

FILE REPORT 56

# Regeneration of Boreal Conifers from Seeds and Cones: A Role for Transported Materials

J.C. Marshall, B. Downie, C.A. Whittle, H. Manning, R.G. Rutledge and B.S.P. Wang



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The views, conclusions, and recommendations contained herein are those of the authors and should be construed neither as policy nor endorsement by Natural Resources Canada or the Ontario Ministry of Natural Resources.

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### **REGENERATION OF BOREAL CONIFERS FROM SEEDS AND CONES:** A Role for Transported materials.

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### ABSTRACT

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Higher levels of seedlings germinated from seeds or cones on scarified soil compared to those sown on undisturbed soil. Naked seeds showed germination within 120 days of sowing and the dimensions of the scars had a significant effect on germination ratios. Remarkable variation in germination success was observed in jack pine and black spruce seed that were sown in adjacent replicate scars located close together on plots with little physiographic variation. In the scars where live seedlings were observed to persist, the seedlings had clearly grown from beneath wind- or rain-borne materials such as soil, leaves, or other debris. The effect of transported materials was simulated using known amounts of wood chips, sawdust, perlite, vermiculite, or local soil. Higher Seeding ratios were observed in scars covered by organic or mineral materials on sites where most uncovered seed perished during the first season. Hand picked cones, or a mulch of cone-bearing delimbing wastes commutated by a hammer mill, produced a small number of new seedlings in the first season and in the season or year following application. With time, black spruce cones displayed seedlings establishment ratios significantly higher than those of naked seed while pine-cone seeding ratios were comparable to those of naked seed.

#### INTRODUCTION

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Seedling establishment from direct seeding seems to vary with the seedbed, site, climate and timing of seed application (Fleming and Mossa, 1994). Germling establishment can vary sharply between closely adjacent but apparently similar microsites. The absence of data to explain and predict the nature and effect of microsite variability on germling establishment remains a serious hindrance to the design of protocols for seeding black The role of variations in temperature, humidity, soil moisture, and spruce on uplands. light regime remain to be quantified. The presence of organic material on top of the mineral soil may prevent direct contact of the seed with mineral soil and thus hinder establishment (Fleming and Mossa, 1995). However, the role of materials transported overtop of naked seed has not been clarified. Herein, high levels of seedling establishment were observed from beneath wind or rain transported materials and hence the hypothesis that natural transport may account for some the variation in direct seeding success was Experiments with hundreds of seeds, or cone equivalents, sown in closely adjacent tested. replicate scars designed to limit variation in temperature, humidity, light and soil moisture, were used to examine the effect of transported materials on seedling establishment. Seeds buried beneath organic or mineral materials had greater seeding ratios compared to uncovered seed on the exposed soil surface. Cones sown in scars were apparently capable of releasing viable seeds over time resulting in germination of seeds in the second season after many seedlings from the naked seed had perished.

### **MATERIALS AND METHODS**

### PLANT MATERIALS

Whole cones for testing the hammer mill were obtained from mixed collections of the provincial seed plant in Angus, Ontario. Purified, heat-extracted seed-standards (≥97% viable seed) were obtained from mixed collections of seeds obtained from the Petawawa National Forest Research Institute (PNFI) in Chalk River, Ontario. Cone-bearing-branches for field trials were collected from local slash piles at cut overs near Shinning Tree and Iroquois Falls Ontario.

### HAMMER MILL

Cone-bearing branches were commuted to a mulch with a 30-cm-drum, Farm King hammer mill powered by a 5 hp, triple-phase, electric motor running at 3200 rpm with 4:1 gear ratios to produce mill speeds of 875 rotations per minute. A full hopper of cone bearing branches were commuted on each run. Hammers were of factory standard length and square cut. The hammer mill was set up and tested using hand picked cones with the screen sizes indicated.

