

Regeneration ecology of sugar maple (*Acer saccharum*): seedling survival in relation to nutrition, site factors, and damage by insects and pathogens

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Abstract: The possible regeneration failure of sugar maple (*Acer saccharum* Marsh.) as part of their decline has been not well explored using field studies. We sought to clarify the roles of maternal effects and dynamics of early-season survival in contributing to the previously documented pattern of larger seedlings and higher seedling densities on a Ca-treated watershed (CAL) at Hubbard Brook Experimental Forest. We used a reciprocal seed planting experiment at four sites, two sites per watershed blocked by elevation. Regardless of maternity, sugar maple seedlings planted in CAL had higher survival than seedlings in the reference watershed. However, this advantage was not as clearly linked to the Ca amendment as in our previous work, probably, in part, because Ca availability has decreased over time. Maternal effects on seed chemistry and some seedling traits were detected, but these were not strong determinants of survival. Site was a good predictor of early seedling survival with litter layer depth, pathogen prevalence, and soil chemistry all contributing to the explanatory power of site. The strength of Ca-addition effects on sugar maple regeneration from seed depends on initial soil characteristics, application amounts, and interactions of the amendment with other factors such as leaf litter cycling, weather, and pathogens.

Résumé : La possibilité que des problèmes de régénération de l'éérable à sucre (*Acer saccharum* Marsh.) contribuent au dépérissement de cette espèce n'a pas suffisamment été explorée à l'aide d'études sur le terrain. Nous avons cherché à clarifier de quelle façon les effets maternels et la dynamique de la survie en début de saison contribuent au patron précédemment documenté et caractérisé par des semis plus gros et de plus fortes densités de semis dans un bassin versant traité avec du Ca à la forêt expérimentale de Hubbard Brook. Nous avons réalisé une expérience réciproque de plantation de semis dans quatre stations à raison de deux stations par bassin versant situées à différentes altitudes et considérées comme des blocs. Peu importe la maternité, les semis d'éérable à sucre plantés dans le bassin versant traité avec du Ca avaient un taux de survie plus élevé que les semis plantés dans le bassin témoin. Cependant, cet avantage n'était pas aussi clairement relié à l'amendement en Ca que lors de nos travaux précédents, probablement en partie parce que la disponibilité du Ca a diminué avec le temps. Des effets maternels sur les caractéristiques chimiques des graines et sur certains caractères des semis ont été détectés mais ils n'avaient pas une grande influence sur la survie. La station était un bon prédicteur de la survie initiale des semis et la profondeur de la couche de litière, la présence de pathogènes et les caractéristiques chimiques du sol contribuaient tous au pouvoir explicatif de la station. L'ampleur des effets d'un apport de Ca sur la régénération de l'éérable à sucre à partir de graines dépend des caractéristiques initiales du sol, de la quantité qui est appliquée et des interactions de l'amendement avec d'autres facteurs tels que le recyclage de la litière de feuilles, les conditions météorologiques et les pathogènes.

[Traduit par la Rédaction]

Introduction

Decline of sugar maple (*Acer saccharum* Marsh.) in eastern North America has been linked to Ca nutrition including effects of soil Ca on growth of mature trees (Long et al. 2009), saplings (Kobe et al. 2002), and seedlings (Juice et al. 2006). The balance of other base cations, particularly Mg, Mn, and Al, has also been shown to be important in sugar maple ecology (St. Clair et al. 2008) and there appear

to be critical thresholds for these elements (Long et al. 2009). The nature of soil chemistry effects on regeneration ecology of sugar maple remains incompletely understood, particularly in the early stages of seedling establishment in the forest.

In the late 1990s, sugar maple decline was documented in Hubbard Brook Experimental Forest (HBEF) in northern New Hampshire as evidenced by relatively slow growth rates and unusually high mortality of mature sugar maple

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trees (Siccama et al. 2007). In 1999, a watershed-scale study was initiated to investigate responses of the forest ecosystem to calcium silicate addition and return of soil Ca status and pH to preindustrial levels (Peters et al. 2004). The treatment was effective in raising soil pH, cation-exchange capacity, base saturation, and Ca status, particularly the Ca to Al ratios in the soil (Cho et al. 2010). Previously, Juice et al. (2006) demonstrated that seedlings and mature trees of sugar maple benefited from the Ca addition experiment in HBEF, including improved foliar Ca to Mn ratios and crown health of mature trees.

Juice et al. (2006) found marked differences in the density, survivorship, and growth of the 2003 cohort of maple seedlings in the Ca-treated watershed (CAL). However, the mechanisms underlying these patterns require clarification. In particular, higher seedling densities on Ca-treated soils (Juice et al. 2006) may have resulted from greater seed production, from higher germination rates, from maternal nutritional effects on the seed quality, from higher early-season survival of seedlings, or from a combination of these effects. Here, we combine long-term data on sugar maple seed production with a reciprocal planting experiment to address the following questions: (i) was there an increase in seed output on CAL, (ii) were seeds and seedlings from CAL sites larger and more likely to have balanced nutrient ratios for base cations, (iii) was emergence and survival highest for seedlings from CAL seed planted in CAL soil and lowest for reference seed planted in reference soil, and (iv) were there individual seedling traits that could be used to predict survival or did survival vary most strongly at the site scale? In addition, we discuss observations on the main agents of seedling mortality.

Methods

Study area

The HBEF is located in north-central New Hampshire, USA (43°56'N, 71°45'W). Detailed descriptions of climate, hydrology, topography, and vegetation of the HBEF are presented in Likens and Bormann (1995). Soils are moderately well-drained acid Spodosols (Haplorthods) of sandy-loam texture formed from glacial till. Thick (average = 6.9 cm) organic horizons, with pH ranging from 3.4 to 3.8, overlie bouldery mineral soil. Overstory vegetation is dominated by northern hardwoods: sugar maple, American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britt.), which comprise over 90% of the forest basal area. The HBEF is mostly second-growth forest developed following logging in the late 19th and early 20th century.

