APP

Indicators of population viability in red spruce, *Picea rubens*. I. Reproductive traits and fecundity

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Abstract: Red spruce (Picea *rubens* Sarg.) has experienced a substantial decline across most of its range in eastern North America over the past century and probably also in the disjunct Ontario populations where it now occurs only in small isolated stands. Measurements of cone and seed traits from natural populations were used as indicators of the reproductive and genetic status of red spruce across the northern margins of its range in Canada. Cone and seed traits were quantified to provide reproductive benchmarks for assessing and monitoring population viability. Reduced fecundity and seedling height growth were observed in some of the smallest Ontario populations, suggesting some inbreeding depression in both vegetative and reproductive components of fitness. Nevertheless, the reproductive status of these small isolated Ontario populations compared favorably with the much larger, more extensive Maritime populations in Nova Scotia and New Brunswick. Significantly higher proportions of aborted (nonpollinated) seeds and lower proportions of tilled seeds suggested poorer pollination conditions in the Maritimes in 19%. The proportion of empty seed, which was used to estimate inbreeding levels, was significantly and negatively related to seedling height growth. In the short-term, the Ontario populations, which probably represent relatively recent remnants of a broader past distribution, generally appeared to be quite resilient to the effects of small population size on fecundity and progeny fitness. In the longer term, continuing decline in population sires and numbers may be expected to erode reproductive success and genetic diversity through the effects of inbreeding, genetic drift, and changes in mating behavior. The reproductive indicators described here have general validity for assessing and monitoring reproductive and genetic aspects of population viability in conifers.

Key words: *Picea rubens*, reproductive success, reproductive fitness indicators, inbreeding, population viability, conservation.

Résumé : Au cours du demier siècle, l'épinette rouge *(Picea rubens* Sarg.) a connu un déclin substantiel sur l'ensemble de son aire de distribution dans l'est de l'Amérique du Nord, et probablement également dans les populations disjointes de l'Ontario, où on ne la retrouve que dans de petites stations isolees. Les auteurs ont utilisé la mesure des cônes et des caractères des graines de populations naturelles comme indicateurs des statuts génétiques et reproducteurs de l'épinette rouge, le long de ses limites nordiques au Canada. Ils ont quantifié les caractères des cones et des graines afin d'établir des points de reference pour évaluer et suivre la viabilité des populations. Ils ont observt une reduction de la fécondité et de la croissance en hauteur des plantules dans certaines des plus petites populations de l'Ontario, ce qui suggère une depression par consanguinid dans les caractères d'adaptation végétatifs ainsi que reproductifs. Cependant, le statut reproductif de ces petites populations isolées de l'Ontario se compare favorablement avec celui des populations beaucoup plus grandes des Maritimes, en Nouvelle-Écosse et au Nouveau Brunswick Des proportions significativement plus élevées de graines avortées (non-pollinisées) et de plus faibles proportions de graines pleines suggèrent que de mauvaises conditions de pollinisation seraient survenues en 1996, dans les Maritimes. La proportion de graines creuses, qui a été utilisée pour évalué le taux d'autofécondation, est significativement et négativement reliée à la croissance des plantules en hauteur. A court terme, les populations d'Ontario, qui représentent probablement les vestiges récents d'une plus vaste distribution dans le passe, semblent bien réagir aux effets d'une population réduite sur la fécondité et l'adaptation de la progéniture. A long terme, on pourrait s'attendre à ce qu'un déclin continu des populations, en nombres et en dimensions, mine le succès de reproduction et la diversite génétique par ses effets de consanguinité, par la derive génétique, et par des changements du comportement reproducteur. Les indicateurs reproductifs

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utilisés ici sont généralement valables pour évaluer et suivre les aspects reproductifs et génétiques de la viabilité des populations chez les conifères.

Mots clés : *Picea rubens*, succès reproducteur, caractères des cônes et des graines, fécondité, vigueur des plantules, indicateurs d'adaptation reproductive, consanguinité, viabilité des populations, conservation.

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Introduction

Red spruce (*Picea rubens* Sarg.) has historically been an important and characteristic component of the Acadian Forest Region of eastern Canada (Rowe 1972). The geographical range of red spruce extends along the Appalachian Mountains from North Carolina and Tennessee to Prince Edward Island in the north (Blum 1990). However, red spruce has declined to a point where it is becoming increasingly uncommon across large portions of its former range. Current site occupancy of red spruce has been estimated at between one-tenth (Korstian 1937) and one-fifth of its former extent (Gordon 1996) in terms of population sizes, numbers, and geographical distribution. Red spruce has a highly fragmented distribution across most of the range depicted in Fig. 1 (adapted from maps presented by Blum 1990; Gordon 1994). It occurs primarily as isolated montane remnants across much of the southern half of its Appalachian distribution (Korstian 1937; DeHayes and Hawley 1992; Hawley and DeHayes 1994; Gordon 1994), where it is associated with the high atmospheric moisture conditions of cloud-capped upper slopes (Johnson and Siccama 1983) and relatively undisturbed forest conditions (McGee and Birmingham 1997). The area of continuous distribution and the center of the range is now limited to a small portion of southeastern Quebec, southern portions of New Brunswick, Nova Scotia, and the northern New England states (McLaughlin et al. 1987). Adverse environmental, atmospheric, and climatic changes have been associated with red spruce decline (Johnson and Siccarna 1983; Friedland et al. 1984; McLaughlin et al. 1987; Hamburg and Cogbill 1988; Eagar and Adams 1992; Strimbeck et al. 1995). Forest harvesting by clear-felling and a long history of selective removal, particularly during the 1800s, have clearly eroded population sizes and numbers (Korstian 1937; Seymour 1992; Gordon 1994, 1996; Leak and Smith 1996).

In Canada, red spruce extends from the Atlantic coast of Nova Scotia across the uplands of southeastern New Brunswick and the lower St. Lawrence River Valley into the Laurentian Mountains of Quebec. The westernmost extension of red spruce occurs as a disjunct and highly fragmented metapopulation associated with the Algonquin Highlands of southern Ontario. This population is characterized by small, widely scattered, isolated stands or patches separated both by geography and the prevailing winds from the center of red spruce distribution in the New England states and the Maritime provinces of Canada. Red spruce is now recognised as a declining and rare species in Ontario and there is growing interest in its conservation and restoration. The decline of red spruce is occurring at a time when anticipated climate warming suggests a possible range expansion northwards and a greater ecological and commercial role for red spruce in the northern parts of its range. The loss of red spruce forests reduces silvicultural options for timber and habitat management based on partial harvesting systems at a time of increasing public demand for management alternatives to clear-felling.

Seed traits, such as the number of filled seeds per cone, seed efficiency (Owens 1995), the ratio of filled seed weight to cone weight (a measure of reproductive efficiency), the proportions of filled seed, empty seed, and aborted ovules per cone, and seedling growth performance, provide measures of reproductive fitness and success in conifers. Measures of reproductive traits along with genetic diversity measures can serve as indicators of reproductive and genetic status for assessing and monitoring the viability of populations at risk because of small population size, low within-population densities, and population isolation resulting from forest fragmentation (Mosseler and Rajora 1998). However, adequate benchmark information on these reproductive traits is generally lacking in conifers, and for red spruce in particular (MacGillivray 1967; Schopmeyer 1974; Powell 1975). Our objective was to establish some reproductive benchmarks in red spruce and to use this information to compare seed yields, seed quality, and progeny fitness among the small, isolated populations of Ontario and the larger, more extensive Maritime populations as a contribution to understanding the reproductive status of this species across the northern margins of its range.

