0.0001**
	Spruce	42.7 (22.3)	22.5 (6.9)		
K	Fir	9,356.0 (1,213.3)	6,815.9 (850.5)	**<0.0001***	0.1117
	Spruce	9,189.5 (1,210.7)	10,721.9 (1,616.3)		
Mg	Fir	1,272.0 (155.6)	1,360.7 (280.5)	**<0.0001**	**0.0032**
	Spruce	892.6 (107.5)	1,181.4 (257.6)		
Mn	Fir	433.5 (91.4)	331.9 (134.0)	**<0.0001**	0.0927
	Spruce	677.8 (202.4)	632.3 (188.8)		
Mean cation ratio (mass balance) per treatment					
Ca:Al	Fir	14.2 (4.7)	39.2 (17.5)	**<0.0001***	**<0.0001**
	Spruce	55.4 (20.8)	121.9 (57.3)		
Ca:Mn	Fir	9.1 (2.7)	19.4 (8.6)	**<0.0001***	**<0.0001**
	Spruce	2.1 (0.7)	3.0 (0.7)		
Mg:Mn	Fir	3.0 (0.6)	4.5 (1.4)	**<0.0001**	**<0.0001**
	Spruce	1.4 (0.4)	2.0 (0.5)		

For Sleepers River spruce, $n = 11$. *P*-values for differences between species are shown in boldface for significant values; *asterisks* indicate a significant species × site effect

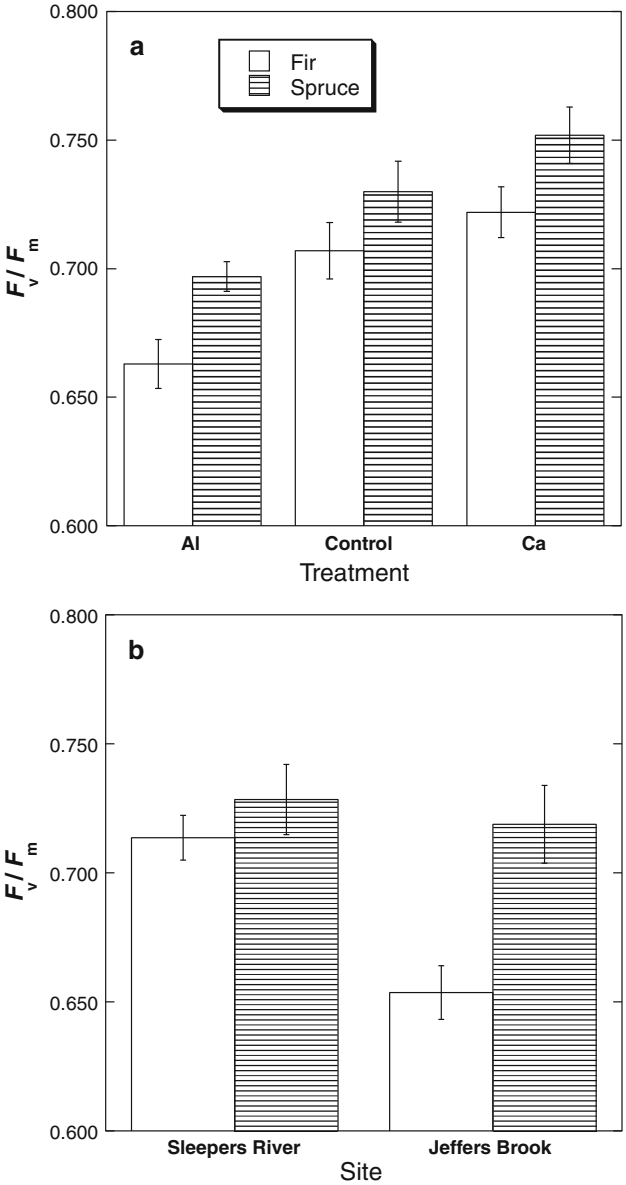

Fig. 1 Dark-adapted chlorophyll fluorescence (F_v/F_m) of fir and spruce saplings, **a** by treatment at NuPert, and **b** by site for Sleepers River and Jeffers Brook. Error bars are standard errors ($n = 12$)

were consistently lower in balsam fir when compared to red spruce, while glucose, raffinose, sucrose, and total sugar concentrations were higher in fir when compared to spruce; there were no species differences in foliar xylose (Table 4). No treatment effects were observed for any of the sugars.