Calcium silicate treated watershed (CAL)

In October 1999 (soon after leaf fall), powdered and pelletized wollastonite (CaSiO₃) was applied by helicopter to Watershed 1 (hereafter referred to as "CAL") with a uniform delivery rate of 0.85 Mg Ca/ha (Peters et al. 2004). By summer 2002, the treatment had increased pH in the O_{ie} horizon of CAL to an average of 4.98 (Groffman et al. 2006). A smaller but significant increase was also observed in the O_a horizon (4.22), while no significant response was detected in the upper mineral soil (4.36; Groffman et al. 2006). The treatment effects on forest floor Ca availability

appeared to peak in 2002–2004 and may now be decreasing based on declining Ca in the foliage of spinulose wood fern (*Dryopteris carthusiana* (Vill.) H.P. Fuchs; J.D. Blum, personal communication (2010)) and declining forest floor pH (P. Groffman, unpublished data).

In November 2006 following a mast event, the reciprocal planting experiment was conducted between CAL, the calcium silicate treatment, and Watershed 3 (hereafter referred to as "REF"), an untreated reference watershed (Table 1). All of the seed germination and seedling ecology results are based on this experiment. However, the seed production and the seed chemistry results are based on the long-term monitoring efforts in CAL and in two nearby untreated forest stands (hereafter referred to as "litter sites"; see Seed collections section).

Seed rain and chemistry

As part of the long-term monitoring program at HBEF, we measure sugar maple seed rain using seed trap arrays (Fahey et al. 2005). Seed rain was estimated for an 11-year period (1998–2008) from 95 litter traps (0.1 m² catch area) positioned at three elevations: Low, 525 m (three sites); Mid, 585 m (three sites); and High, 725 m (two sites). The traps were located in three areas: the Ca-treated watershed (CAL) and two nearby long-term reference areas (litter sites): litter site 1 (Litter1, 1.25 km west of CAL) and litter site 2 (Litter2, 1 km west of CAL). In each year, all seeds and leaves were collected, sorted, and weighed from the baskets after full leaf-off in early November. To normalize for variation in sugar maple abundance, seed rain was expressed as the number of samaras per 100 leaves.

Seeds from these collectors from the 2006 sampling were also used to measure differences in seed chemistry between CAL ($n = 3$) and litter stands ($n = 5$) that spanned the elevation gradient (500–800 m). Seeds were dried, counted, weighed, and opened (scored for eaten, aborted, or filled) and the embryos were removed. The embryos were dried whole and weighed and then ground and processed for chemical analysis. Samples were powdered in a ball mill and analyzed for concentrations of Al, Ca, Fe, K, Mg, Mn, P, and Si following combustion of 0.1 g subsamples at 550 °C for 4 h. After adding 0.5 mL of 50% H₂O₂, samples were reheated to 550 °C for 2 h. Ash was dissolved in 5 mL of 50% ultrapure HNO₃ and brought to 10 mL final volume with distilled water. Solutions were analyzed by plasma spectroscopy (Spectro Analytical, Kleve, Germany).

Reciprocal planting experiment

In autumn of 2006, 18 experimental seed plots (1 m²) were established in each of four sites by collecting fallen seeds using mesh screens. The four sites were low elevation in CAL (525 m) and REF (560 m) and high elevation in CAL (730 m) and REF (725 m). Within each site, nine randomly located plots were planted with 100 seeds per plot for each seed source within elevations (e.g., plots at CAL-Low received seeds from CAL-Low (S1, $n = 9$) or from REF-Low (S3, $n = 9$)).

Seedling emergences

In early to mid-May 2007, all seedlings that emerged from the planted seeds in the plots were uniquely identified.

Table 1. Comparison of the two watersheds used in this study of sugar maple (*Acer saccharum*) seedling emergence and survival at Hubbard Brook Experimental Forest, New Hampshire.

	Watershed	
	CAL (Ca addition)	REF (reference)
Treatment	October 1999 Ca addition	None
Area (ha)	11.8	42.4
Slope (°)	18.6	12.1
Aspect	158° SE	203° SW
Elevation range (m)	488–747	527–732
Streamwater chemistry (average values 2003–2008)		
Ca (mg/L)	1.28	0.78
Sulfate (mg/L)	4.53	4.01
Nitrate (mg/L)	0.41	0.18
Dissolved Si (mg/L)	6.26	4.74

Note: Values were obtained from online data for the watersheds (<http://www.hubbardbrook.org/>).

Seedling specific data were taken at 14-day intervals for May–October 2007 (12 observation times), May–October 2008 (14 observation times), and three observation times in 2009 (mid-May, mid-July, and mid-October). At each observation time in 2007, leaf expansion class, leaf number, and leaf damage estimates (visual percentage) were recorded for each seedling. Leaf expansion stages were scored. Damaged leaves were inspected to determine the causal agent, which with time could be assigned to several categories. These complex data were simplified as either fungal or insect related for statistical analysis, but details of our observations and the prevalence of specific agents are provided in the Discussion.

In 2008, bud position (apical bud, top side bud, or lateral side bud), leaf size class, leaf number, and leaf damage class (1–4) and agents were recorded. For each seedling, we calculated the number of leaf-on days in 2007 and 2008. In May 2009, bud position, leaf number, and leaf expansion were recorded. In July 2009, only alive or dead was recorded, and in October 2009, leaf status was also noted.

Seedling collections

Seedlings were collected for plant dimensions, biomass, and chemical analyses from CAL and REF. In July 2007, 10 seedlings were collected from each of eight stands in CAL and six in REF where seedlings were being tracked for a concurrent project (N.L. Cleavitt et al., in preparation). In CAL, three stands were near CAL-High, four stands were near CAL-Low, and the eighth stand was at midelevation (630 m). In REF, three stands were near REF-High, two were near REF-Low, and the sixth stand was at midelevation (625 m). Whole seedlings were pooled by stand, dried at 60 °C for 48 h, finely ground, and analyzed by the same methods as for the seed embryos (described under Seed collections section above).

In both 2007 and 2008, seedling final leaf size was measured in 10 size classes and converted to square centimetre values by creating a standard curve for leaf area. In October 2007, stem diameters were measured just below the cotyledon scar for 50 seedlings per site. In October 2008, seedling diameters were taken for all surviving seedlings and stem elongation in 2007 and 2008 was also measured.