Materials and methods

Red spruce populations and sampling procedures

Cones were collected from 15-20 dominant and codominant red spruce trees from five stands or populations in the Maritime provinces of Nova Scotia and New Brunswick and from five stands in Ontario (Fig. 1; Table 1). The sampled populations were all located within a similar range of latitude and elevation. Average tree heights were similar across the range, but the stand average and maximum tree diameters were much larger in Maritime populations and the stand average and maximum ages were higher in the Maritimes. The Ontario red spruce populations had a reasonably good cone crop in 1996 with many of the dominant and codominant mature trees bearing a collectable cone crop. However, the Maritime region experienced a relatively poorer cone crop that may have been related to a heavy cone crop in the previous year (1995).

The Ontario populations consisted of small, remnant stands or patches, often with fewer than 40-50 widely spaced trees per stand within upland hardwood forest mixtures. These small stands were isolated from the nearest neighboring stands, separated by several kilometres (e.g., Haliburton Forest, Bruton-Clyde Reserve, and Centennial Ridges) and in some cases by much longer distances (Fig. 1) that probably restrict pollination, seed dispersal, and gene flow among stands (e.g., Gloucester Township and Blythe Township). The largest and most extensive population exists in the Bruton-Clyde Red Spruce Reserve in Algonquin Park, where cone collections were made from a small patch of red spruce that was

Fig. 1. Location of the red spruce study populations across the Maritime and Ontario regions. RL, Rossignol Lake; AL, Abraham Lake; QR, Quiddy River; HR, Hurlett Road; BB, Blowdown Brook; GT, Gloucester Township; HF, Haliburton Forest; BC, Bruton Clyde Reserve; CR, Centennial Ridges; BT, Blytbe Township.

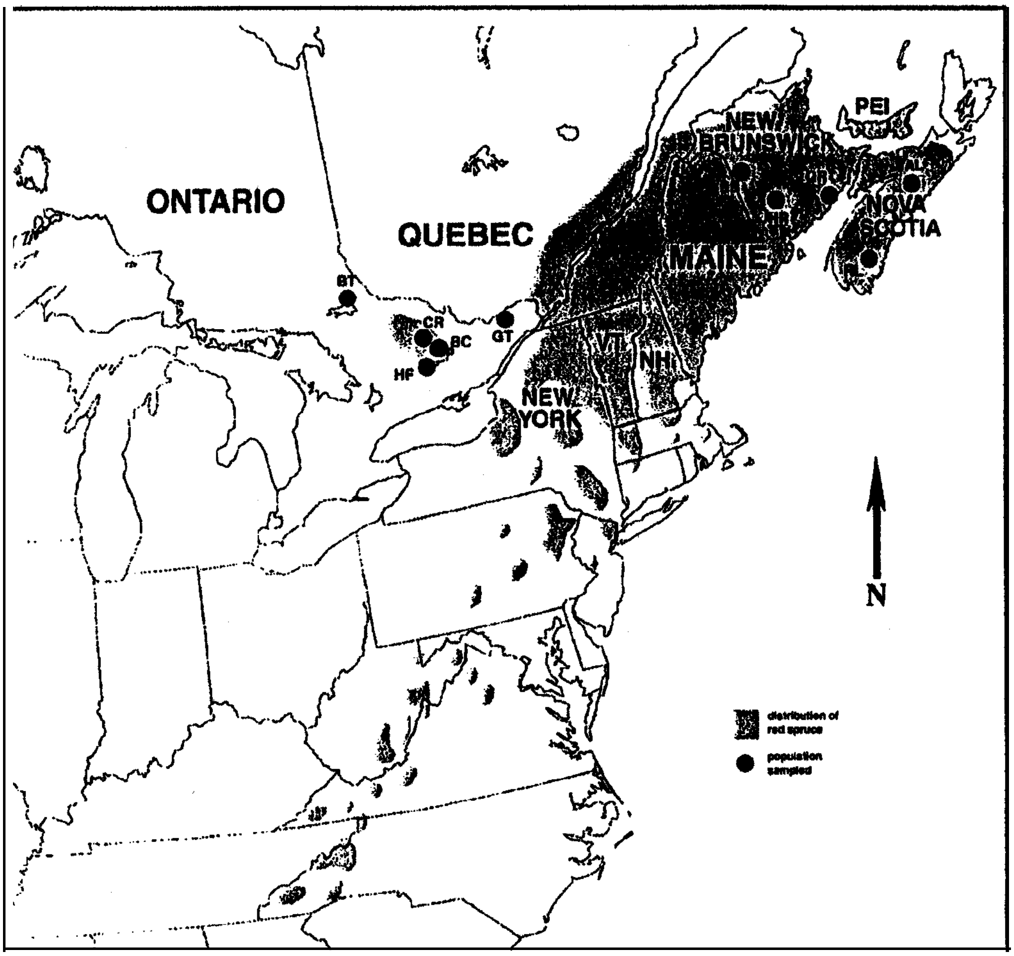


Table 1. Geographic coordinates, number of trees sampled, and mean (and range) for height, diameter at breast height, and tree age for sampled red spruce populations.

Location of populations	Latitude (W)	Longitude (N)	Elevation (m)	Number of trees sampled	Heigbt (m)	Diameter at breast height (cm)	Age (years)
Maritimes							
Rossignol Lake, N.S.	45°08	65°14′	100	15	26 (23-29)	46 (31-66)	136 (100-245)
Abraham Lake, N.S.	45°10′	62°38	185	15	24 (20-29)	46 (35-79)	210 (70-296)
Quiddy River, N.B.	45°31′	65°12′	100	15	21 (19-23)	46 (37–67)	140 (70-177)
Hurlett Road, N.B.	46°07	66°39′	185	20	18 (12-22)	36 (19-49)	133 (93-182)
Blowdown Brook, N.B.	46°41′	67°36′	380	20	23 (19-29)	35 (29–45)	109 (83-155)
Average					22.6	42.0	146
Ontario							
Gloucester Township	45°21′	75°32′	80	16	27 (23-32)	35 (22-50)	95 (80-130)
Haliburton Forest	45°13	78°35′	185	15	23 (10-33)	34 (15–50)	114 (48-180)
Bruton Clyde Reserve	45°17′	78°17	460	16	23 (19–33)	34 (24-51)	118 (70–188)
Centennial Ridges	45°34′	78°25	510	15	19 (11-30)	28 (22–43)	88 (60-148)
Blythe Township	46°32′	79°32′	380	17	23 (21-28)	41 (33-50)	121 (85-152)
Average					22.1	34.4	107

part of a highly fragmented continuum of similar small patches associated with a steep gorge covering an area of about 60 ha and consisting of a total population estimated at fewer than 1000 trees. The southern townships of Algonquin Park and some of the immediately adjacent townships to the west contain the highest concentration of red spruce in Ontario. These forests have been regularly harvested since the 1800s and the existing red spruce probably represents only a scattered remnant of a more continuous, past distribution throughout this area (Gordon 1957, 1976). The smallest population in Gloucester Township consists of 36 widely scattered mature trees within a mixedwood stand. Small population sizes in Ontario forced us to limit our sampling to between 15 and 20 cone-bearing trees per population in order to sample all cone bearing trees within our defined stand boundaries and to keep sample sizes relatively uniform for reproductive assessments based on analyses of variance in cone and seed traits.

