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Seed Size, the Only Factor Positively Affecting Direct Seeding Success in an Abandoned Field in Quebec, Canada

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Abstract: Direct tree seeding is potentially an economical technique for restoring forests on abandoned fields. However, the success of tree establishment depends on many factors related to species and seed characteristics, environmental conditions, competition and predation. We compared seedling emergence, survival and growth of six tree species of different seed sizes in a forest restoration project of abandoned fields. Species were seeded in plots with and without herbaceous vegetation and with and without protection from bird and mammal predation. Yellow birch (*Betula alleghaniensis*) did not emerge in all treatments, paper birch (*Betula papyrifera*) and tamarack (*Larix laricina*) had a seedling emergence rate lower than 1%, and sugar maple (*Acer saccharum*) had a low overall emergence rate of 6%. Seedling emergence reached 57% for northern red oak (*Quercus rubra*) and 34% for red pine (*Pinus resinosa*), but survival of oak after one year was much higher (92%) than pine seedlings (16%). Overall, protection from birds and mammals and elimination of the herbaceous vegetation cover had no detectable effects on seedling emergence, survival and height. Nonetheless, red oak seedlings growing in the presence of vegetation had a smaller diameter and shoot biomass and a larger specific leaf area. We conclude that only large seeded species, such as oak, should be used for forest restoration of abandoned fields by direct seeding in our region.

Keywords: tree; direct seeding; seedling emergence; survival; growth; seed size; competition; predation

1. Introduction

Over recent decades, industrialisation and intensification of agriculture in occidental countries have led to agricultural fields in marginal environments being abandoned [1–4]. In these fields, herbaceous or shrub communities may inhibit tree establishment for many years [5,6], and tree recruitment is dominated by a few wind-dispersed pioneer species [7,8]. In both temperate and tropical ecosystem, direct seeding has been proposed as a time- and cost-effective technique to restore abandoned fields; indeed, tree plantations may cost 1.5 to 2.5-times the cost of direct seeding [9–13]. However, many endogenous factors, such as the type and density of competing vegetation, the presence or not of predation, soil type and exogenous factors, such as climatic conditions, interact with seed size and shade tolerance to affect the overall success of this technique [10,14–17]. Although many studies have been conducted to investigate the success of direct seeding as a way to restore abandoned fields, few have attempted to evaluate the interacting effects of herbaceous vegetation (both positive and negative) and predation across a wide range of tree species in temperate ecosystems (but, see [14–16]).

Effects of vegetation (both positive and negative) on seedling emergence vary with plant litter [18] and surrounding vegetation types [16]. In general, grasses have more negative effects and are stronger competitors than forbs [19]. For examples, *Poa pratensis* communities constitute a less conducive environment to tree seedling emergence, except for *Crataegus mollis*, while many other types of vegetation were not found to have an impact on the emergence of *Acer saccharum* [16]. Although herbaceous vegetation can have a positive, neutral or negative effect on seedling emergence, it usually has a negative impact on seedling growth [19]. In the studies conducted by de Steven [14,20], even if herbaceous cover had a positive effect on the emergence of species with larger seeds (*Fraxinus americana* and *Acer rubrum*), it had negative impacts on seedling survival and growth of all five species tested [20].

Seedling predation can be more important than competition as a factor affecting overall tree survival in seeding experiments. In the Northeastern US, Gill and Marks [15] showed that seedling mortality was essentially due to predation by voles rather than herbaceous competition and that predation was more important under herb cover. Vegetative cover, in fact, increases the probability of seedling damage caused by small rodents, because it decreases the risk of small mammals being seen by their own predators [21]. Meadow voles (*Microtus pennsylvanicus*) are important seedling predators, whereas white-footed mice (*Peromyscus leucopus*) are large seed consumers [22,23]. Fortunately, their negative impacts are rarely simultaneous, because mice are less abundant in fields when vole populations are high, and they are more active when vole density is low [22,23]. *Muridae* and *Cricetidae* are not the only types of seed and seedling predators; squirrels, rabbits, deer, cattle, wild boars, birds and insects, such as ants, may also affect the success of seedling establishment via predation [24–27]. The wide range of predator sizes probably explains in part why Moles *et al.* [28] found no significant relationship between seed mass and seed predation in their literature review.