Plot traits

At CAL-Low and REF-Low, plot traits assessed included litter depth and composition, nearest canopy maple to plot center, and number and identity of herb species present in plots. Litter depth and leaf composition were recorded at each of four sides of every plot in June 2007.

Statistical analyses on group comparisons

To test for differences in seed rain, a generalized linear model including site within location (CAL, Litter1, and 2) as a random effect and year and elevation as fixed effects was used to test for main effects and the possible interaction between elevation and year. The model was run in SPSS version 18 for Windows. To examine differences in seed and seedling chemistry, we used two-tailed *t* tests on the group means.

Seedling initial development stage, seedling emergence, and all other individual seedling traits were compared with a linear mixed model to accommodate the unbalanced nature of different numbers of seedlings in each plot (SPSS 2005). The model included site and seed source as main fixed effects with their interaction. Plots nested within site were included as a random effect, and the significance of plot was determined by comparing the difference in $-\log$ -likelihood for a model (linear mixed model) with and without this effect included against the χ^2 distribution ($df = 1$). For the counts of emerged seedlings, the count data were square root transformed prior to analysis. All linear mixed models had unstructured covariance and were run in SPSS version 18 for Windows.

Survival analysis

We used nonparametric maximum likelihood estimators to quantify the survivorship of sugar maple seedlings for each treatment and then compared survival curves using weighted log-rank tests. For practical reasons, we could not determine the exact date of death of the seedlings in this longitudinal field study. Thus, our data included two kinds of censoring. Some seedlings ($n = 96$) survived the entire 3 years (right censored). For the remainder ($n = 2216$), we knew only the interval in which they died (i.e., interval censoring). As noted above, the length of the intervals ranged

from 14 days during the growing season to more than 5 months over the winter.

We followed the recommendation of two recent reviews of the analysis of interval-censored data (Gómez et al. 2009; Fay and Shaw 2010) and used Turnbull's (1976) generalization of the Kaplan–Meier estimator to calculate the survival functions of sugar maple seedlings from the different treatments. To compare survival curves, we used weighted log-rank tests that employ a permutation procedure when there are many samples (as in our case). Survival analyses were implemented in R statistical language (<http://www.r-project.org/>) using the “interval” statistical library provided by Fay (2010).

Logistic regressions for seedling traits related to survival

Logistic regressions were used to examine seedling traits related to survival. Full models including site, seed source, seedling traits, and their two-way interactions were run using conditional forward selection. Significant variables in the model were determined based on Wald statistics (the ratio of estimated coefficient and standard error squared) (Hosmer and Lemeshow 1989). Seedling traits included for survival overwinter 2007–2008 were leaf expansion stage in May 2007, leaf expansion size in August 2007, leaf area damaged (percentage) in late August 2007, leaf lost (binary), and the leaves-on days. Seedling traits included for survival into October 2009 were leaf size in August 2008, seedling diameter in October 2008, bud position in 2008, stem length grown in 2007, stem length grown in 2008, number of leaves in 2008, and leaf-on days in 2008. Seedling traits were examined for collinearity following the recommendations of Hosmer and Lemeshow (1989). Logistic regressions were run in SPSS version 18 for Windows.

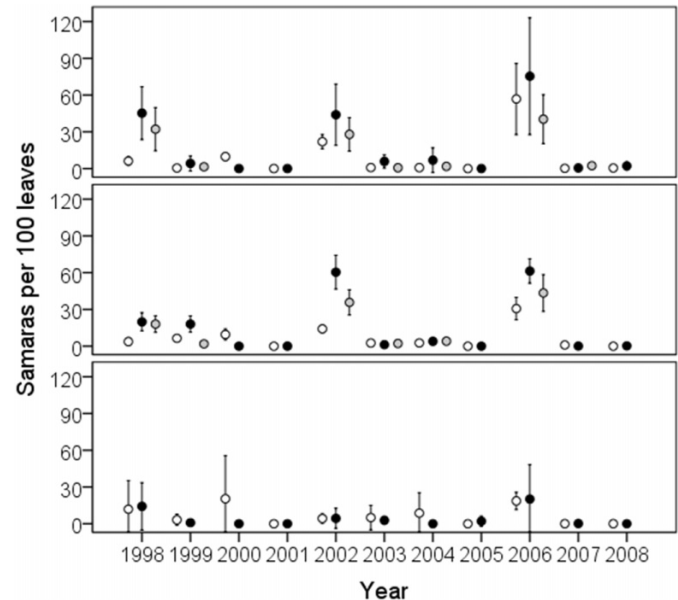
Results

Seed rain and chemistry

We observed mast events for sugar maple at HBEF in 1998, 2002, and 2006. These events varied considerably across stands and between years, with the 2006 mast event being of greater magnitude than the 1998 and 2002 events (Fig. 1). Samara production in nonmast years averaged 2.4 ± 2.5 of leaf litter production (per 100 leaves) across stands. The three mast years were greatest at the low (39.4 ± 17.2) and midelevations (32.5 ± 16.3) and less than half this amount at high elevation (12.2 ± 7.5). A generalized linear model comparing seed rain data from CAL and Litter1 (note that Litter 2 was missing high-elevation data) detected significant differences between years ($\chi^2 = 327.4$, $df = 10$, $p < 0.001$) and elevations ($\chi^2 = 29.1$, $df = 2$, $p < 0.001$), but there were also significant interactions between years and elevations ($\chi^2 = 100.4$, $df = 20$, $p < 0.001$) and no conclusive evidence of a Ca effect (Fig. 1).

The Ca treatment resulted in seeds that were chemically different from seeds from the litter sites (Table 2). Seeds from the three CAL sites together had significantly lower concentrations of Al, K, and Mg and significantly higher concentration of Si (Table 2). However, there was no significant difference in seed size, nor was there a difference in Ca concentration. In general, seed traits in CAL were more variable than in the litter sites (e.g., the coefficient of varia-

Fig. 1. Sugar maple (*Acer saccharum*) seed rain from litter basket collections from three areas across three elevations. The three areas are Watershed 1 (CAL, Ca addition area) and two untreated litter collection areas Litter1 and Litter2. Litter2 does not have sugar maple present on the site at high elevation, accounting for the lack of gray points in this part of the graph. Error bars show ± 1 SE from the mean.



tion of Ca concentration was 22% in CAL versus 4% in the litter sites) (Table 2).