The Maritime populations generally consisted of large, extensive stands that normally consisted of several thousand mature trees capable of contributing to the reproductive pool. The Nova Scotia red spruce populations at Abraham Lake and Rossignol Lake represent relatively undisturbed “old growth” forest stands dominated by red spruce of all ages (including trees presumed to be well over 300 years old) on sites that have not been harvested for some time-if ever; whereas most of the other populations have been affected by some selective harvesting in the past. Although trees from the Maritime populations were generally older and larger in diameter (Table 1), the most distinguishing features at the population level were the larger stand sizes, greater stem densities, and greater prominence of red spruce as a proportion of the forest. For instance, the Nova Scotia red spruce stands contained several thousand mature stems, at stem densities of more than 100 mature trees per hectare. Thus, Maritime stands could be expected to experience a much larger pollen pool, favoring increased levels of outcrossing and gene flow.

Cones were collected at their natural ripening time during September 1996 by climbing trees and sampling the entire upper tree crown. Five samples of four undamaged cones (20 cones/tree) with no external signs of physical defects or insect infestation were separated into individual paper bags from each parent tree at the time of cone collection. Cones were stored in a rodent-proof storage facility to allow for after-ripening, drying, and natural opening under ambient conditions.

Seed extraction and processing

Seeds with developed or partially developed, rotund (e.g., enlarged) seedcoats (sensu Bramlett et al. 1977) were collected by hand-shaking each cone sample in a plastic container. Cones were then soaked for 2 h in warm tap water and oven dried at 40°C for 20 h; this was followed by a second shaking treatment. Seed wings were removed from the seeds manually by rubbing in a moist cloth bag. Subsequently, seeds were removed from the seedlot by aspiration. Seeds with developed seedcoats were separated from the remaining debris by hand sorting and were then stored in plastic vials at 4°C for further processing. A count of seeds with developed or enlarged seedcoats was made prior to separating filled seed from empty seed by flotation in 100% ethanol. A sample of 100 empty (floating) seeds was mushed to verify that no filled seeds were lost through the flotation procedure. The total number and weight of filled seeds in each sample were determined. The number of flat, aborted or unpollinated seeds (e.g., those without enlarged seedcoats) was calculated by subtracting the number of developed seeds (those with enlarged or rotund seedcoats, whether filled or empty) from twice the number of fertile cone scales (Bramlett et al. 1977). The number of fertile cone scales was counted, and proportions of aborted ovules, empty seeds, and filled seeds were calculated in relation to twice the number of fertile cone scales. Following seed extraction, each four-cone sample was oven dried at 105°C for 18 h and weighed to the nearest 0.01 g to obtain the oven-dry cone biomass. Reproductive efficiency was calculated as the ratio of filled seed weight to dry cone weight.

To assess the effects of inbreeding on seed production, we calculated the proportion of developed seeds that were empty (e.g., our quotient of inbreeding depression). The ratio of the number of filled seeds to the number of seeds with enlarged seedcoats was calculated and used as an estimate of outcrossing. Both of these calculated quantities were based on the assumption that empty, full-sized or rotund seeds are largely the result of the deleterious genetic effects of inbreeding on embryo development (e.g., embryo abortion). The strongest evidence for the dramatic effects of inbreeding on empty seed formation in *Picea* comes from controlled self-pollination studies (Mergen et al. 1965; Coles and Fowler 1976; Fowler and Park 1983) that clearly demonstrate that the majority (e.g., generally more than 80%) of empty seeds result from the expression of deleterious genetic effects owing to the increased homozygosity resulting from inbreeding. Potential nongenetic causes for the development of empty seeds include unfavourable clitic effects on pollen germination or ovule development and insect predation within the developing seed (Andersson 1965; Sarvas 1968).

Seed germination was tested using four replicates of 84 seeds per tree (open-pollinated family or seedlot from all populations sampled) sown under glasshouse conditions. Germinated seeds were counted 28 days after sowing, and survival was assessed after 95 days. The young germinants were surveyed for chlorophyll-deficient types to determine the frequency of this recessive trait (Cram 1960; Fowler 1965; Bramlett and Popham 1971) in the parental population and for estimating inbreeding levels in natural populations (Squillace and Kraus 1963). Height growth in the seedling progeny was measured to the nearest cm at 169 days after sowing.

Statistical analyses

Variation in cone and seed traits was assessed based on five samples of four cones per sample per tree for 1.5 trees per population from five Ontario populations and five Maritime populations (e.g., three from New Brunswick and two from Nova Scotia) using the following ANOVA model:

$$[1] \qquad Y_{ijkl} = u + R_i + P_{(l)j} + T_{(j)k} + e_{(ijk)l}$$

where Y_{ijkl} is the seed or cone trait from the lth cone sample (I = 1...5), from the kth tree ($T_{(j)}$ = 1 . ..15), from the jth population ($P_{(l)j}$ = 1...5) within the ith geographic region (R = Ontario or Maritime Canada); u is the parametric mean of the population, and $e_{(ijk)l}$ is the experimental error. The ANOVA for cone and seed traits was based on the GLM program developed by the SAS Institute Inc. (1996) using a fixed effects model.

Covariate regression analysis using region was used to examine seedling height response to reproductive traits (e.g., proportion of aborted or empty seed) in a way analogous to the analysis of covariance of female effects presented by Major and Johnsen (1996), using the model $Y_{ij} = B_0 + B_{0i} + B_1X_{ij} + B_{1i}X_{ij} + e_{ij}$, where Y_{ij} is the seedling height of the jth tree (or population) of the ith region, B_0 and B_1 are average regression coefficients, B_{0i} and B_{1i} are region coefficients, X_{ij} is the independent variable (reproductive trait), and e_{ij} is the error term. In this analysis, three sources of variation were identified: (*i*) reproductive trait (covariate), (ii) region, and (iii) region × covariate. Significant region effects indicate differences in region means (i.e., differences in B_{0i} coefficients, if B_{1i} coefficients are similar) and significant region × covariate effects indicate diffices in the slopes (B_{1i} coefficients) between regions.

Results

Rates and patterns of diameter growth from observations on increment cores taken while determiniig tree ages (Table 1) displayed variable patterns of radial growth over the life span of individual trees. Normally, a period of very slow growth over the first 20-30 years after seedling establishment was followed by variable-length periods (e.g., between 15 to 30 years) of relatively rapid growth followed again by alternating, variable periods of growth suppression and rapid growth. These patterns were particularly evident in the older trees from Maritime stands. Many trees in the Abraham Lake population and at other locations across the northern limits of the range in Nova Scotia also had prominent frost cracks on the lower boles of mature trees, indicating that trees in these northern coastal locations may also be experiencing other climatic stresses. This type of stem damage was not observed in any of the other populations sampled For instance, the most northerly population in Blythe Township, Ontario, regularly experiences extended periods of temperatures below -40°C during winter without sustaining such damage.