The objectives of this study were: (1) To measure seedling emergence, survival and growth of six tree species of different seed size and shade tolerance directly sown in an abandoned field; and (2) To evaluate the effects of herbaceous vegetation, predation and their interactions on seedling emergence, survival and growth. We hypothesize that seedling emergence and survival rates will increase with increasing seed size. We suppose that vegetation will enhance seedling emergence of the shade tolerant species evaluated in our study (sugar maple), have no impact on the emergence of the large-seeded mid-shade tolerant species (red oak) and have a negative effect on the emergence of small-seeded shade intolerant species. We also hypothesize that vegetation cover will have neutral or negative effects on seedling survival and growth and that predation will be higher under a vegetation cover.

2. Methods

2.1. Study Area

The study took place in three abandoned fields located in southwestern Québec (Canada), in the agricultural zone of Laval (45°40'N; 73°43'W), north of Montréal. The region is characterized by a humid continental climate. During the study, the average annual temperature recorded at the Montréal weather station was 8 °C, with monthly means of 23 °C in July and −10 °C in January [29,30]. The annual precipitation varied between 1194 mm (2010) and 1281 mm (2011) [29,30]. A 3.5 ha forest, dominated by *Acer saccharinum* and composed of *Fraxinus nigra*, *Fraxinus pennsylvanica*, *Populus deltoides* and *Thuja occidentalis* separates the first two fields from the third. The surficial deposits are mainly of glacial origin. A stony clay loam soil is found on the first two fields, while the soil in the third field is a clay loam [31]. Fields one and three have an area of about 9000 m², while the second field is bigger, with an area of about 23,000 m². The three abandoned fields had a similar past land use and were cultivated for at least 25 years. In the early 2000s, cultivation stopped, but the sites were mown once or twice a year, until the fall of 2009. This mowing prevented tree and shrub establishment and promoted ruderal herbaceous vegetation. Fields had sufficiently similar conditions to establish a split-plot design of three randomized blocks (see next section), with one block per field. However, herbaceous vegetation differed somewhat between blocks. In the first one, the vegetation was principally dominated by grass species (*Poaceae* spp. and *Cyperaceae* spp.), *Solidago* spp., *Trifolium repens* and *Trifolium pratense*. The vegetation in the second plot was also dominated by graminoids, but associated species were *Taraxacum officinale*, *Sonchus oleraceus* and *Medicago lupulina*. In the third plot, *Solidago* spp. were as important as grass species, and *Taraxacum officinale*, *Sonchus oleraceus*, *Medicago lupulina* were also abundant. Total ground coverage by all species was around 70% in each plot.

2.2. Experimental Design

The study took place between May, 2010, and August, 2011. In each field, a 9 × 9 m experimental block was installed at least 20 m from the forest, trees bordering the fields and a road, to avoid edge effects. Experimental blocks were divided into four plots following a split-plot design, where the main factor was “protection” (protection of seeds and seedlings from predation or no protection), and the subplot factor was “vegetation” (intact herbaceous vegetation or bare soil). Protection from rodents

was achieved using a surrounding enclosure fence of 6 mm mesh and 120 cm high. The bottom part of the fence was curved, nailed into the ground and covered with stones and soil instead of buried, due to soil compaction. Fishing lines were placed on the top of the protected area, every 10 cm, to prevent birds from entering into these plots. Fishing lines had no effect on light transmission; percentages of photosynthetic photon flux density (%PPFD) were similar in plots with or without protection. Deer could have jumped into the protected area, breaking fishing lines, but no sign of deer damage was seen.

Vegetation on half of the protected and unprotected plots was maintained. On the other half, all vegetation was weeded by hand before the beginning of the experiment. From June, 2010, to August, 2011, vegetation was regularly cut by scissors in the sown locations instead of weeded by hand, to avoid soil disturbance around seeds and small seedlings. In rows between sown locations, vegetation was weeded by hand until the end of August, 2010. By this time, almost all seedling emergence occurred; thus, coconut fiber mulches were put between sown locations to reduce weeding time.