Ascorbate peroxidase and glutathione reductase activity

Foliar APX activity in samples collected from NuPert was significantly higher in red spruce than balsam fir ($P = 0.0027$), and its activity increased across the Al-control-Ca addition gradient ($P < 0.0001$; Fig. 2). Similarly, GR activity was significantly higher in spruce than in fir ($P = 0.0234$; Fig. 3). However, there was no evidence of treatment differences ($P = 0.7278$).

Correlations among foliar measurements

At NuPert, F_v/F_m, foliar APX activity, and foliar Ca:Al ratios of balsam fir and red spruce were all strongly positively correlated with each other (Table 5). APX activity was not measured at Sleepers River or Jeffers Brook, but F_v/F_m and foliar Ca:Al ratios were also positively correlated at those sites.

Table 4 Foliar sugar concentrations from balsam fir and red spruce at NuPert), with standard deviations given in parentheses ($n = 12$)

Sugar	Species	Mean concentration (mg/g fresh weight) per treatment			*P*-values	
		Al	Control	Ca	Species	Treatment
Fructose	Fir	16.6 (3.1)	17.5 (2.3)	18.0 (1.8)	**<0.0001**	0.9204
	Spruce	42.7 (8.4)	43.3 (8.1)	41.1 (6.2)		
Glucose	Fir	73.5 (12.4)	74.8 (7.3)	73.7 (10.1)	**<0.0001**	0.8180
	Spruce	61.4 (11.7)	60.4 (6.3)	57.2 (4.3)		
Raffinose	Fir	18.9 (4.9)	20.3 (3.6)	20.7 (3.2)	**<0.0001**	0.8878
	Spruce	3.8 (1.1)	3.4 (1.5)	3.4 (1.4)		
Stachyose	Fir	0.0[a]	0.0[a]	0.0[a]	**<0.0001**	0.6496
	Spruce	2.0 (0.5)	1.9 (0.4)	2.1 (0.7)		
Sucrose	Fir	48.7 (9.8)	52.2 (5.7)	50.6 (5.5)	**<0.0001**	0.9066
	Spruce	16.4 (7.3)	14.2 (8.4)	18.5 (10.2)		
Xylose	Fir	0.46 (0.15)	0.40 (0.10)	0.41 (0.17)	0.1553	0.3299
	Spruce	0.39 (0.18)	0.99 (1.56)	0.43 (0.16)		
Total sugars	Fir	158.2 (26.5)	165.2 (13.3)	163.4 (10.4)	**<0.0001**	0.9638
	Spruce	126.7 (19.9)	124.2 (10.4)	122.7 (8.7)		

P-values for differences between species are shown in boldface for significant values