Very little of the genetic variation in the number of fertile cone scales or seed weight in red spruce was attributable to a regional effect (Tables 2 and 3). Statistically significant regional differences were detected in cone length and cone biomass (dry weight), but these differences were relatively small. However, regional differences were more prominent in the proportions of aborted, empty, and filled seeds per cone and in reproductive efficiency. Significant and large differences among populations and among trees within populations were present in all cone and seed traits ($p < 0.001$). With the exception of the proportion of aborted seed, most of the genetic variation and differences in cone and seed traits occurred among populations and trees within populations. Significant, positive relationships were generally observed between the number of filled seeds and seed weight, and the various cone size parameters (Table 4) across both regions. However, relationships between either tree age, tree height, or diameter and seed yields per cone, average seed weight, and seed quality parameters such as germination rate and seedling survival were generally weak and not siguificant.

Cone traits were similar between regions with the exception of cone dry weight, which was almost 12% lower in the Maritimes (Table 3). Average 1000-seed weight was also similar for both regions. Maritime populations had significantly higher proportions of aborted seeds (0.43 and 0.25 for the Maritimes and Ontario, respectively) and a lower proportion of filled seeds per cone (0.23 and 0.34, respectively).

To compare some reproductive traits in the Maritime and Ontario regions in approximately the same pollination environment, an adjustment was made for the proportion of aborted seed using covariate regression analysis. The ratio of filled seeds to developed seeds in relation to the proportion of aborted seed indicated an interaction between region and the proportion of aborted seed ($p = 0.025$) (Fig. 2). Further analyses of this relationship showed no significant relationship for the Ontario region ($p = 0.99$) but showed a siguificant negative slope for the Maritime region ($p = 0.001$). As the proportion of aborted seed decreased, the proportion of

Table 2. ANOVA (mean sum of squares and significance lev)r cone and se traits in re ruce.

Source of variation	df	Cone length[a]	Cone dr weight	No. of fertile cone scales	Weight of 1000 seed	Proportion aborted see	Proportion empty seed	Proportio filled see	Inbreedi quotient	Reproducti efficiency
Region	1	4.98**	8.28***	4.5ns	1.6ns	438.4***	56.1***	29.8***	32.4***	213.8***
Population within region	4	4.53***	2.82***	123.7***	420.8***	9.4***	35.6***	84.6***	28.8***	104.4***
Tree within population	70	5.38***	1.01***	202.3***	76.3***	3.2***	4.3***	5.3***	5.1***	2.5***
Experimental error	64–655	0.66	0.12							

Note: ns, not significant at $p = 0.05$; ***, significant at $p = 0.0001$

[a]Values are stated at 10^{-2}.

Table 3. Population means ± SE (and range) for cone and seed traits measured in Maritime and Ontario red spruce stands.

Region and population	Cone length (cm)	Oven-dry cone weight (g)	No. of fertile cone scales	Weight of 1000 seeds (g)	Proportion of Ovules aborted	Empty seeds	Filled seeds	Inbreeding depression quotient	Reproductive efficiency
Maritimes									
Rossignol Lake	3.29 ± 0.05c	1.74±0.06c	54.4±0.9c	3.351±0.058a	0.44±0.02b	0.28±0.01c	0.28±0.02b	0.50±0.02d	0.060±0.003b
	(2.39–4.64)	(0.762.96)	(37.5–70.5)	(25144.196)	(0.18-0.91)	(0.04-0.51)	(0.04059)	(0.24-0.78)	(0.010–0.129)
Abraham Lake	3.33±0.04c	1.76±0.04c	57.1±0.5b	3.100±0.075b	0.52±0.02a	0.33±0.01b	0.14±0.01d	0.73±0.02a	0.030±0.003d
	(2.73–4.14)	(1.13-2.82)	(46.2–66.8)	(1.467-3.910)	(0.28-0.94)	(0.06-0.61)	(o-0.43)	(0.33-1.00)	(0–0.090)
Quiddy River	3.60±0.03a	1.98±0.04a	58.0±0.5ab	3.345±0.042a	0.46±0.01 b	0.34±0.01b	0.20±0.01c	0.63±0.02c	0.040±0.002c
	(3.13-4.65)	(1.36-3.10)	(47.2-71.8)	(2.440–4.152)	(0.26-0.77)	(0.16–0.53)	(0.02-0.46)	(0.34-0.97)	(0.0003-0.096)
Hurlett Road	3.48±0.06b	1.88±0.07b	58.8±0.9a	3.301±0.068a	0.35±0.01c	0.30±0.01bc	0.35±0.02a	0.48±0.02e	0.073±0.003a
	(2.23-4.85)	(0.75–3.50)	(44.2-78.0)	(2.086-4.490)	(0.16-0.54)	(0X3-0.56)	(0.09-0.65)	(0.20-0.86)	(0.022-0.122)
Blowdown Brook	3.15±0.04d	1.44±0.05d	57.7±0.7ab	2.641±0.062c	0.37±0.01c	0.44±0.02a	0.19±0.01c	0.70±0.02b	0.043±0.003c
	(2.254.06)	(0.64–2.48)	(44.8–68.0)	(1.174–3.450)	(0.16–0.64)	(0.19-0.74)	(0.01-0.45)	(0.36–0.99)	(0.002-0.105)
Average	3.37±0.02A	1.76±0.03B	57.3±0.3A	3.134±0.031A	0.43±0.01A	0.34±0.01B	0.23±0.01B	0.61±0.01A	0.049±0.002B
	(2.23-I.85)	(0.64–3.50)	(37.5–78.0)	(1.174-4.490)	(0.16–0.94)	(0.04-0.74)	(o-0.65)	(0.20-1.0)	(O-0.129)
Ontario									
Gloucester Township	3.21±0.04d	1.72±0.05c	55.2±0.8c	2.945+0.058d	0.29+0.01a	0.56+0.01a	0.15+0.01e	0.79+0.01a	0.030±0.002d
	(2.45-3.97)	(0.89-2.84)	(41.2-72.2)	(1.833-4.178)	(0.06–0.70)	(0.23-0.78)	(O-0.37)	(0.58-1 .0)	(0–0.065)
Haliburton Forest	3.37±0.05c	2.04±0.06b	59.1±0.8a	2.841 +0.045e	0.30+0.01a	0.34+0.01d	0.36+0.01c	0.48+0.01c	0.063±0.002c
	(2.26-4.28)	(O-72-2.85)	(37.8-71 .5)	(2.132–4.000)	(0.04-0.58)	(0.14-0.65)	(0.20–0.56)	(0.22-0.70)	(0.020-0.115)
Bruton Clyde Reserve	3.64±0.05a	2.09±0.05ab	57.2±0.8b	3.235+0.050b	0.22+0.01c	0.33+0.01d	0.45+0.01a	0.42+0.01d	0.081±0.002a
	(2.73-4.81)	(1.29-3.00)	(46.2–75.0)	(2.430–4.076)	(0.06-0.41)	(0.14-0.54)	(0.18–0.69)	(0.19-0.70)	(0.031-0.130)
Centennial Ridge	3.48±0.04b	2.00±0.05b	60.1±0.6a	3.666+0.060a	0.25+0.01 b	0.44+0.02b	0.31+0.01d	0.58+0.02b	0.070±0.003b
	(2.73–4.46)	(1.14-3.03)	(48.8-72.0)	(1.967–4.660)	(0.1 I-0.43)	(0.17-0.67)	(0.08–0.60)	(0.24–0.90)	(0.027-0.147)
Blythe Township	2.89±0.03e	2.12±0.04a	55.3±0.4c	3.025+0.039c	0.20+0.01c	0.39+0.02c	0.41+0.01b	0.48+0.02c	0.064±0.002c
	(2.26-3.38)	(1.52–2.85)	(47.2-66.5)	(2.370-3.709)	(0.03-0.43)	(0.1 l-0.71)	(0.16+0.58)	(0.20–0.80)	(0.030–0.108)
Average	3.32±0.02B	1.99±0.02A	57.4±0.3A	3.142+0.027A	0.25+0.01B	0.41+0.01A	0.34+0.01A	0.55+0.01 B	0.061±0.001A
	(2.26-4.81)	(0.72–3.03)	(37.8–75.0)	(1.833–4.660)	(0.03-0.70)	(0.1 I-0.78)	(0–0.69)	(0.19-1.0)	(0–0.146)