2.3. Seeding

Each subplot contained ten sown locations per species, spaced 30 cm apart. The six species used in this study were chosen, because they are native to the area, and they differed in seed size and shade tolerance (Table 1). They were: paper birch (*Betula papyrifera*), yellow birch (*Betula alleghaniensis*), tamarack (*Larix laricina*), red pine (*Pinus resinosa*), sugar maple (*Acer saccharum*) and northern red oak (*Quercus rubra*). Seeds were prepared and provided by the Centre des semences forestières de Berthier (CSFB) from the Quebec Ministry of Natural Resources. Usually, all of these species are sown in the spring, except acorns of northern red oak, which are sown in the autumn. Acorns were thus placed in a cold room (4 °C) from November, 2009, to May, 2010, so they could also be sown in the spring. Yellow birch seeds needed stratification for 21 days, while sugar maple seeds spent two weeks in cold water, followed by stratification for 3.5 weeks. Other species did not require special treatment preceding spring seeding. Seed germination in the laboratory, determined by professionals from the Quebec Ministry of Natural Resources, was more than 80% for all species (Table 1). Seeds were kept in a dark cold room (4 °C) until seeding from 25 to 27 May, 2010. The number of seeds in each sown location decreased with seed size, as the production of seeds in nature is usually higher for small-seeded species than for large-seeded species. Five seeds of paper birch and five of yellow birch were used (total of 600 seeds/species), four seeds of sugar maple, red pine and tamarack (total of 480 seeds/species) and three acorns of red oak (total of 360 acorns). Seeds were buried at a depth of 0.5 to 5 cm, depending on seed size and following recommendations given by professionals from the Quebec Ministry of Natural Resources.

Table 1. Characteristics and seed germination in the laboratory of the six tree species tested.

Species	Species development in natural conditions	Average dry seed mass (g)	Shade tolerance	Germination in laboratory
<i>Betula papyrifera</i> Marsh. (Paper birch)	Fast-growing tree, often established after fire or logging; develops best on well-drained sandy loams	0.00045	Low	82%
<i>Betula alleghaniensis</i> Britton (Yellow birch)	Slow-growing tree, often found on moist well-drained soils	0.0012	Intermediate	88%
<i>Larix laricina</i> (Du Roi) K. Koch (Tamarack)	Fast-growing tree, often found on wet to moist organic soils	0.0027	Low	81%
<i>Pinus resinosa</i> Ait. (Red pine)	Moderate to fast growing tree, usually established following fire; often found on sandy soils	0.01	Low	93%
<i>Acer saccharum</i> Marsh. (Sugar maple)	Slow-growing tree; grows on a variety of sites and soils	0.06	High	88%
<i>Quercus rubra</i> L. (Northern red oak)	Moderate to fast growing tree; found on a variety of soils	3.14	Intermediate	88%

Species development in natural conditions [32]; average dry seed mass [33] (for red oak: [34]); shade tolerance [32,35]; rates of germination in the laboratory [33].

2.4. Measurements

2.4.1. Seedling Emergence, Survival and Growth

Seedling emergence, survival and height were measured every week between 21 June and 14 July, 2010, at the end of July and August, 2010. Sampling was also conducted once in May, 2011, and twice in June, 2011, to measure seedling height and seedling survival after the winter and to see if new seedlings emerged one year after seeding. Seedling emergence refers to the proportion of seedlings emerging in a sown location. We use the term proportion of live seedlings as the number of seedlings emerged per number of seeds buried. Seedling survival for 2010 was calculated as the proportion of live seedlings at the end of August, 2010, out of the proportion of live seedlings at the end of June, 2010. Seedling survival after one year is calculated as the proportion of live seedlings in June, 2011, out of the maximal proportion of live seedlings in 2010. Height was measured as the distance between the soil surface and the apical meristem, but for red pine, we measured height until the end of the needles.

2.4.2. Red Oak Seedling Measurements

Additional measures were taken on red oak seedlings in August, 2011, to measure the effects of competing vegetation on different variables related to growth. At this time, pine and maple seedlings were not sufficiently abundant to make comparisons between treatments. To avoid intraspecific competition between oak seedlings, we kept only one seedling per sown location at the beginning of July 2011. We randomly chose among averaged size seedlings in good shape. Height, diameter, shoot biomass and specific leaf area (SLA) were measured on five red oak seedlings randomly chosen in

each subplot ($n = 60$ seedlings). No belowground measures were taken, due to the high compaction of the soil. Specific leaf area (SLA) was measured following the method described by Cornelissen *et al.* [36]. Two healthy leaves per seedling were taken and were scanned the day of collection. Leaf area was calculated using the Winfolia software (Régent Instruments, Québec, QC, Canada). Leaves were dried at 70 °C for at least 48 h before their mass was measured. We also calculated relative height growth rate (RGR_H) using the formula:

$$RGR_H = \frac{\ln(H2) - \ln(H1)}{t}$$

where $H2$ corresponds to height in August 2011, $H1$, height in August 2010, and t , time between both measures, which is one year.