### SEED TESTING

Cones or cone parts were heated 3x at 60°C for 24 h, vigorously agitated, and the extracted seeds combined. Live-seed counts of heat extracted cones were made on elevated kimpacs set in plastic trays and incubated in a growth chamber with a 5 mM solution of benlate (a commercial antifungal compound). Seed from mechanically produced mulches was screen-purified and live-seed counted on by incubating for several weeks on peatmoss in open trays saturated with the solution of benlate. Seed for x-rays analysis were prepared

with an atmosphere of saturated chloroform in an upright 50 ml beaker covered by an inverted 150 ml beaker that also enclosed cotton balls soaked with 15 ml of chloroform and the seed were equilibrated for 3 hours at room temperature. The seeds resting on Kodak x-ray film were then exposed to a an ionizing radiation source after the method described by the International Rules for Tree Seed Testing (International Seed Testing Association, 1993).

### FIELD TRIALS

In order to limit the effect of site, hundreds of seeds were distributed on each of the replicate scar that were located close together on small plots, i.e. ≤ 10m x 10m, that displayed limited physiographic variation. The rationale for this design is that variation of in humidity, soil moisture, temperature, and light would be reduced by establishing multiple test scars in close proximity on an even surface. Plots were selected to minimize variation in slope, soil type and vegetation type. Experimental plots were arranged as Latin squares. Each replicate treatment scar received 150-300 black spruce seed or 125-250 jack pine seed as indicated. Trials were established in April, May June, July or October 1994 & 1995 as indicated. The upland experimental sites selected were challenging locations for direct seeding of black spruce.

# Iroquois Falls Experimental Site

Seeds were distributed on 25 cm long scars of the indicated width and 2.5 depth made with a flat shovel and hoe on elevated, slow growing moss over shallow bedrock. Competing vegetation was removed by hand. The site was located in the Abitibi Model Forest about 22 km north of the Abitibi River bridge on the Northwest Industrial Road

from Iroquois Falls, Ontario, Canada (Map 1a).

### Shinning Tree Experimental Site

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Seeds were distributed on 25 cm<sup>2</sup> application spots in power disc trencher rows, or intervening hillocks, in a fresh sandy loam 1 km south of the junction of Highway 144 and the main entrance of the E.B. Eddy timber limits near Shinning Tree, Ontario, Canada (Map 1b).

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### RESULTS

### **PREPARATION OF CONES**

Cones for regeneration studies were prepared by mechanically commutating conebearing foliage, or by hand-picking cones, collected from local slash piles. A hammer-mill platform that supported a variety of speed settings, retention screen sizes and hammer types was assembled to mechanically prepared cone-bearing foliage for this purpose.

Mechanical Preparation of Black Spruce Cones using retention screen sizes of  $\geq 2.5$ cm and mill speeds of 875 rpm produced mostly intact cones. Retention screens of 2 cm or less removed many cone scales and thus released some live seed. The viability of some black spruce seed released by mechanical extraction as measured by x-ray analysis (not shown) was also confirmed by germination tests (Figure 1a).

Mechanical Preparation of Jack Pine Cones using retention screen sizes of  $\geq$  3.5 cm combined and mill speeds of 875 rpm produced c. 50% intact cones but most cones were damaged using screen sizes of less than 3.5 cm (Figure 1b). In contrast to black spruce, virtually all seed released from cones at reduced screen sizes were damaged as measured by x-ray analysis and confirmed by germination tests.

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# EFFECTS OF SCARIFICATION

Seeds or cones sown on scars in sandy soil and on elevated peat, showed greater stocking compared to seeds sown on the directly adjacent, undisturbed soil surface (Figure 2). Significantly greater numbers of 120 d black spruce seedlings were observed in narrow rectangular trenches that in square scars (Figure 3). In general seeding success was found to depend on the presence and type of scarification.

# OBSERVED NATURAL VARIANCE IN SEEDLING SUCCESS AND TRANSPORTED MATERIALS

Similar replicate scars in small plots selected to minimize variability in surface gradient and soil type often displayed remarkable variation in the germination of seeds. A minority of the scars were stocked with a high number of seedlings by the application of naked seed but adjacent scars of similar depth and character one meter away were observed to contain an order of magnitude fewer germlings or found completely empty. For example, 300 black spruce seed sown over each replicate scar in the same small plot, displayed 120 d seedlings that varied from a maximum of 39 to a minimum of zero with a modal value of 3 and were not normally distributed as measured by the Shapiro-Wilk test (Figure 4a). Similarly, the jack pine data depicted in Figure 4b shows a scar with greater than 50 successful seedlings directly adjacent to empty scars. This unusual pattern of variation indicated that some important environmental factor(s) varied sharply between similar, handmade scars only a few meters apart. Visual observation of highly productive scars of both species revealed that successful seedlings were growing from below rain- or wind-borne mineral or organic materials.