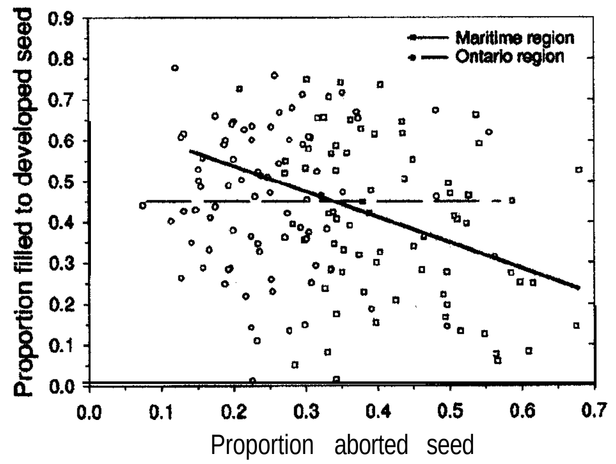
Note: Means with different letters are significantly different (at $p = 0.05$) as determined by Tukey's test. Lower case letters are used for comparisons within regions; whereas upper case letters are used for comparisons among regions.

Table 4. Pearson's correlation coefficients for cone and seed traits in Maritime and Ontario populations of red spruce.

Region and trait	No. of fertile cone scales	Cone length	Cone dry weight	Reproductive efficiency"	Number of filled seeds per cone	Seed efficiency	Weight of 1000 seeds
Maritime3							
Filled seed per cone	0.28***	0.36***	0.40***	0.92***		0.97***	0.35***
1000 seed weight	0.22***	0.58***	0.63***	0.32***	0.35***	0.32***	
Ontario							
Filled seed per cone	0.24***	0.22***	0.37***	0.86***		0.96***	0.12*
1000 seed weight	0.15**	0.42***	0.31***	0.30***	0.12*	0.08ns	

Note: ns, not significant at $p = 0.05$; *, significant at $p = 0.05$; **, significant at $p = 0.001$; ***, significant at $p = 0.0001$.
"Ratio of filled seed weight to cone weight.

Fig. 2. The ratio of filled to developed seeds in relation to the proportion of aborted seeds for individual red spruce trees from the Maritime and Ontario regions. Equations from the analysis of covariance are as follows: y = 0.660 – 0.625x for the Maritime region and y = 0.455 (r = 0.316) for the Ontario region.



filled to developed seeds increased. Thus, given an approximately similar pollination environment (proportion of aborted seed equal to Ontario (0.25)) the ratio of filled to developed seeds for the Maritime and Ontario regions became 0.51 and 0.45, respectively. Following this, the adjusted proportion of filled seed would be 39 and 34% rather than 23 and 34% for the Maritime and Ontario regions, respectively.

Despite these differences in pollination, there was still a significantly higher proportion of empty seeds in the Ontario region than in the Maritime region (0.41 versus 0.34), suggesting lower rates of post pollination abortion in the Maritimes. Using the proportion of empty seed as an estimate or surrogate for inbreeding depression, seedling height showed a significant negative relationship to proportion of empty seed ($p = 0.004$) (Fig. 3A), demonstrating the effects of inbreeding depression on traits related both to growth and fecundity. Covariate regression analysis of individual tree data within populations indicated no regional effect ($p = 0.159$) or differences in slope ($p = 0.632$). When data were pooled by population, covariate regression analysis indicated no regional effect ($p = 0.362$) and a significant negative slope ($p = 0.032$) (Fig. 3B). Rossignol Lake had the lowest proportion of empty seed (Table 3) along with the greatest seedling height (Table 5); whereas Gloucester Township, which is the smallest and most isolated of all the populations studied, bad the greatest proportion of empty seed and the lowest seedling height, suggesting possible inbreeding ef-

Fig. 3. Seedling height in relation to the proportion of empty seed of red spruce from the Maritime and Ontario regions for individual trees (A) and populations (B) as identified in Table 1. Equations from the analysis of covariance are as follows: y = 20.1 – 4.26~ (r = 0.339) for Fig. 3A and y = 20.97 – 6.73x (r = 0.674) for Fig. 3B. RL, Rossignol Lake; AL, Abraham Lake; QR, Quiddy River; HR, Hurlett Road, BB, Blowdown Brook, GT, Gloucester Township; HF, Haliburton Forest; BC, Bruton Clyde Reserve; CR, Centennial Ridges; BT, Blythe Township.

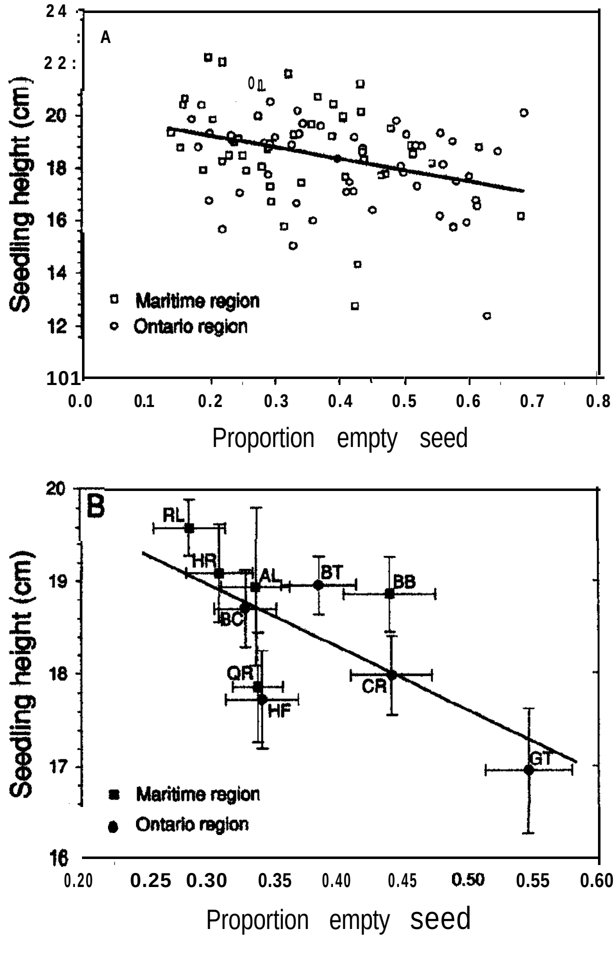


Table 5. Red spruce seed germination, seedliig survival, seedling height, and frequency of chlorophyll-deficient germinants under glasshouse conditions.