2.4.3. Environmental Measurements: Soil Moisture and Light

During the summer of 2011, two sown locations per species were randomly chosen to take environmental measurements. Soil volumetric water content was measured at three occasions using a TDR-200 probe (Spectrum Technologies Inc., Plainfield, IL, USA) with 12 cm rods. A mean value was obtained from two measurements taken in the middle of each sown location. The first series of measurements was taken on 15 June, two days after a rain of 10 mm and three days after a rain of 30 mm. The second measurements were taken on 12 July, after a light rain of 2 mm, and the third measurements were taken on 18 July, after a light rain of 1 mm. Photosynthetic photon flux density (%PPFD) was measured using point quantum sensors (LI-COR, Inc., Lincoln, NE, USA). Measurements were taken at the end of July, 2011, on cloudy days, following the method described in Messier and Puttonen [37].

2.5. Data Analysis

Linear regressions were used to test the relationship between seedling emergence and seedling survival as a function of seed size. Repeated measure ANOVAs, following a two-way split-plot design (described above), were computed to evaluate the effect of protection treatment, vegetation treatment, time and their interactions on the proportions of live seedlings and on soil volumetric water content. Analyses of variance of the proportions of live seedlings between treatments were also performed by date, following the same two-way split-plot design. Variances of seedling survival and height on 25 August, 2010 and 15 June, 2011 were analysed using the same statistical model. Blocks ($n = 3$) were considered as a random effect, and analyses were performed separately by species, due to the large differences between the proportions of live seedlings and seedling heights. Yellow birch, paper birch and tamarack were not included in the data analysis, because their seedling emergence was nonexistent or negligible. ANOVA was also used to compare light levels (%PPFD), which were log transformed to achieve normality. Finally, ANOVA was used to compare five growth variables of red oak seedlings between treatments following the same two-way split-plot design. For all analyses, we computed Tukey *post hoc* tests when a variable was found to be significantly affected by a treatment ($p \leq 0.05$). Statistical analyses were conducted using JMP software (version 10.0) [38].

2.6. Second Seeding Experiment

In 2011, we conducted a second seeding experiment to compare rates of seedling emergence between 2010 and 2011 for our best performing species (red oak, red pine and sugar maple) and to test another large-seeded species, bur oak (*Quercus macrocarpa* Michx.; dry seed mass of 6.15 g [34]; mid-shade tolerant species [35]). Once again, seed germination in the laboratory was higher than 80% for all species [33]. Unfortunately, we could not use the same site, because the fields were full of small trees planted in the summer of 2010. We thus used another site, located in the same area. The site was an abandoned old field recently disturbed by a residential development. Competing vegetation was sparse and dominated by *Equisetum* species, grass species and some *Cornus stolonifera* shrubs. The soil was composed of clay loam [31].

Four experimental blocks were established in the field. Each block contained eight sown locations per species (for a total of 32), and 4 seeds by sown location were buried (for a total of 128 seeds/species). No vegetation or predation control was conducted. Seeding was done on 19 May and 25 May, 2011. The proportions of live seedlings were monitored four times in the summer of 2011 and once in the spring of 2012.

3. Results

3.1. Establishment Success

Seedling emergence and, to a lesser extent, seedling survival increased with seed size ($R^2 = 0.63$, $p < 0.0001$; $R^2 = 0.47$, $p = 0.0006$, respectively). Despite having sown 600 seeds, no yellow birch seedlings emerged. Only one paper birch seedling emerged, in a plot with vegetation and protection, and it measured 15.5 cm in the summer of 2011. Five tamarack seedlings emerged at the beginning of July, but they died a few days after emergence. Seedling emergence of red pine reached a maximum of 34% at the end of June 2010 (Table 2). Over the summer, the needles of several pine seedlings changed color, from green to red-brown and decreased in length, until those pine seedlings died. Between the end of July and the end of August, 2010, 22% of the pine seedlings decreased in height (measured until the end of live needles) and the average height declined from 4.2 to 4.0 cm. The same tendency was observed between August, 2010, and June, 2011: The average live height decreased from 4.0 to 3.8 cm, and 45% of the pine seedlings were smaller in 2011 than in August, 2010. At the end of the first growing season, only 52 pine seedlings out of 163 (survival of 32%) were still alive, while in the spring, 2011, the proportion of live seedlings was only 5.5%.

Table 2. Seedling emergence, survival and height of red pine, sugar maple and northern red oak.