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# THE EFFECT OF SIMULATED TRANSPORTED MATERIALS

The effect of materials that might be transported over seedbeds on the subsequent germination of seeds were simulated using a known depth of material. Black spruce seeds that were sown onto scars and subsequently covered by a layer of wood chips produced greater numbers of seedlings after 120 days. Black spruce seed were found to germinate maximally under 1.3 cm deep layer of wood chips (Figure 5a). Similarly, greater numbers of seedlings were detected after the first season when jack pine seeds were sown in the summer and subsequently covered with wood chips. Jack pine seeds germinated optimally under a 0.64 cm thick layer of wood chips (Figure 5b).

## FIELD TRIALS OF CONES VERSES FREE SEEDS

The efficacy of seeds contained within cones was compared to that of heat-extracted seeds and seeds covered by simulated transported materials. Seeds were compared to both hand-picked cones and mechanically-milled cone-bearing-foliage. However, there was no significant difference in seeding ratios between mechanically-produced cone-mulch and hand-picked cones. Hence for the purpose of statistical analyses germination results from these two cone sources were averaged together in this study.

Black spruce seed were compared to cones on a sandy upland site and on elevated peat. One hundred and twenty days after sowing on sandy soil, spruce seeds covered by wood chips showed greatest seeding ratios with a smaller number of seedlings observed from free seed with detectable seeding ratios from intact cones (Figure 6a). However with time uncovered seedlings on peat or sand, perished more rapidly compared to covered seed. A

small number of new seedlings were observed in scars treated with cones in the season after sowing (Figure 6b). After two seasons all black spruce seedlings on the Iroquois falls site had perished.

Jack pine seed were compared to cones on sandy soil and after 120 d the seeds covered with woodchips showed higher seeding ratios than free seeds but with low seeding ratios observed from cones (Figure 7a). However, one year later some of the seedlings from free seeds perished while new seedlings were produced in scars treated with cones (Figure 7b). Thus with time, pine cones produced seeding ratios were at least comparable to those of free seeds but less than those of seeds covered by simulated transported materials. Many jack pine seeds covered with chips still remained at the end of the second season on the shinning tree site. Most uncovered jack pine had perished at the end of the second season on the shinning tree site and by the end of the first season on the Iroquois Falls site (See below).

# THE NATURE OF TRANSPORTED MATERIALS

The Iroquois Falls location was selected to test the protective effect of different mineral and organic materials on the basis of the higher mortality rates observed on this site. The nature of the covering material's effect on seedling establishment was examined by applying 1.5 cm of biotic and abiotic materials with different physical properties over 250 jack pine seed (Figure 8). Jack pine seeds covered by perlite, saw-dust, vermiculite, soil, or wood chips all showed greater seeding ratios compared to uncovered seed after the first season. However, seeds covered with fresh peat moss appeared wilted and germinated poorly. In summary, scarification was necessary for regeneration from seeds or cones. The dimensions of the scar had a significant effect on 120 d seeding ratios. Scars treated with seeds that were covered by a thin layer of organic of mineral materials displayed the greatest seeding ratios, scars treated with cones displayed low but more stable seeding ratios while those of uncovered seed declined most rapidly with time.