Seedling emergence

In all, 2312 seedlings were tracked across three of the four sites (out of 5400 planted seeds, 43% of seeds produced seedlings with an upright hypocotyl that could be tagged). At the fourth site, high elevation on the reference watershed, all seedlings were dead by the third survey time (June 2007).

There were significant site differences in emergence by watershed ($F_{3,32} = 38.87$, $p < 0.001$) with higher emergence at CAL-Low for both seed sources (Fig. 2). The maternal (seed source) effect on seedling emergence was also significant ($F_{1,104} = 4.78$, $p = 0.031$) and was consistent across sites, with REF seed always slightly lower than CAL seed (Fig. 2). There was also significant variation between plots within sites ($\chi^2 = 23.104$, $df = 1$, $p < 0.001$). We detected no interaction between site and seed source. Of the plot traits measured, only litter depth varied between the sites ($t_{2,34} = -3.262$, $p = 0.003$). For the low-elevation sites, the litter layer in REF was nearly twice as deep as in CAL. Note that differences in emergence at the plot scale were negatively correlated with litter layer depth (Pearson's $r = -0.545$, $df = 36$; $p = 0.001$) (Fig. 2 inset).

Seedling chemistry and growth

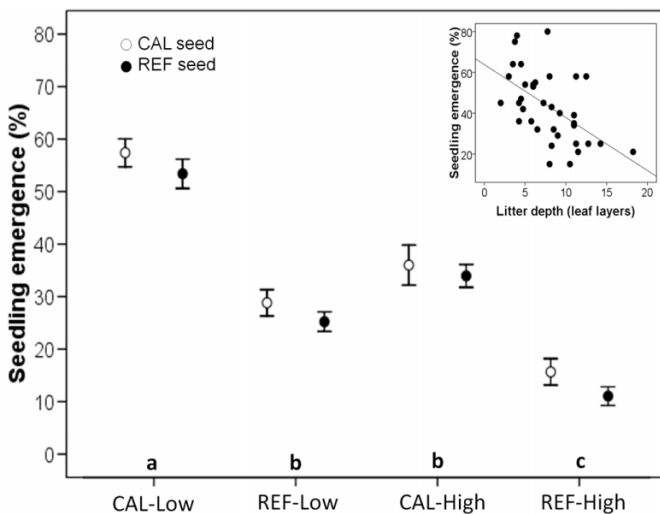
The Ca treatment resulted in some differences in seedling chemistry (Table 3). Seedlings from CAL had significantly lower Mg concentration and higher Si concentration (t tests between sites, all $p < 0.003$) (Table 3). Soon after emergence in May 2007, seedling development stages were best

Table 2. Comparison of sugar maple (*Acer saccharum*) seed traits and chemistry for seeds collected in October 2006 from CAL (CaSiO₃-treated watershed) and reference areas at Hubbard Brook Experimental Forest, New Hampshire.

Seed variable	Seed source	
	CAL (Ca addition)	Litter sites
Seed mass (mg)	113.1 (17.6)	103.6 (4.5)
Embryo mass (mg)	51.1 (8.1)	40.9 (5.6)
Embryo (% of seed mass)	45.1 (0.1)	39.6 (5.6)
Filled seeds (%)	46.0 (4.5)	43.2 (5.0)
Al (μg/g)*	2.29 (0.32)	4.76 (1.59)
Ca (μg/g)	4789 (1059)	4364 (186.8)
Fe (μg/g)*	50.53 (1.80)	48.42 (4.10)
K (μg/g)*	15297 (1034)	17197 (601.8)
Mg (μg/g)*	2647 (222.3)	3110 (53.0)
Mn (μg/g)	493 (73.6)	528 (98.9)
P (μg/g)	6988 (230.2)	7534 (555.8)
Si (μg/g)*	105.2 (7.9)	34.0 (7.9)

Note: An asterisk indicates a significant difference ($p < 0.05$) between the seed sources for those variables, and values are presented as mean (1 SD). Seeds were pooled within collections areas to obtain sufficient mass for the analyses, and sample size for collection areas was $n = 3$ sites, average number of seeds used to represent a site = 95 ± 37 for CAL and $n = 5$, seed average = 109 ± 63) for litter sites.

Fig. 2. Sugar maple (*Acer saccharum*) seedling emergence in relation to seed source, site, and litter depth (inset) in a northern hardwood forest, New Hampshire. The litter depth data are from the two low-elevation sites. CAL and REF are abbreviations for Ca added and reference watersheds, respectively. Low and High refer to the elevation classes. Error bars show ± 1 SE from the mean ($n = 9$). The letters above the site abbreviations denote significant differences between sites.



described by differences between sites ($F_{3,32} = 4.17, p = 0.032$) and plots within sites ($\chi^2 = 5.339, df = 1, p < 0.025$) (Fig. 3). There was no significant maternal effect or interaction term. The average life stage was significantly advanced in both CAL sites relative to either of the REF sites and REF-High was significantly behind the other three sites (Fig. 3). In both 2007 and 2008, stem diameters were highly correlated with leaf sizes (2007: $n = 152$, Pearson's $r =$

Table 3. Comparison of sugar maple (*Acer saccharum*) seedling traits and chemistry for seedlings collected in late spring (June to early July) 2007 from CAL (CaSiO₃-treated watershed) and REF (reference watershed) at Hubbard Brook Experimental Forest, New Hampshire.