Species	Date of maximum emergence	Maximum emergence (%)	Seedling survival in 2010 (%)	Seedling survival in 2011 (%)	Proportion of live seedlings in June 2011	Height in June 2011 (cm)
Red pine	21 June, 2010	34.0 ± 17.6	32.1	16.3	5.5 ± 5.9	3.8 ± 0.6
Sugar maple	6 July, 2010	5.8 ± 4.6	71.4	57.1	3.3 ± 3.1	11.5 ± 0.7
Red oak	25 August, 2010	57.2 ± 13.8	100	91.7	52.5 ± 14.8	10.6 ± 0.2

Seedling emergence of sugar maple reached a maximum of 5.8% at the beginning of July 2010 (Table 2). After one year, 57% of maple seedlings were still alive and their average height was 10.9 cm. Red oak emerged later than the other species, but had excellent survival. The maximum seedling emergence of 57.2% occurred at the end of August 2010 and as no oak seedlings died during the first growing season, we estimated its survival to be 100% in 2010. In the spring, 2011, the proportion of live seedlings was 52.5%, for a seedling survival of 92%, and their average height was 10.6 cm. Only one new red oak seedling and one new sugar maple seedling emerged in the spring, 2011.

In the second experiment, seedling emergences were 65.6% for red oak, 14.1% for sugar maple and only 3.9% for red pine, while seedling survival for each species after one year were 98.8%, 83.3% and 20.0%, respectively. Seedling emergence of bur oak was 60.9% in 2011. Only three bur oak seedlings died between 2011 and 2012, but 28 new seedlings emerged in the spring of 2012, for a maximum of 82.8% seedling emergence.

3.2. Effects of Treatments

Repeated measures ANOVA showed no effect of treatments “protection” and “vegetation” on the proportions of live seedlings with time (Figure 1). Analyses of variance calculated by date showed only one significant effect. In the middle of July 2010, the proportion of live pine seedlings was higher in plots without vegetation (27.0%) than in plots with vegetation (7.8%) ($F_{1,2,22} = 44.33$, $p = 0.0165$; Figure 1a). Mortality of pine seedlings started earlier in plots with vegetation, but this difference disappeared after two weeks. No differences in variances were observed during the other weeks. Vegetation and protection treatments had no effect on seedling survival and seedling height of the three species. Moreover, no sign of predation on seeds or seedlings were seen in any plots, except for one sugar maple seed in a plot without vegetation and without protection.

Figure 1. Mean percentage of live seedlings in response to treatments “vegetation” and “protection”, between 21 June, 2010 and 15 June, 2011 for (a) red pine; (b) sugar maple; and (c) red oak. Sub-figures (a to c) ordered by species seed size.