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#### DISCUSSION

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#### **Effects of Scarification**

Distributing seeds or cones over unscarified soil had little beneficial effect and scarification to the mineral soil enhanced establishment of pine and spruce seedlings after 120 days (Figure 2). In general, seeding ratios were in agreement with the results of Losee (1961). The higher seeding ratio of black spruce sown in slits compared to square scars may indicate that a large area of mineral soil exposed to the drying air around the seed application spot reduces the suitability of the microsite for seedling establishment (Figure 3). The greater presence of seedlings on mineral-soil scars compared to test spots on undisturbed surface materials indicates that the presence of organic surface materials somehow inhibits seedling establishment. However, the increase in seeding ratio on scars covered with wood chips compared to uncovered scars indicates that the mere presence of organic material does not seem to inhibit germination per se. The success of seeds germinated beneath wood chips (Figures 5&6) is consistent with the interpretation that main detrimental effect of organic layers is the prevention of seed-contact with the mineral soil (Fleming and Mossa, 1994). However, seedlings in scars treated with wood chips did display greater chlorosis compared to the seedlings covered with mineral materials or the seedlings from uncovered seed that survived. This chlorosis may indicate that the presence of dead wood has some latter effects on seedling health (Haavisto and Atkinson, 1995). In contrast, Long (1946) indicates that in black spruce the presence of dead wood is correlated with long term seedling success.

#### Natural Variability in Germination Success

The unequal distribution in the number of seedlings that resulted from equal amounts of naked seed sown in similar, hand-made micro-sites only a few meters apart on sites selected for the absence of steep physiographic gradients indicates that some important factor varied sharply between microsites (Figure 4). However, it is doubtful that soil moisture or temperature, air temperature or humidity and light intensity or duration could vary so sharply between these similar, closely-spaced microsites on a uniform surface. Hence, perhaps some other factor(s) are primarily responsible for the large observed variation in germination success. In scars with high seeding ratios the seed were buried beneath, and germinated through, a layer of mineral or organic materials that were transported over the seed bed after sowing. These observations were interpreted to suggest that the variation in the random transport of natural materials by wind or water onto exposed scars might represent a key source of variation between hand-made microsites. A role for transported materials is consistent with the importance of scar geometry and microrelief noted in other studies (Sutherland and Foreman, 1995; Van Damme and Bax, 1991). Perhaps the sloughing of materials onto the naked seed may provide more complete and lasting hydration by protecting seed from the soil-air interface. The interpretation that transported material is a key factor germination success leads to the testable hypothesis that artificially covering seeds will enhance seedling establishment.

The small thickness (0.5 - 1.5 cm) of manually applied seed-cover required to produce a sharp increase germling establishment is consistent with the depth of materials that were observed to have been naturally transported onto successful seedbeds. Seeding ratios increased with the application or mineral or organic materials and this could be interpreted to suggest the effect is, at least in part, abiotic. However, the range of seeding ratios that was observed with an equal volume of different materials suggests that each material may have a different optimal depth. Seeds covered with different materials that ranged in albedo from white perlite to dark soil all showed increases in germling establishment compared to naked seed, but the increase was greater in seed covered by perlite or sawdust than fine mineral soil. The non-specific enhancement from these different materials may imply they all serve to protect seeds from the dryness of the soil surface at least in part. This interpretation is consistent with the poor protective effect of porous peat moss. No chlorosis was observed in germlings rooted in beds covered with soil, perlite or vermiculite compared to seedlings rooted through wood chips. The observation that a thin surface covering of soil elicits close to the full benefit of other more expensive covering such as perlite, vermiculite, wood chips or sawdust may have the greatest practical significance since a small amount of mineral soil is often available at many cut-overs.

#### Cones as a Unit of Dispersal

Cones produced few seedlings in the first year but still provided some seed for new seedlings in the second year after most or all uncovered seed had apparently perished. The small number of newly germinated seedlings from cones in the second season and the disappearance of the previously germinated cone-seeds implies that seeds are released from cones over time, germinate when conditions are favourable and die when conditions change. The germination of new seeds from cones in the second season, the more rapid demise of uncovered free seeds with time, the persistence of covered seedlings and the success of seedlings sown in slits are all consistent with an important role for periodic dehydration

stress in early seedling mortality on upland sites.