[a] Below detection

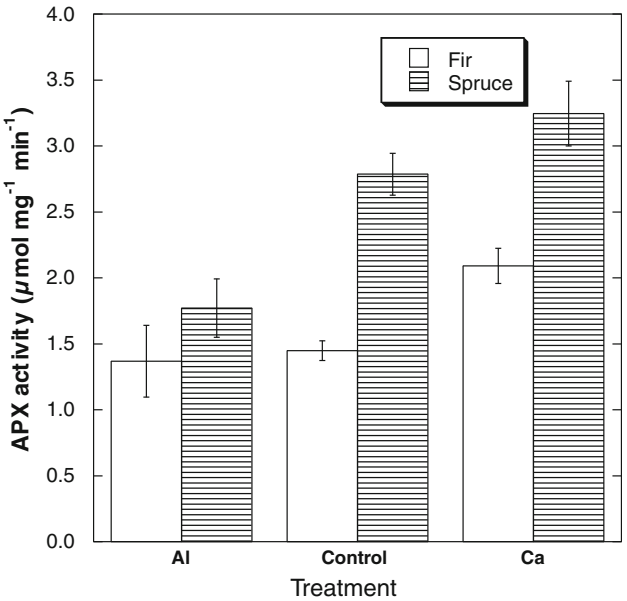

Fig. 2 Foliar ascorbate peroxidase (APX) activity (μmol mg^{-1} min^{-1}) of fir and spruce saplings at NuPert. Error bars are standard errors ($n = 6$)

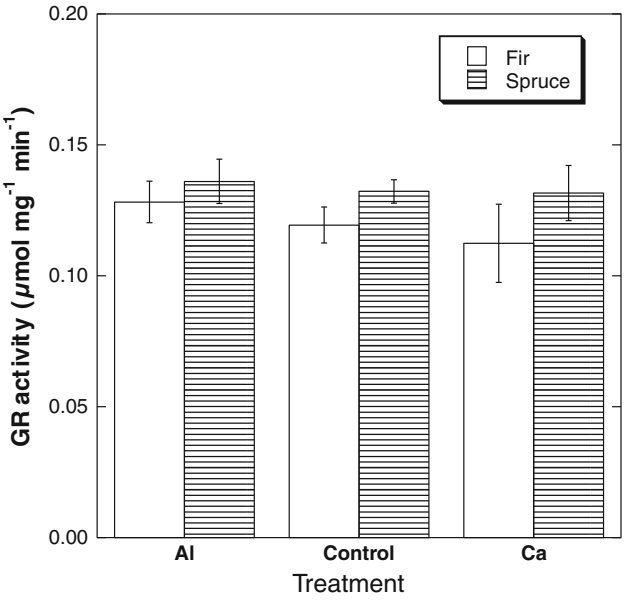

Fig. 3 Foliar glutathione reductase (GR) activity (μmol mg^{-1} min^{-1}) of fir and spruce saplings at NuPert. Error bars are standard errors ($n = 6$)

Discussion

Our study sites include both manipulated (NuPert; Al-control-Ca) and natural (Jeffers Brook and Sleepers River) gradients in Ca and Al nutrition, which were reflected in foliar Al and Ca contents and Ca:Al ratios (Jeffers Brook and Sleepers River: Table 3) and root Ca and Ca:Al ratios (NuPert: Table 2). Although overall trends in foliar nutrition mirrored those found in roots, with the exception of Mn, treatment differences for foliar cation means were not generally significantly different at NuPert, possibly because (1) transpirational uptake and incorporation of foliar cations was more limited for these understory saplings, and (2) foliar samples were partially bulked and had a slightly reduced sample size, leading to lower statistical power. Despite limited evidence of foliar

Table 5 Correlations among foliar measurements (combined for balsam fir and red spruce) at NuPert, Sleepers River, and Jeffers Brook

	NuPert		Sleepers river	Jeffers brook
	F_v/F_m	Ca:Al	F_v/F_m	F_v/F_m
Ca:Al	$r = 0.55$		$r = 0.46$	$r = 0.65$
	$\mathbf{P = 0.0053}$		$\mathbf{P = 0.0270}$	$\mathbf{P = 0.0015}$
	$n = 24$		$n = 23$	$n = 21$
APX	$r = 0.52$	$r = 0.67$	–	–
	$\mathbf{P = 0.0094}$	$\mathbf{P = 0.0003}$		
	$n = 24$	$n = 24$		

At NuPert, data were aggregated by plot before analysis; correlations were performed on individual saplings at Sleepers River and Jeffers Brook. *r* Pearson correlation coefficient, *P* associated *P* value, and *n* number of pairs in the correlation

cation differences, we found higher APX activity and F_v/F_m values with greater Ca and Ca:Al ratios associated with treatment (NuPert) or differences among native sites (Jeffers Brook and Sleepers River) for both balsam fir and red spruce. The correspondence of treatment-associated differences in root nutrition, particularly Ca and Ca:Al, and foliar physiology could indicate that changes in foliar function initiated from signals and responses at the root level. This pattern is particularly well-established for Al, which is either excluded from or sequestered in roots in an effort to reduce its phytotoxicity (Marschner 2012).