Region and population	No. of families tested	Percent seed germination at day 28”	Percent survival at day 95	Height at day 169 (cm)	Frequency of chlorophyll-deficient genotypes^b	
					Parent trees	Progeny arrays^c
Maritimes						
Rossignol Lake, N.S.	15	74.1*b*	72.6*bc*	19.6*a*	3/15	161842
Abraham Lake, N.S.	12	74.66	73.7*ab*	19.7*a*		
Quiddy River, N.B.	15	70.1*c*	70.5*bc*	17.4*bc*	1115	3/302
Hurlett Road, N.B.	15	79.4*a*	78.3*a*	19.1*ab*	1/15	1/296
Blowdown Brook, N.B.	16	78.5*a*	77.0*a*	18.9*ab*		
Maritime region	73	75.3*A*	74.4*A*	18.9*A*	5/73 (0.034)^d	
Ontario						
Gloucester Township	15	77.7*a*	75.8*a*	17.0*c*	4/15	3611287
Haliburton Forest	14	69.1*c*	70.0*c*	17.7*bc*		
Bruton Clyde Reserve	14	79.4*a*	77.9*a*	18.2*ab*	1/14	1/296
Centennial Ridges	15	63.81	64.1*d*	18.0*bc*	1/15	1/226
Blytbe Township	16	71.4*bc*	71.9*bc*	18.5*ab*		
Ontario region	74	72.38	71.9*B*	17.9*B*	6/74 (0.041)”	

Note: Means with different letters are significantly different (at $p = 0.05$) as determined by Tukey's test. Lower case letters are used for comparisons within regions; upper case letters are used for comparisons among regions.

‘Percent seed germination on day 28 after sowing under glasshouse conditions.

“Chlorophyll deficiency expressed in young germinants as either albino or virescent conditions.

‘Progeny arrays from parents heterozygous for chlorophyll deficiency.

^{d}Allele frequencies are given in parentheses.

fects at the Gloucester Township site. Both data sets showed similar equations with the population data showing a slightly steeper slope.

Using the ratio of filled to developed seeds as an estimate or surrogate of outcrossing rate, seedling height from both regions displayed a positive linear regression response to higher ratios of filled to developed seeds (Fig. 4A). Covariate regression analysis indicated no differences in slope between the Maritime and Ontario regions ($p = 0.826$) but indicated a significant region effect ($p = 0.008$); this indicated that the Maritime populations had seedling heights that were approximately 0.9 cm greater across a given proportion of filled to developed seeds. When data were pooled by population, covariate analysis indicated a significant relationship between seedling height and the ratio of filled to developed seeds ($p = 0.055$), as well as a significant regional effect ($p = 0.030$) (Fig. 4B). Equations were similar using both data sets. From the Maritime region, the Hurlett Road and Rossignol Lake populations had among the highest ratios of filled to developed seeds and the greatest seedling heights. From the Ontario region, the Bruton Clyde Reserve population had the highest ratio of filled to developed seeds and among the highest seedling heights. The Abraham Lake and Gloucester Township populations had some of the lowest ratios of filled to developed seeds and seedling heights for the Maritime and Ontario regions, respectively, and also displayed the greatest variation in seedling height.

Red spruce seed germination measured 28 days after sowing under operational conditions in a glasshouse was generally between 70 and 80%, as was seedling survival at 95 days (Table 5). Significant differences ($p = 0.05$) were found in seed germination, survival, and height at day 169 among trees within populations and among populations within regions. Values for Ontario populations were significantly lower than those for Maritime populations for all of these measures of seedling vigour. The frequency of a recessive gene for chlorophyll deficiency was 0.041 in the Ontario region and 0.034 in the Maritime region (Table 5). In Gloucester Township, Ontario, 4 of the 15 trees were heterozygous (carriers) for this recessive, lethal chlorophyll deficiency. In these four heterozygous carriers for the chlorophyll deficiency trait at Gloucester Township, levels of inbreeding calculated using this gene marker (Bramlett and Popham 1971) were estimated to be 11.2% (e.g., 36/1287 × 4) and as high as 14.4% in one tree (GT#6).

Discussion

The proportion of developed seeds that were empty reflects the relative effects of inbreeding on fecundity. This is based on the assumption that empty, full-sized or rotund seeds are largely the result of the deleterious genetic effects of inbreeding on embryo development (e.g., embryo abortion). The strongest evidence for the dramatic effects of inbreeding on empty seed formation in *Picea* comes from controlled self-pollination studies (Mergen et al. 1965; Coles and Fowler 1976; Fowler and Park 1983) that clearly demonstrate that the majority (e.g., generally more than 80%) of empty seeds result from the expression of deleterious genetic effects resulting from increased homozygosity. Furthermore, Mergen et al. (1965) found no full-sized seeds in 99 unpollinated white spruce cones, indicating that pollen is required for seedcoat development in spruces. However, other investigations of seed development in *Picea abies* (Andersson 1965; Sarvas 1968; Mikkola 1969) and *Picea sitchensis* (Owens and Molder 1980*a*) have observed full-sized, empty seeds developing in the absence of any evidence of pollination. Nevertheless, under normal pollination levels in natural populations, the effect of nonpollination on empty seed formation is believed to be relatively minor in comparison with the failure of pollination to lead to fertilization or the deleterious genetic effects of inbreeding that lead to embryo abortion (Sarvas 1968, Andersson 1965). Mikkola (1969) and Sarvas (1968) estimated that 5–10% and 10–15%, respectively, of empty seed was produced in the absence of evidence of pollination. Nevertheless, the stimulus of pollen, even ungerminated pollen or that from another species, is recognized as providing an important stimulus for subsequent ovule development in spruces (Mikkola 1969). Other potential nongenetic causes for development of empty seeds includes unfavourable climatic effects and insect predation within the developing seed (Andersson 1965; Sarvas 1968). These studies (Sarvas 1968; Mikkola 1969; Mergen et al. 1965; Fowler and Park 1983) suggest that our assumption that all empty, full-sized seeds result from embryo abortion may lead to an over estimation of these deleterious genetic effects by up to 20% in spruces. Nevertheless, we believe that calculations of inbreeding effects based on proportions of tilled and empty seeds can be used to estimate relative differences in levels of inbreeding (or outcrossing) in spruces.

The large variation in traits related to tilled seed production among populations and trees within populations is consistent with the important effects of genotype and environmental (primarily climatic) conditions on pollination and fertilization success (Burdon and Low 1973; Krugman et al. 1974; Owens and Molder 19806; Owens and Blake 1985; Owens 1995). Cool, wet weather during pollination can reduce pollination success and viable seed yields. These observations on environmental effects are consistent with the relatively high numbers of nonpollinated or aborted ovules observed in Maritime trees. A large cone crop in 1995 in the Maritimes may also have adversely affected the 1996 cone crop.

The early ovule abortion that produces flattened, undeveloped seeds may result from either the absence of pollen and (or) adequate pollen germination (Mergen et al. 1965; Mikkola 1969; Bramlett et al. 1977; J.N. Owens, personal communication) or insect feeding (Schowalter and Sexton 1990). Neither the presence nor the impact of insect feeding in outwardly undamaged cones was observed or assessed, yet there are several seed pests that are not detectable by examining the external condition of cones but could cause seed damage nonetheless (J. Sweeney, personal communication). For instance, potential predation by species such as the seed bug *(Leptoglossus occidentalis)* could result in empty seeds. This species has been expanding its range eastward and has been reported in southwestern Ontario, but it has not been observed or reported throughout the geographical range covered by this study (Gall 1992). Schowalter and Sexton (1990) showed that such insect feeding had little impact on the development of empty seeds. Therefore, we assumed that any effects on empty seed production owing to the occurrence of seed insect feeding were either absent or consistent across populations and regions.