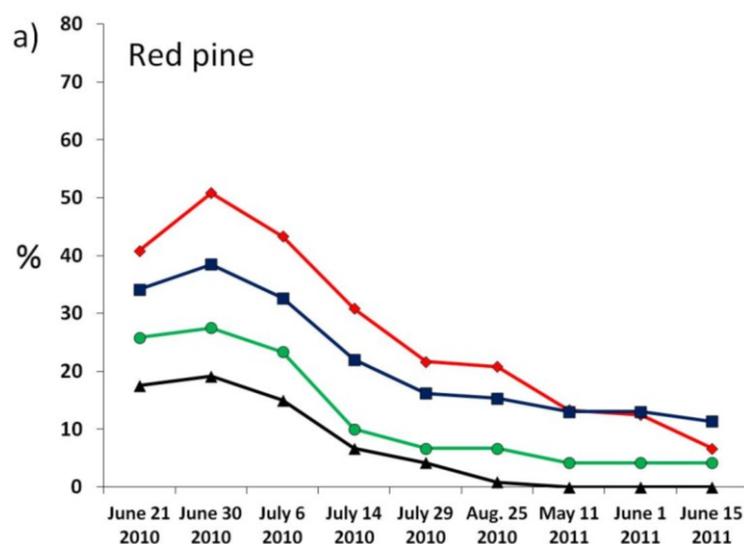
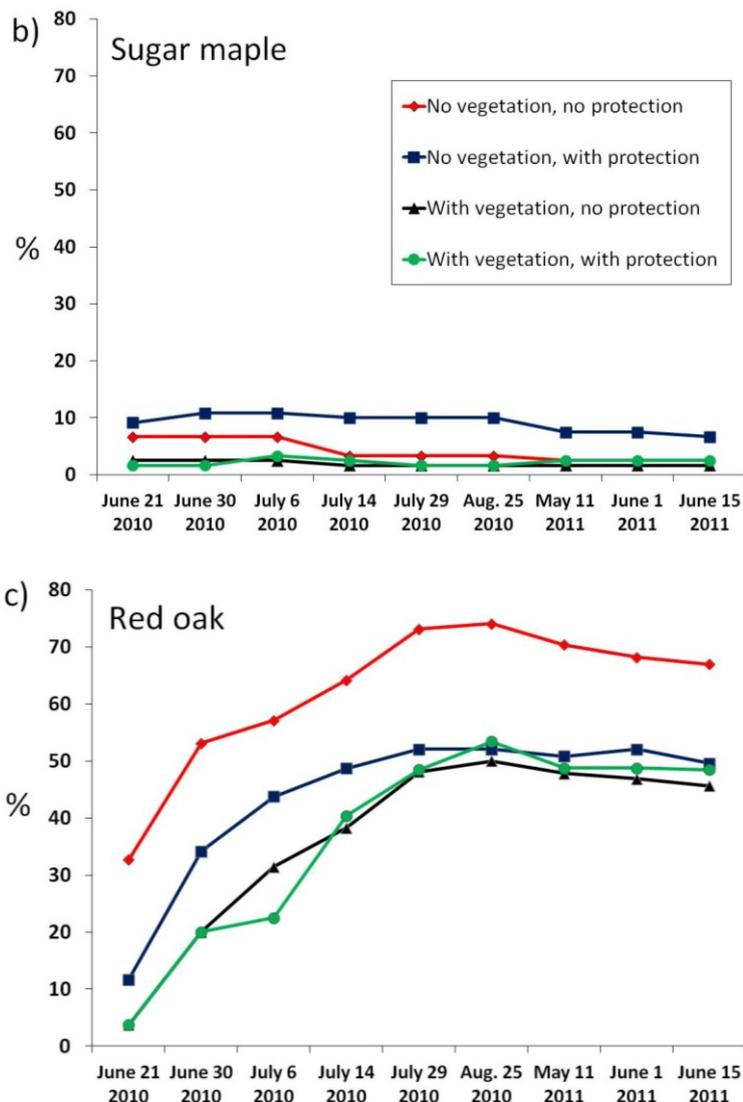


Figure 1. Cont.



At the end of the second summer, only red oak seedlings were numerous enough to make growth comparisons between treatments. *Post hoc* tests in the analysis of variance of five growth variables showed that vegetation significantly reduced red oak diameter and shoot biomass and increased specific leaf area (Table 3), while the protection treatment had no effect. Seedling height and relative height growth rate did not differ between treatments. Vegetation also had a negative effect on soil moisture ($p \leq 0.05$). Soil volumetric water content was higher in plots without vegetation, and this difference increased with time (Table 4). Repeated measure ANOVA showed an interaction between vegetation and dates (time) for soil moisture ($p < 0.0001$). Percentage of photosynthetic photon flux density (%PPFD) was lower under vegetation (23.1%) than on bare soil (88.3%) ($F_{1,1.97} = 3066.28$, $p \leq 0.05$).

Table 3. Effects of the presence of vegetation on five variables related to the growth of red oak seedlings in August, 2011.

Growth variables	No vegetation	Vegetation	$F_{df}; p$ -values
Height (cm)	10.65 ± 0.58	12.53 ± 0.57	$F_{1,1.98} = 7.82; p = 0.1086$
RGR _H (cm cm ⁻¹ yr ⁻¹)	0.50 ± 0.03	0.45 ± 0.03	$F_{1,1} = 0.0005; p = 0.9860$
Diameter (mm)	3.51 ± 0.19	2.64 ± 0.19	$F_{1,1.11} = 118.47; p = 0.0486$
Shoot biomass (g)	1.36 ± 0.08	0.78 ± 0.07	$F_{1,1.85} = 29.42; p = 0.0381$
SLA (mm ² ·mg ⁻¹)	14.75 ± 0.53	17.13 ± 0.53	$F_{1,1.96} = 21.24; p = 0.0456$

Values are the means ± standard errors (SE). Means and SE in bold and italics are significantly different at $p \leq 0.05$ according to ANOVA and *post hoc* tests. Measurements were taken at the end of August, 2011.

RGR_H = relative height growth rate for one year; SLA = specific leaf area.

Table 4. Soil volumetric water content (%) within the first 12 cm of soil in the summer of 2011.