Seedling variable	Seedling source	
	CAL (Ca addition)	REF (reference)
Stem mass (mg)	25.9 (9.4)	22.0 (6.8)
Leaf mass (mg)	51.3 (18.7)	38.8 (14.4)
Root mass (mg)	10.9 (3.4)	10.4 (1.2)
Root:shoot (mass)	0.47 (0.23)	0.51 (0.18)
Al (μg/g)	254.6 (156.9)	118.7 (100.9)
Ca (μg/g)	7446 (1789)	6434 (793.6)
Fe (μg/g)*	336 (217)	93.9 (22.4)
K (μg/g)	9506 (1152)	10800 (1676)
Mg (μg/g)*	1442 (221.5)	2307 (174)
Mn (μg/g)	481 (221)	736 (386)
P (μg/g)*	2443 (553)	3226(604)
Si (μg/g)*	648 (230)	166 (108)

Note: An asterisk beside a variable indicates a significant difference between the seedling groups ($p < 0.05$; see text for statistic details). Seedling mass and chemistry values are derived from the pooled samples of 10 seedlings (separate collections for dry masses and chemistry) from each collection area to obtain sufficient mass for the analyses, and sample size for collection areas was $n = 8$ for CAL and $n = 6$ for REF. Values are presented as mean (1 SD).

0.717, $p < 0.001$; 2008: $n = 148$, Pearson's $r = 0.619, p < 0.001$).

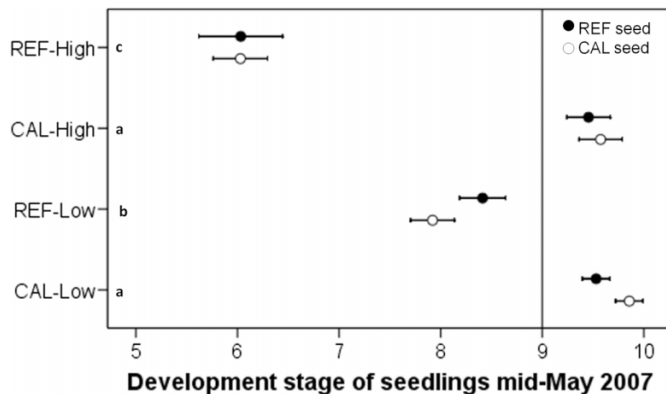
Seedling survival

The general pattern of the survivorship curve for the first growing season shows high mortality between emergence and mid-July (Fig. 4). This time encompassed the early developmental stages leading to full expansion of the primary leaves such that 68.5% of the seedlings had reached primary leaf expansion at the time of greatest seedling mortality and there was about a 40% chance that a seedling would live to late June (Fig. 4). By August when the seedlings had dropped their cotyledons and 99% of the population had reached full primary leaf expansion, mortality leveled out and remained at 20% probability of survival through the leaf-off period (Fig. 4).

Three-year survivorship varied significantly by site (weighted log-rank tests for survival analysis, $p < 0.001$), with both the watershed ($p < 0.002$) and elevation ($p < 0.001$) being significant predictors (Fig. 5). Survivorship was higher in the CAL sites and at lower elevation (Fig. 5). Seed source was not a significant predictor of survival and the curves almost completely overlap (Fig. 5 inset). Late-autumn and overwinter mortality were higher in the first year than in subsequent years (Fig. 5).

The best logistic regression model for predicting survival over the 2007–2008 winter (model fit: $\chi^2 = 107.91, df = 3, p < 0.001$) included leaf area damaged by August 2007 (Wald: 62.69, $df = 1, p < 0.001$), number of days with green leaves in 2007 (Wald: 29.82, $df = 1, p < 0.001$), and leaf stage at initial observations in May 2007 (Wald: 23.97, $df = 1, p < 0.001$). Seedlings that died were at younger stages at the first observation time and had greater leaf damage and a shorter season with leaves on (Fig. 6). Neither site nor seed

Fig. 3. Development stages of sugar maple (*Acer saccharum*) seedlings on the first observation dates (11–15 May 2007) in a northern hardwood forest, New Hampshire. The reference line at stage 9 denotes the first stage of primary leaf expansion. CAL and REF are abbreviations for Ca added and reference watersheds, respectively. Low and High refer to the elevation classes. Error bars show ± 1 SE from the mean ($n = 9$). The letters beside the site abbreviations denote significant differences between sites.



source was a significant predictor of overwinter survival in the model.

For the third season, the model for predicting survival during the overwinter period and through the 2009 season (model fit: $\chi^2 = 33.00$, $df = 2$, $p < 0.001$) included the number of leaf-on days (Wald: 15.41, $df = 1$, $p < 0.001$) and leaf size in 2008 (Wald: 16.31, $df = 1$, $p < 0.001$). Seedlings that died had smaller leaves (average size 7.62 ± 1.08 cm²) than those that lived (10.33 ± 0.97 cm²) and leaves that were on for less time (122 ± 15 days) than those that lived (131 ± 11 days). Neither site, seed source, nor any of the variables from the 2007 season was relevant to survivorship in the third year.

Discussion

Sugar maple seedlings planted in CAL had a better chance of survival than seedlings planted in REF, and these differences were not linked strongly to maternal effects. In large part, the seedlings from both seed sources responded similarly to differences between the watersheds, although maternal effects on seed chemistry and seedling emergence were detected. Site advantages to sugar maple seedlings growing in CAL were weaker and not as definitively linked to the Ca addition as in our previous work where striking differences in chemistry, size, and health of seedlings were found (Juice et al. 2006).

We suggest several nonexclusive explanations for these results. First, the Ca treatment effect on CAL appears to be diminishing. Since being added in 1999, the pelletized wollastonite has been dissolving, with the Ca moving into deeper soil layers. The treatment effects on surface soil horizons appear to have peaked from 2002 to 2004 and seem to be decreasing especially in the forest floor horizon. For example, pH of the O_{ie} was between 4.5 and 5.0 in 2002–2004 and decreased to 4.0–4.5 in 2005–2006; analogous values for the O_a were 4.0–4.3 in 2002–2004 and 3.5–4.0 in 2006 (P. Groffman, unpublished data). Consequently, the differ-

ence in forest floor pH between CAL and various untreated reference sites has decreased in recent years. Similarly, Ca availability in the forest floor has decreased based on declining Ca concentrations in fern foliage (J.D. Blum, personal communication (2010)). Thus, the shallow roots of seedlings planted in 2006 may not have as much access to Ca enrichment as those germinating in 2003 at the peak Ca availability. This explanation would support the interpretation that relatively large increases in soil Ca availability are necessary to favor strong sugar maple seedling response on acid soils.