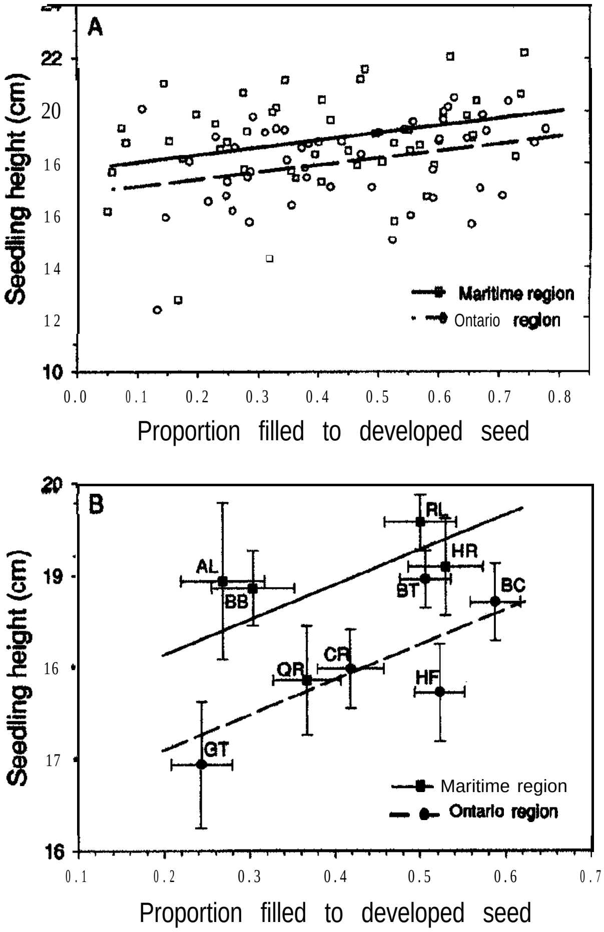


Fig. 4. Seedling height in relation to the ratio of filled to developed seeds of red spruce from Maritime and Ontario regions by individual trees (A) and populations (B) as identified in Table 1. Equations from the analysis of covariance for Fig. 4A are as follows: $y = 17.75 + 2.76x$ for the Maritime region and $y = 16.83 + 2.76x$ ($r = 0.369$) for the Ontario region. Equations from the analysis of covariance for Fig. 4B are as follows: $y = 17.34 + 3.77x$ for the Maritime region and $y = 16.35 + 3.77x$ ($r = 0.767$) for the Ontario region. RL, Rossignol Lake; AL, Abraham Lake; QR, Quiddy River; HR, Hurlett Road; BB, Blowdown Brook; GT, Gloucester Township; HF, Haliburton Forest; BC, Bruton Clyde Reserve; CR, Centennial Ridges; BT, Blythe Township.

The relatively poor cone crop across the Maritime region, the unusually large portion of total variance (e.g., a variance component of 5 1.3%) in aborted seeds attributable to the regional effect, and the significant regional difference in the proportion of aborted seeds appear to be the result of poor pollination conditions and (or) a poor pollen cone crop. The negative relationship between the ratio of filled to developed seeds and the proportion of aborted seeds in the Maritimes (Fig. 2) indicates that some individuals had very low levels of pollination and that a better pollination environment may have improved the ration of filled to developed seeds (Mikkola 1969). The absence of a similar relationship in the Ontario region suggests that the pollination environment was probably close to optimum. Poor pollination conditions would most likely affect coastal Maritime populations such as Abraham Lake and Quiddy River, which are often affected by cool, wet conditions during the pollination period. These populations had the highest proportions of seed abortion among the Maritime populations.

Inbreeding effects were most clearly demonstrated by the relationships between seedling height and both the proportion of empty seeds and the ratio of filled to developed seeds, Seedling height displayed a 13.7% decrease in relation to proportions of empty seeds ranging from 0.15 to 0.70 (Fig 3A). This demonstrates a consistent impact of inbreeding depression on both reproductive success and growth. Across all populations, the proportion of empty seeds and inbreeding depression was highest, and the proportion of filled seeds and reproductive efficiency were lowest (Table 3), in the smallest and most isolated Ontario population in Gloucester Township; consistent with our hypothesis that inbreeding effects on fecundity would be most apparent in such small, isolated populations. Similarly, seedling height increased an average of 11% from a filled to developed seed ratio of 0.1 to 0.8 across all individuals for both regions. As an estimate of outcrossing level, the increase in the ratio of filled to developed seeds clearly showed the positive relationship to seedling vigor.

While the frequency of the recessive chlorophyll deficiency trait (Table 5) was too low to adequately assess either population or individual inbreeding levels, the trend towards relatively higher levels of inbreeding in the Gloucester Township population corresponded with estimates of inbreeding obtamed from seed data, depressed seedling growth performance, and allozyme-based estimates of population mating behavior (Rajora et al. 2000). Positive correlations among seed weight and traits related to filled seed production (Table 4) and cone size traits (e.g., cone weight, cone length, and number of fertile cone scales) are consistent with similar relationships reported in spruces and other conifers (Andersson 1965; Sarvas 1968; Stoehr and Farmer 1986; Mosseler et al. 1992). These relationships may be important in assessing reproductive success and progeny fitness (Table 6) because higher seed yields per cone and larger seeds tend to produce more vigorous seedlings (Andersson 1965; Sarvas 1968; Dunlap and Barnett 1983).

Although red spruce and black spruce can hybridize under natural conditions, the level and role of introgression are likely to be minor under the characteristic habitat and ecological conditions of the parental species (Manley 1972; Gordon 1976; Manley and Ledig 1979; Morgenstern et al. 1981). In a study of *Picea rubens* × *Picea mariana* hybridization in Ontario, Gordon (1976) found very few intermediate types. This corresponds with our own observations in natural populations of red spruce where intermediate types were sometimes observed in situations (e.g., Halibut-ton Forest, Bruton-Clyde Reserve, and Blythe Township populations) where upland red spruce came into contact with lowland sites containing black spruce (cf. Manley 1972). In contrast to field and laboratory observations based on morphological traits, molecular marker-based approaches have suggested a much broader hybrid zone extending over several thousand kilometres in eastem Canada (Perron and Bousquet 1997).

Hybrid indices have been developed for separating red spruce and black spruce from their hybrids (Manley 1971; Gordon 1976; Bobola et al. 1996; Perron et al. 1995). The rangewide uniformity observed in the number of fertile cone scales suggests that this trait may also be a useful diagnostic morphological feature for separating the parental species and hybrids and that there was little evidence of introgression in Ontario red spruce. The comparatively larger cone length observed in Ontario red spruce also suggests a limited effect of introgression with black spruce. The maximum length for cones measured by Gordon (1976) from 352 black spruce trees was 28 mm, whereas red spruce cones ranged from 25 to 49 mm,. The average cone lengths from only 10 of the 150 red spruce trees measured from 10 different populations (based on measurements of 20 cones per tree) were less than 28 mm. Five of these trees came from the most northwesterly population in Ontario at Blythe Township. A similar clinal effect in cone length was observed in the most northerly Maritime population at Blowdown Brook, which also had the shortest average cone length (31.5mm). The minimum cone length measured in our sample was 24 mm and the maximum was 45 mm. Seed weight also appears to be a potentially useful morphological feature, but it can also be under some environmental control related to plant nutrition and site quality (Burdon and Low 1973). In only 3 of 150 trees sampled did the average weight of 1000 seeds fall below the maximum value (2.0518 g) measured by MacGillivray (1967) for black spruce, indicating a minimal impact of introgression in Ontario red spruce.