Date	Precipitation during the last 3 days (mm)	No vegetation	Vegetation	$F_{df}; p$ -values
15 June, 2011	41	22.5 ± 0.4	21.1 ± 0.4	$F_{1,2.08} = 21.42; p = 0.0405$
12 July, 2011	2	22.4 ± 0.4	18.6 ± 0.4	$F_{1,1.96} = 31.14; p = 0.0321$
18 July, 2011	1	23.8 ± 0.5	17.3 ± 0.5	$F_{1,2} = 83.39; p = 0.0117$

Values are the means (%) ± standard errors (SE). Means and SE in bold and italics are significantly different at $p \leq 0.05$ according to ANOVA and *post hoc* tests. There was a positive interaction between dates (time) and vegetation treatment ($p < 0.0001$).

4. Discussion

4.1. Establishment Success and Seed Size

Our field study confirmed the importance of seed size for seedling emergence and seedling survival in abandoned fields, despite high germination rates (>80%) of all species in laboratory trials. Other studies have also shown major differences between germination in controlled conditions (for example, a greenhouse) and seedling emergence in an abandoned field [14,17,39,40], which is a harsh environment with wide fluctuations in temperature and moisture and often with compacted soils [14,16]. In our experiment, the lightest seeded species did not emerge even in the bare soil plots. Under natural conditions, the germination capacity of yellow birch is only about 20% [32]. Tests conducted, in an agricultural field at the government nursery have shown that seedling emergence attained only 20% for paper birch and 39% for yellow birch [33]. Seedling emergence for these two species was lower than for species with higher seed mass, even if these soils were watered regularly and free of competition. This further confirms that larger seeded tree species emerge and survive better in the field than smaller seeded species.

A lack of soil moisture may be one of the main factors causing the failure of seedling emergence. However, according to Burton and Bazzaz [16], temperature may be more important than moisture for the emergence of temperate biome species, and larger seeded species can germinate at a broader range of temperatures than smaller seeded species. June, 2010, was a rainy month (158 mm of rain; the normal is 83 mm), and July was also not drier than usual (precipitation of 97 mm; normal of 91 mm) [29,41]. On the other hand, July was a particularly warm month, with 15 consecutive days of

maximum temperatures over 27.5 °C, including an important heat wave (>30 °C) from 5 July to 9 July [29]. Some species, like *Fraxinus excelsior* and *Acer pseudoplatanus*, do not emerge when the daily maximum soil temperature exceeds 25 °C [42]. Germination of *Acer saccharum* also drops as temperatures increase; it is optimal at 1 °C and does not germinate well at temperatures above 10 °C [32]. The warm temperatures experienced at the beginning of the first growing season and the late period of seeding (at the end of May instead of late April) may explain the failure of sugar maple emergence, as well as other species.

The heat wave period corresponded with the moment where the five tamarack seedlings emerged and died and the moment where pine seedlings began to slowly perish (Figure 1a). Almost 70% of the pine seedlings died in the first growing season. Then, half of the remaining pine seedlings died during the first winter. Gill and Marks [15] also observed similar patterns of desiccation and frost heaving on young pine seedlings. The low emergence of pine seedlings in the second experiment may be explained by the alkaline nature of the soil [31]; red pine is known to grow better when the soil has a pH between 5.1 and 5.5 [32].

Like our experiment, other studies have obtained a better survival for oak than for pine seedlings [43,44]. The large nutrient reserves and energy stock in their acorns and their ability to rapidly develop a long taproot presumably allow young oak seedlings to survive short periods of drought or other stresses [32,43,45,46].

4.2. Effects of Vegetation

No statistically significant effect of vegetation was observed on seedling emergence, survival and height of pine, maple and oak seedlings. Our hypothesis that the emergence of shade tolerant maple seedlings would be enhanced by a vegetation cover was not confirmed. Nonetheless, past studies have shown that an intact vegetation cover may enhance emergence of red maple (*Acer rubrum*) and have a facilitating effect on the survival of sugar maple seedlings [14,47]. Herbaceous vegetation protects shade tolerant *Acer* seedlings from heat and desiccation stresses by providing a shadier environment, with lower light levels and, thus, lower leaf temperatures [15,47].