A second factor contributing to the more limited responses in the present study (versus Juice et al. 2006) could be differences between the reference forests for the two studies. The reference watershed for this study (REF, Watershed 3) has 29% higher weathering rates and consequently more available Ca than the reference site (Watershed 6) used by Juice et al. (2006) (Bailey et al. 2003). In other words, the difference in “baseline” Ca supply may have created a greater contrast in resource availability in Juice et al. (2006). The Ca application rate in this study (0.85 Mg/ha) was much lower than the majority of published Ca addition studies showing long-term effects (e.g., Moore and Ouimet 2006). However, a recent study from Quebec also used low application rates (0.4 and 0.8 Mg/ha) and has similarly found that effects of the Ca addition may be both more transient and more dependent on the soil status preapplication (Ouimet et al. 2008).

Third, factors other than Ca nutrition may be important in determining seedling emergence and early survival. Certainly, young sugar maple seedlings are vulnerable to a host of biological enemies, many of which vary among sites and years for poorly understood reasons (e.g., see Survivorship details section below) (Gardescu 2003).

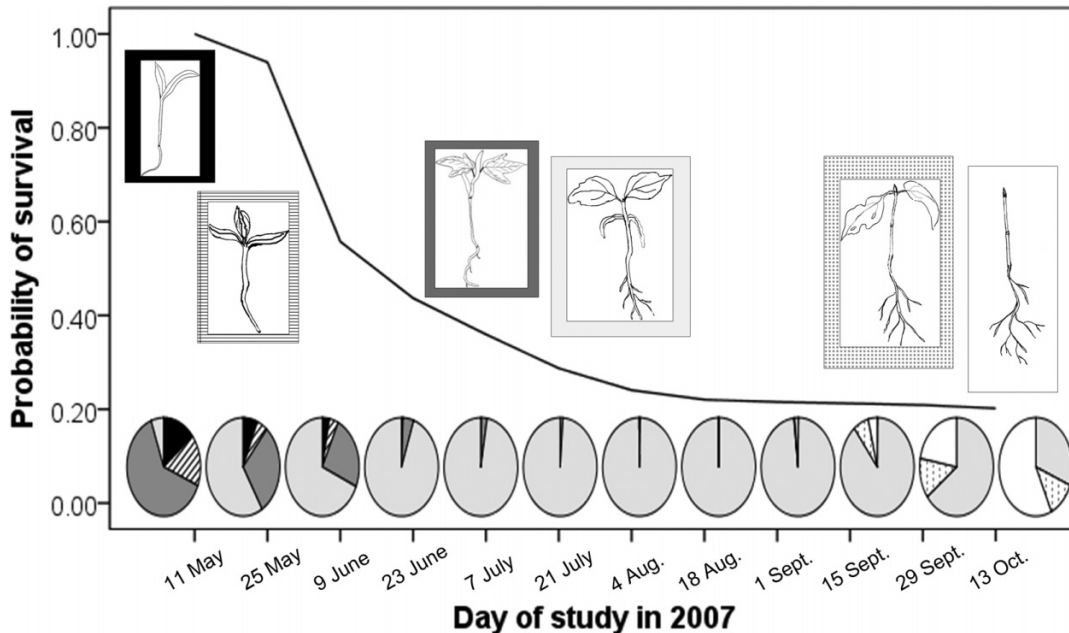
Maternal effects

Maternal effects in plants should be most obvious during the seed and early seedling stage when the largest portion of character variation is under maternal control either through structure, composition, or biomass of the seed (Roach and Wulff 1987). Based on this study and Long et al. (1997), we suggest that maternal effects on sugar maple seed rain are most likely through an increase in seed number during mast years rather than through an increase in seed biomass or masting frequency. Maternal effects on seed chemistry and seedling emergence were detected; however, these effects were not strong determinants of seedling survival and probably were relatively unimportant in explaining higher seedling densities seen by Juice et al. (2006) on the Ca-treated watershed.

The nature of the maternal effects may be environmental (e.g., nutrition effects) or genetic (inherited effects) (Roach and Wulff 1987). Nutrition effects were reflected in significantly lower contents of Mg and K in seeds from trees on CAL (Table 2). Seeds from REF had significantly lower emergence across sites, suggesting that seeds from CAL had some maternal advantage at this early stage (Fig. 2).

The extent to which the chemistry of seeds (maternally determined) contributes to the chemistry of young seedlings is not precisely known. Based on the altered chemistry of seeds shown here, nutrient imbalances for base cations re-

Fig. 4. Summary of survival and life history transitions for sugar maple (*Acer saccharum*) seedlings at three study sites in northern New Hampshire. The generalized survival curve describes survivorship of more than 2200 tagged seedlings and the pie charts depict changes in the percentage of seedlings in six life history stages through the first season. The “matting” of the life history sketches corresponds to that pattern in the pie chart slices with the youngest stages starting at 12 o’clock and proceeding clockwise.



sulting from wollastonite addition appear to be passed on through the seeds (Tables 2 and 3). Moore and Ouimet (2006) have reported that liming improved Ca and N nutrition but caused imbalances of P, K, and Mg in the mature foliage of trees in their study. Seedlings (St.Clair et al. 2008) may be more susceptible to negative impacts from nutrient imbalances than mature trees that have a larger pool of stored nutrients and can access a larger soil volume.

In our seedlings, the ratios of Ca, Mg, and K to Al and Fe were two- to fourfold lower in seedlings from CAL than in seedlings from REF (Table 3); however, whether this resulted from differences in seed concentrations of these elements and therefore maternal nutritional effects is unknown. The Mn concentrations and respective cation ratios remain favorable in CAL seedlings in this study (Table 3). The biological significance of these nutrient differences for sugar maples are not well explored and much more is known about the importance of ratios between Ca, Mg, and Mn (e.g., review by St.Clair et al. 2008). Moore and Ouimet (2006) concluded that despite causing imbalances in P, K, and Mg, the positive effects of liming suggested the primacy of Ca in nutrition of sugar maples. Quantitative differences in nutrient concentrations may not have much ecological relevance if the majority of the seeds and seedlings remain within critical ranges for these nutrients or if the nutrients are not limiting on the soil type studied.