The periodic fluctuations in diameter growth observed in red spruce were similar to patterns described in Appalachian red spruce populations (Van Deusen 1990, Reams 1995; Stephenson 1995). These fluctuations can probably be attributed to stand disturbances resulting in increased light availability for short periods of time (e.g., of 15-30 years duration). Despite its high shade tolerance and association with relatively undisturbed forest conditions, red spruce appears to be very responsive to increased light levels (Blum 1990), provided that relatively high atmospheric moisture conditions can be maintained. The ability of red spruce to withstand prolonged periods of suppression under low light conditions and its apparent ability to respond to increased light levels at virtually any age following disturbance (Korstian 1937) make it difficult to predict tree age based on stem diameter in “old growth” red spruce forests (McIntosh and Hurley 1964) or to use such age-size relationships in predicting site index (Blum 1990). However, as a long-lived, shade-tolerant species, red spruce seems well suited to dendrochronological studies of stand disturbance events (Reams 1995). The long-lived nature of red spruce gives it an important competitive advantage over its primary coniferous competitors, such as balsam fir (*Abies balsamea* L.), white spruce (*Picea glauca* (Moench) Voss), and black spruce (*Picea mariana* (Mill.) BSP). Despite long periods of suppression under a closed forest canopy, red spruce can outlive these competitors (McIntosh and Hurley 1964; Davis 1966). This was most evident in the relatively undisturbed old growth forest remnants in Nova Scotia where most of these competitors, including such dominant trees as white pine (*Pinus strobus* L.), were breaking-up and dying out as a result of advanced age, leaving red spruce and eastern hemlock (*Tsuga canadensis* (L.) Carriere) to dominate these relatively undisturbed forests.

Red spruce appears able to endure extended periods of very low winter temperature without experiencing damage to its foliage or stems (e.g., Blythe Township, Ontario population) but may be less tolerant of the mid-winter thaws fol-

Table 6. Potential indicators of reproductive and genetic status in trees based on cone and seed traits.

Population viability criteria'	Indicators
Reproductive status	Cone weight
	Number of filled seeds per cone
	Seed efficiency (per cone ratio of number of filled seeds to number of fertile ovules)
	Proportions of filled, empty, and aborted seeds per cone
	Reproductive efficiency (ratio of filled seed weight to cone weight)
	Seed quality (seed weight, germination rate, and germination speed)
	Seedling vigour (percent seedling survival and growth performance)
	Inbreeding quotient (ratio of empty seeds to seeds with developed se&oats)
	Inbreeding estimates based on morphological marker genes (e.g., albino frequencies)
Genetic status	Genetic variances
	Presence of rare alleles
	Gene frequencies and genetic drift
Minimum viable population size	Population size (numbers of mature individuals)
	Within population density
	Population distribution at the landscape level (fragmentation)

lowed by subsequent freezing events (Strimbeck et al. 1995) that are more common in maritime environments. The increased climatic variability projected for the Maritime provinces (Shaw 1997) may result in increased winter damage, such as that observed at Abraham Lake, to stems of red spruce.

The decline of red spruce in the more continental parts of its range in Ontario and Quebec may have been hastened by clear-felling practices that probably favored the more aggressive balsam fir, some of the less shade-tolerant congeneric associates of red spruce such as black spruce and white spruce, and the many shade-tolerant hardwood species that compose mixedwood stands (Gordon 1976, 1994, 1996; Seymour 1992). The selective removal and (or) loss of red spruce from the upland shade-tolerant hardwood forest mixtures described by Leak and Smith (1996) for the northeastern states of the United States is also evident across the Canadian range, where clearcut harvesting of upland hardwood forests probably reduces the high atmospheric moisture conditions required for optimal growth (Fowler et al. 1988; Blum 1990). The upland forests in the vicinity of Blowdown Brook in central New Brunswick best illustrate this situation where only isolated individuals or patches of red spruce now exist in hardwood forests that probably contained much higher proportions of red spruce in the past. In this area, red spruce has now been relegated to steep gorges that were largely inaccessible to economical harvesting. On these upland forest types, the coppicing ability of hardwoods enables them to recapture sites following clearcutting at the expense of conifers such as red spruce, which do not maintain persistent or sufficient soil seed banks from which to re-establish themselves (Frank and Safford 1970). In the absence of advanced regeneration and (or) a viable seed source, red spruce can be largely eliminated following the clearing of upland forests, particularly as one moves inland away from the high atmospheric moisture conditions of coastal areas.

Reproductive traits such as filled seed yields per cone (reproductive success), reproductive efficiency, proportions of aborted ovules, empty seeds, and filled seeds, seed germination rate, early seedling survival, and seedling vigour represent useful measures for assessing the reproductive status of trees in populations at risk because of small population sizes, low within-population densities, and population fragmentation levels that may hinder dispersal and gene flow among populations. The measures of reproductive fitness and success reported here can also be used to make inferences about the genetic status of populations since levels of fecundity reflect inbreeding levels, tolerances to inbreeding depression, and support the maintenance of gene pool diversity. Some of the potential indicators of population viability that can be derived from seed analyses are summarized in Table 6. These indicators can also be used to estimate minimum viable population parameters such as population size, stem densities within populations, and levels of population fragmentation in relation to their effects on reproductive fitness. Furthermore, these reproductive traits are relatively easily measured, are scientifically meaningful with respect to reproductive fitness assessments, and may provide useful indicators for monitoring biological processes important to population viability analysis in trees (Mosseler and Rajora 1998).

Conclusions

If we accept measures on reproductive traits obtained from the larger, more extensive Maritime populations of red spruce-despite relatively poor pollination conditions-as benchmarks of reproductive capacity, then we may conclude that the small, isolated stands of the disjunct red spruce populations of central Canada have generally been able to maintain their reproductive output at close to optimum levels. The Ontario populations probably represent remnants of what was once a more continuous distribution of red spruce that existed prior to European settlement. Our results suggest that in the short-term, such small remnant stands can maintain surprisingly high levels of reproductive success. However, in the longer term, these small population remnants may experience reduced fecundity as they adjust to the effects of reduced pollination levels and inbreeding depression on fecundity. This process of adjustment to higher levels of inbreeding may result in losses of genetic diversity through inbreeding and genetic drift (e.g., the chlorophyll-deficiency trait at the Gloucester Township site) in small populations. Such small populations will also be at increased risk of elimination through stochastic events (Shaffer 1981; Gilpin and Soule 1986). The Gloucester Township population may already be showing signs of such reproductive and genetic decline in the quality of its progeny and the absence of natural regeneration. Reductions in viable seed yields per cone, seed vigour, and seedling growth performance are consistent with the effects of inbreeding depression expected in such small, isolated populations where either the amount and (or) quality of pollen may be limited.

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