Some competition for water and light may have occurred, since the diameter and shoot biomass of red oak seedlings were lower and specific leaf area higher when they grew in herb vegetation. On bare soil, where light levels are high, seedlings developed thick leaves of small size (low SLA) to reduce transpiration losses and increase carbon gain [48]. Under vegetation, oak seedlings can reduce their leaf biomass to increase their fine root biomass, due to the lack of water in vegetation plots [49]. Indeed, we observed, like Löff *et al.* [50] and Laliberté *et al.* [51] did, lower soil water content (SWC) in vegetation plots than in weeded plots. In dry conditions, grassland vegetation considerably reduces soil water, increasing the intensity of competition and, thus, decreasing seedling survival, whereas in wet conditions, herbaceous vegetation has a smaller effect on seedling survival [52,53].

The absence of vegetation impact on emergence, survival and height is probably due to the combined effects of variation in the intensity of competition for water and protection by plants from high light levels. In June, 2010, competition for water was probably not a problem, since it was a rainy month (158 mm), and surrounding herb species did not reach their final height. Pine seedling emergence occurred principally at that time when light and soil water content (SWC) were high, even

in vegetation plots. Then, in the summer, 2010, competition for water should have been higher, due to the heat wave and warmer conditions, while taller herb plants should have protected seedlings from high light levels.

4.3. Effects of Predation

There were no apparent signs of seedling predation on our seedlings, even though predation damage by voles and rabbits were seen on almost 15% of hardwood trees (none on conifers) in the nearby plantation around experimental blocks [54]. Rodents can be a major cause of seedling mortality in abandoned fields [15,22], but the abundance of food probably protected the small seedlings from predation. Seed predation by rodents and birds was not a problem, as no difference in seedling emergence was observed between non-protected and protected sites. Acorns were not predated, even if the study took place in a suburban area, where gray squirrels (*Sciurus carolinensis*) are present, perhaps as the acorns were buried. The absence of acorn predation was also reported by Laliberté *et al.* [51] in a similar study. These results do not support our hypothesis regarding a higher predation rate under vegetation cover.

Researchers from the tropics and Australia observed that invertebrates, such as ants, contribute to seed loss [27,55,56]. Andersen [56], for example, showed that ants removed 93% of *Eucalyptus baxteri* seeds (seed mass of 0.0019 g) and 58% of *Casuarina pusilla* seeds (seed mass of 0.0022 g) in an Australian woodland. In Mexico, more than 90% of small-seeded (0.0009 g and 0.0068 g), early successional species were predated by invertebrates [55]. Ants, beetles, molluscs and microbes are seed consumers, but the first two groups may also transport intact seeds [26,57]. Predation and/or removal of seeds by ants may have been responsible for the poor success of our small-seeded species. There were many ants in the field, and many ant eggs were observed during weeding. In the plantation around the experimental blocks, more than 10% of the planted trees growing on a bare soil were surrounded by ant-hills and around 5% for trees growing in vegetation [54].

In the tropics, seed predation by ants may be equivalent or higher than seed predation by vertebrates and ants, like leaf-cutter ants, can also attack seeds of medium size (>0.2 g or >5 mm) [27,55]. However, a study conducted in the UK showed that seed losses attributable to invertebrates were negligible or null when seeds were buried [26]. Indeed, other studies indicated that burying seeds reduces seed loss [55,58,59]. The effect of invertebrate predation has rarely been controlled in direct seeding studies, particularly in Canada (see Hulme [60]), and we are an example of a research bias towards protecting from mammal and bird predation of seeds without considering the possible effects of smaller organisms on small-seeded species. We thus encourage future researchers to consider the potential effect of invertebrate predators, such as ants, in seeding experiments.

5. Conclusions and Restoration Implications

It is difficult to establish with certainty why small-seeded species did not emerge in the field. A lack of appropriate temperature and soil moisture or ants may have been important factors impeding the emergence of small-seeded species, but further research is required to determine the actual causes. Our results confirm other studies about increasing seedling emergence and survival with increasing seed size. Hooper *et al.* [10] and Doust *et al.* [40] also concluded that large-seeded species of medium to

high shade tolerance are good candidates for direct seeding, even under a dense vegetation cover. No effects of vegetation and predation were observed in our study on emergence, survival and height growth of red pine, sugar maple and red oak seedlings, suggesting that both vegetation control and protection against mammal and bird predation are unnecessary in restoration efforts. Red oak and bur oak seem to be promising species for forest restoration of abandoned fields by direct seeding, but other large-seeded species, such as walnut and hickory, should be tested to confirm the potential of using large-seeded species for direct seeding in forest restoration projects.

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Conflict of Interest

The authors declare no conflict of interest.

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