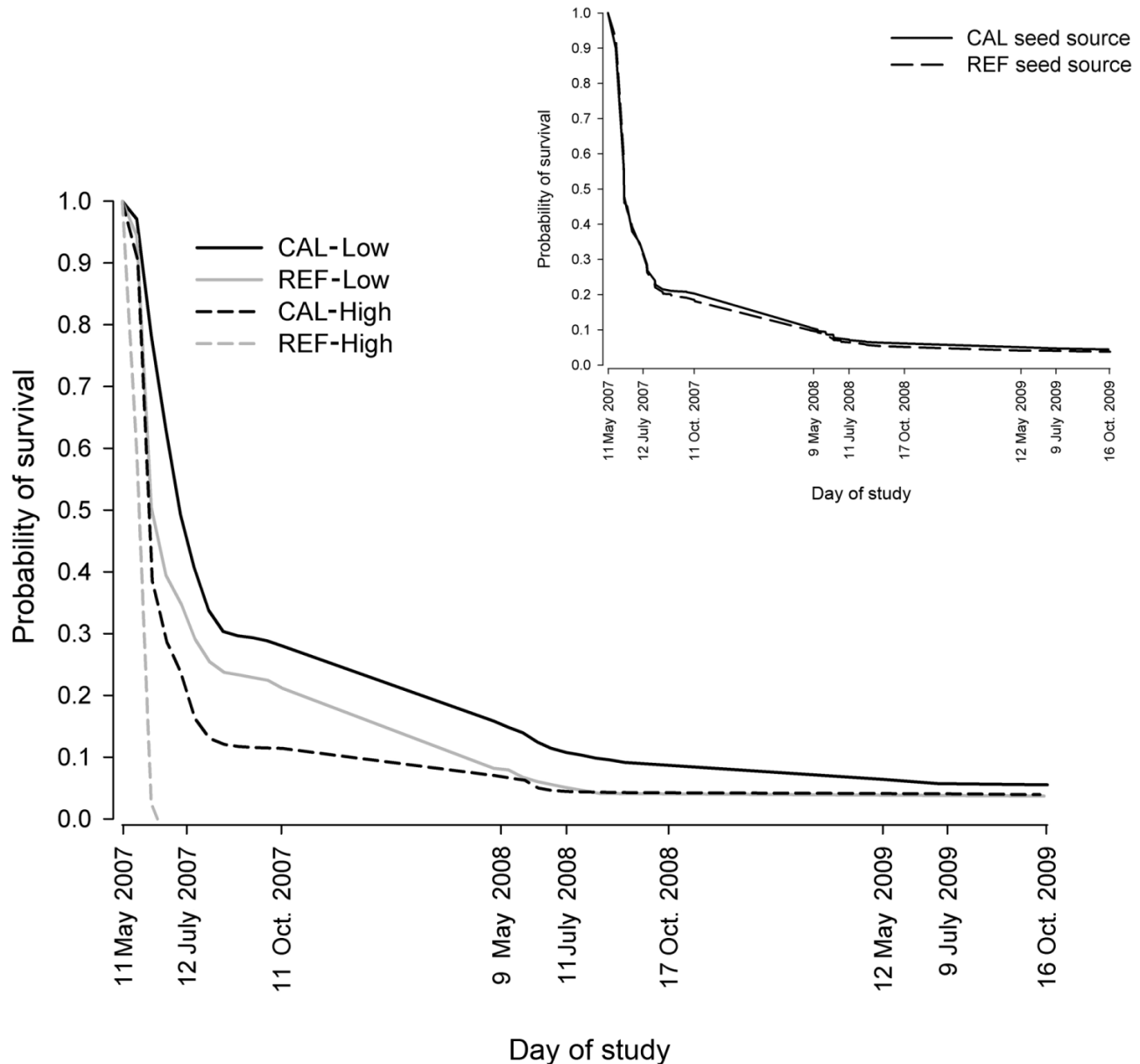
Site effects

Although Ca may play some role in the site effects reported here, the lack of large differences in chemistry and seedling size between the treated and untreated sites together with the apparent importance of site factors besides Ca sug-

gests a more complex role of site in sugar maple regeneration from seed. When Ca was at higher levels earlier in this experiment (2002–2004), any additional site effects were overwhelmed (Juice et al. 2006), whereas now, litter layer depth represents an important difference between the watersheds. It is possible that the thinner litter layer in CAL is an indirect effect of the Ca addition, as a study from Quebec has found a decrease in the depth of the forest floor and an increase in the rate of litter cycling (Ouimet et al. 2008). The thicker litter layer on REF appears to have played an important role by impeding penetration of the seed radicles into the soil, delaying seedling emergence, and producing etiolation of seedlings on this watershed (Figs. 2, 3, and 4). The lower emergence on REF was partly explained by the larger number of seedlings that died before they reached a stage at which they could be tagged (i.e., upright hypocotyl). In early May 2007, plots at REF-Low (14.2%) and REF-High (35.0%) had notably more seedlings that could not be tagged and that subsequently died than plots at CAL-Low (2.8%) and CAL-High (9.3%).

The consistently low seed production at high elevation even in mast years in conjunction with significantly lower seedling survival (Fig. 6) clearly constrains the ability of sugar maple to migrate to higher elevation sites in Hubbard Brook. Moreover, the noted lack of suitable soil types at high elevations in the White Mountain regions (Demers et al. 1998) may limit recruitment more widely. Lower seed production at higher elevations must also partially explain the pattern of decreasing seedling densities with increasing elevation shown previously at the HBEF (Forcier 1973; Juice et al. 2006). However, the ability of sugar maple to migrate to higher elevations will vary with soil type, as evidenced by the recent report of an increase in sugar maple in

Fig. 5. Survivorship curves for the first 3 years of life for sugar maple (*Acer saccharum*) seedlings of the 2007 cohort at four sites differing in soil Ca status and elevation. Plots within each site were reciprocally planted with seed from CAL and REF within elevation (inset). CAL and REF are abbreviations for Ca added and reference watersheds, respectively. Low and High refer to the elevation classes. At REF-High, all seedlings were dead by the fourth observation time.