Calcium nutrition has been shown to have important physiological effects in red spruce. It acts as a second messenger in the development of chloroplasts (e.g., Bowler and Chua 1994; Bowler et al. 1997; Huang et al. 2012). Higher Ca concentrations have also been associated with greater APX activity in red spruce foliage (Halman et al. 2008). APX is an important antioxidant enzyme that repairs photooxidative damage. In the fall, when light levels are high but temperatures are falling, photooxidative damage is more likely, and higher APX activity is required for repair (Gilles and Vidaver 1990; Taiz and Zeiger 2002). Halman et al. (2008) have proposed that lower APX activity in the reference (low-Ca) watershed at HBEF in 2003 relative to the Ca-addition watershed may have contributed to the widespread winter injury to red spruce in the reference watershed. F_v/F_m levels are a sensitive indicator of stress (Baker 2008), and reactive oxygen species (ROS) are produced by high light and other stresses (Apel and Hirt 2004). F_v/F_m, APX levels, and foliar Ca:Al ratios are highly correlated at NuPert, which suggests that higher Ca levels allow greater repair of photooxidative damage. Since F_v/F_m is also a measure of potential photosynthesis, and Boyce (2007) found higher F_v/F_m levels at the Ca-treated HBEF watershed, this suggests that higher foliar Ca concentrations may ultimately lead to increased photosynthesis and growth in both red spruce and balsam fir.

While many of the findings in this study confirm those found by Halman et al. (2008) in the Ca-treated HBEF watershed, there are a number of differences. Although sampling occurred at about the same time of year, we did not find Ca effects on sugar levels at NuPert. While Halman et al. (2008) did not find any Ca effects on APX activity until midwinter; we found them in the fall. We sampled foliage from saplings in the understory, whereas Halman et al. (2008) sampled branches from mature trees in the upper canopy. Differences in maturity, light availability, and microclimate may be responsible for some variability among findings. For example, sugar concentrations may be more limited by light than Ca availability in the understory, while lower temperatures may be experienced first or more intensely in the upper canopy in the fall.

Both APX and GR are involved in the detoxification of oxidants, including those caused by excess light, but APX scavenging often occurs before GR is activated, and GR is not always needed at low oxidant levels (e.g., Polle and Rennenberg 1994). It is possible that APX levels in all the NuPert treatments were adequate for dealing with the levels of photooxidative damage encountered at the time of sampling, so GR levels were not responsive to differences in foliar Ca.

Not surprisingly, the Ca:Al ratios both at NuPert and Jeffers Brook-Sleepers River varied with treatment-induced or natural site-based gradients. Jentschke et al. (1991) found that Al additions displaced not only Ca but also Mg in seedlings of *Picea* abies (L.) Karst. While the Mg foliar concentrations increased from Jeffers Brook to Sleepers River, this was not seen across the Al-control-Ca addition gradient at NuPert (Tables 1 and 2). There were, however, foliar increases in the Ca:Mn and Mg:Mn ratios for both comparisons at the high-Ca sites. High foliar Mn has been shown by St. Clair and Lynch (2004) and St. Clair et al. (2005) to increase photooxidative stress in sugar maple. For both red spruce and balsam fir, lower values of these ratios are associated with lower values of F_v/F_m, indicating higher levels of photooxidative stress.

As noted in an earlier study (Boyce 2007), F_v/F_m values were consistently lower in balsam fir than in red spruce. This was also true for the foliar Ca:Al and Ca:Mn ratios and APX activity. These findings suggest that balsam fir appears more stressed than red spruce, which is surprising because spruce has experienced a well-documented decline throughout the northeastern US (e.g., Adams et al. 1992), whereas fir has not. Greater apparent stress for fir over time (Boyce 2007 vs. this study), space (HBEF watersheds (Boyce 2007) vs. Sleepers River and Jeffers Brook in this study), and a gradient of soil Ca and Al treatments (NuPert) highlight the consistency of this trend. Yet recent trends in

balsam fir basal area and aboveground net primary production (ANPP) in the region are mixed. In the Adirondacks of New York, balsam fir basal area has remained stable or increased from the 1980s to the 2000s (Battles et al. 2003; Bedison et al. 2007), while ANPP in the 1990s at one site decreased (Kulmatiski et al. 2007). Balsam fir basal area near the lower edge of the spruce-fir zone in the Green Mountains of Vermont has decreased since the 1960s, but it has increased at higher elevations (Beckage et al. 2008); at a lower elevation site in northeastern Vermont near Sleepers River, ANPP decreased in the 1990s. van Doorn et al. (2011) resampled 371 plots at the Hubbard Brook Experimental Forest in the White Mountains of New Hampshire 10 years after a 1995/96 survey and found an increase in balsam fir aboveground biomass, consistent with an increase in ANPP found by Kulmatiski et al. (2007) in the 1990s. Thus, the cause(s) for difference in stress level between balsam fir and red spruce is not readily apparent and has not yet appeared to consistently affect growth.

One possibility for contemporary differences in stress between the species is that, after decades of foliar winter injury and decline, remnant spruce may be benefitting from generally milder temperatures that reduce the possibility of winter damage and increase net photosynthetic gain and vigor (Kosiba et al. 2013). Red spruce has a unique capacity to reduce its cold tolerance and photosynthesizes when temperatures moderate during the traditional dormant season (Schaberg et al. 1995, 1998; Schwarz et al. 1997), increasing carbohydrate stores at a time when other species are losing C via respiration (Schaberg et al. 2000a). In addition to retaining extreme cold hardiness and associated low photosynthetic potential during the dormant season (Schaberg and DeHayes 2000; Strimbeck et al. 2008), balsam fir would likely increase its dark respiration as temperatures rise during the fall through spring (as seen in other boreal conifers; e.g., Ögren et al. 1997), putting this species at an added disadvantage. The proposition that warming cold season temperatures might be competitively advantageous to red spruce but potentially detrimental to balsam fir is consistent with the native ranges of these species (Little 1971). Red spruce is a temperate conifer with a more southerly range (suggesting a limitation by the cold), whereas balsam fir is a boreal conifer with a northern range (suggesting a competitive disadvantage in warmer climates). Although range limits and species-specific research are consistent with the possibility that increasing fall through winter temperatures could select for red spruce and against balsam fir, evidence for this in the field is currently lacking.

Another possibility is that balsam fir now has a new source of foliar injury and stress: herbivory by a resurgent moose population. Moose feed on fir but avoid spruce (Pastor et al. 1993; McLaren 1996; McLaren et al. 2000; Christenson 2004), and fir sampled at all sites showed signs of browsing. Browse may create a stress on fir saplings that spruce saplings avoid. Mature balsam fir trees that are tall enough to avoid browsing should be compared to saplings to determine whether this is responsible for the species difference seen in this study.

Broader comparative analyses are needed to determine if differences between these species are driven by improvements in red spruce vigor, increases in stress among balsam fir, or some combination of the two factors. Furthermore, the influences of climate, herbivory and other factors that may alter species health and productivity require specific analysis. Whatever the cause(s) of differences in physiological capacity of red spruce and balsam fir, indications of greater stress among fir on sites low in Ca and with greater Al availability suggest that the influences of cation nutrition on tree physiology extend beyond previously studied examples associated with red spruce, sugar maple and paper birch (*Betula papyrifera* Marsh.) trees (e.g., DeHayes et al. 1999; Juice et al. 2006; Halman et al. 2011).

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