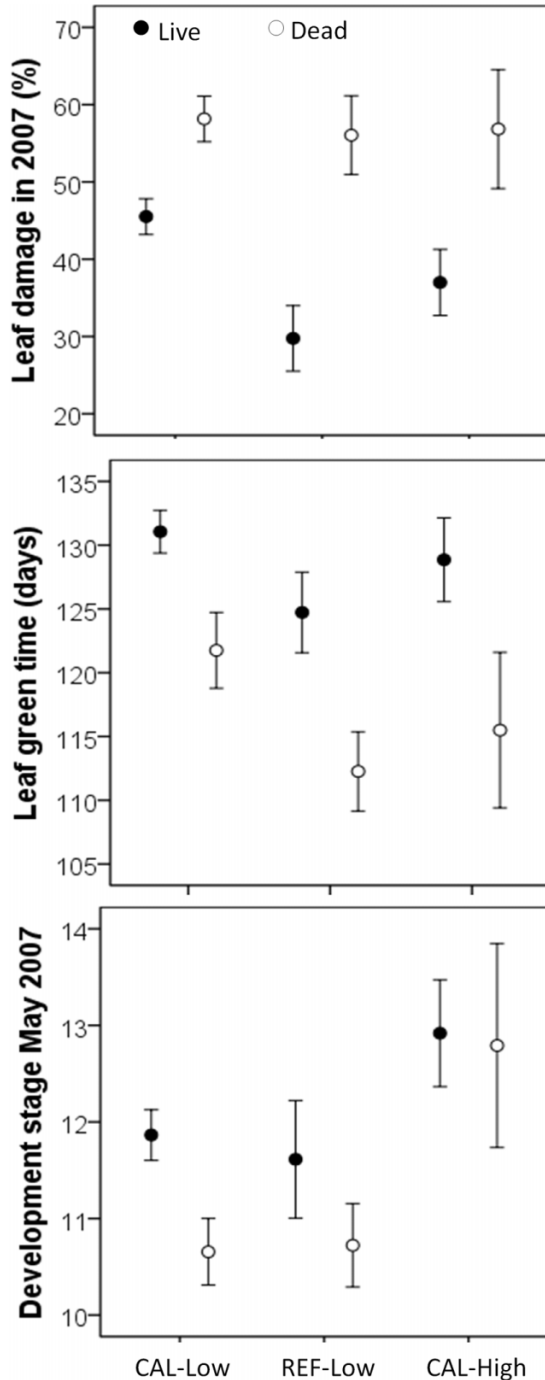
plots above 670 m elevation in the Green Mountains of Vermont (Beckage et al. 2008).

Survivorship details

The temporal patterns of survival were similar across all treatments and are in agreement with previous studies of sugar maple seedling survival (Gardescu 2003; Hane 2003). Mortality was highest at the earliest life stages that precede primary leaf expansion in May and June (Fig. 5). After these high-risk initial stages, overwinter mortality was much greater than mortality during the growing season in the second and third years (Fig. 2). In the earliest stages, our new germinants were killed by several exotic pests. The most

damaging pathogen was a previously undocumented and undescribed species of root and vascular system fungus in the genus *Rhizoctonia* (identified by culturing and sequencing of field samples, Department of Plant Pathology, Cornell University, Ithaca, New York). Seedlings were also lost to pear thrips (*Taeniothrips inconsequens* Uzel.), an exotic insect pest from orchard trees known to cause heavy damage to first-year sugar maple seedlings (Gardescu 2003). Slugs (notably exotic *Arion* spp.) were also an important cause of early-season death, particularly at REF-High (7% of deaths), while pear thrips caused the greatest damage at CAL-High (25% of deaths compared with 9% at REF-High and lower at the low-elevation sites).

Fig. 6. Sugar maple (*Acer saccharum*) seedling traits that served as significant predictors of seedling survival overwinter based on their status in May 2008. CAL and REF are abbreviations for Ca added and reference watersheds, respectively. Low and High refer to the elevation classes. Note that neither site nor seed source was a significant model variable in the logistic regression analysis. Error bars show ± 1 SE of the mean values.



Gardescu (2003) also found that differences in damage agents of sugar maple seedlings varied significantly between study sites and years. These differences in the influence of damage agents can be important determinants of patterns of seedling survival (Packer and Clay 2000; Gardescu 2003).

The extent to which soil Ca status may interact with populations of soil pathogens and insect defoliators is not known but has potentially important implications for long-term impacts of Ca addition on the ability of sugar maple to regenerate. The possibility that Ca addition could increase pathogen loads needs further investigation, particularly given the critical role that pathogens play in determining seedling recruitment (Packer and Clay 2000; Gardescu 2003).

Overwinter survival in young sugar maples has been linked through defoliation experiments to nonstructural carbohydrate reserves (Canham et al. 1999). The link between overwinter survival and prewinter photosynthesis time and photosynthesizing area in this study might be explained by carbohydrate reserves. Although not measured directly, the amount of C assimilated by a seedling is determined in part by season length (days with green leaves) and photosynthesizing area (amount of nondamaged or missing leaf surface area), and these variables were significant predictors of seedling survival for both winters.

The decreasing importance of site in predicting seedling survivorship after the first growing season suggests a shift in the relative importance of extrinsic and intrinsic seedling traits. In the early stages of establishment, outside variables such as site conditions and prevalence of pathogens have an overarching influence on which seedlings will survive. As time progresses, survivorship becomes more predictable based on attributes related to seedling size and growth. However, site factors such as Ca status would continue to have an indirect effect on long-term survival through influences on seedling growth rates. In conclusion, although the importance of Ca to sugar maple is firmly established, many complexities remain, particularly in regards to interactions of Ca amendments with other site factors and the possible implications of using Ca addition as a long-term management tool for sugar maple stands.

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