A significant amount of seeds remain in the cones for at least three years after ripening in black spruce (Millar, 1936) and much longer in jack pine. Schoenike and Hansen (1954) showed that the cones at bottom of 4-year-old slash piles contained 8% viable seed while cones greater 6 feet off the ground contained 12% viable seed. The greater than one- year-old cones collected from typical slash piles used in the present study where found by heat extraction to contain less than 20 (pine) and less than 10 (spruce) live seeds per cone. However with time, the seeds within 5 such black spruce cones were more potent than 300 free seeds and 5 jack pine cones were at least as potent as 250 free seed and cones seem to provide a window of opportunity for seedling establishment that extends beyond the first season. The unusual crown-form of black is spruce is apparently caused by squirrels (*Sciurus hudsonicus*) pruning off twigs that bear partially opened cones (Lebarron, 1948; Jonson, 1956; Lutz, 1956). The results here are consistent with a natural role for cones as a unit of dispersal in black spruce.

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The greater seeding ratio of cones placed on scars compared to cones place on undisturbed soil may indicate that the close proximity of exposed mineral soil to the cones is an important factor in successful dissemination. While the natural capacity of seeds in redistributed slash to reach more distant areas of exposed soil remains to be measured but experiments with mature stands indicate that significant amounts of seed travel more than 30 meters (Vincent, 1965).

#### The Mechanical Preparation of Cones.

The experiments indicate that cone-bearing branches can be mechanically commuted

to a mulch that contains intact cones. However, care must be exercised when setting up the hammer mill to produce cone-bearing-mulches without damaging the seeds. Experiments here indicate that cone-bearing branches should be commuted at low speeds (i.e.  $\leq 850$  rpm) with screens that yield large particle diameters in order to obtain mostly intact cones that contain large amounts of live seed. The process can be monitored by observing the proportion of undamaged cones, and x-ray analysis or germination tests of extracted seeds.

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#### **Economic and Ecological Considerations**

Sustainable forest management should regenerate the native genotype without degrading the site capacity for future productivity (Nyland et al., 1979; Foster and Morrison, 1987; Jeglum, 1990). Forests are often harvested mechanically and regenerated by mechanical scarification followed by aerial seeding using locally collected genetic materials. It is clear that scaring, and thus damaging, some amount of top soil is required for regeneration from ground or air. However regeneration from the air requires large continuous scars from shear blades or disc trenchers and the scarification of extensive surface areas. In contrast, regenerating from ground level would require an appropriate number of small, individual scars.

In jack pine seeds in cones were found with time to be as potent as heat extracted seeds. Thus, the prospect of developing a method to use the cones in de-limbing waste as a means of regenerating the native genotype with little damage may seem attractive. Manual cone planting may reduce costs associated with the transportation, cataloguing, and heat extraction of cones, mechanical site preparation, and aerial seeding. However, the cost of hand picking cones, manually scaring the soil and distributing the cones would preclude

the use of unionized labour in and thus limit the applicability of this method. Furthermore, in jack pine where the cones are distributed over a large volume of foliage the cost of milling the cone-bearing foliage costs would be prohibitive for the mechanisation of this process.

The results indicate that in black spruce, seeding from ground level with mechanically produced cones could provide some regeneration of on sites that resist direct seeding. Black spruce cones are concentrated in a small volume at the apex and thus the cost of hand picking cones may be avoided by mechanical milling the of cone tops. It seems possible to develop a complementary mechanical means to apply the mulch to small individual scars. A mechanical means to scarify small areas and apply the mulch of black spruce cones may prove more robust than ground-level precision seeding on broken terrain. Such a method could complement the mechanical methods of timber harvesting. Together, the results presented herein are consistent with the development of means to regeneration boreal conifers from ground level using de-limbing wastes that will ensure the renewal of the native genotype and help preserve the potential of the site for future productivity.

#### Summary

Transported materials that cover seeds after sowing play an important role in the success of seedlings on mineral soil scars. The preliminary experiments presented here indicate that seeding ratios might be markedly enhanced by sowing seeds onto scars and subsequently covering the seed with a variety of mineral or organic materials. A similar effect was observed in the spring, summer or fall. Seeds from cones were found to germinate slowly with time and in black spruce higher seeding ratios were observed from

cones than from free seed in the season after application. With time, scars treated with jack pine cones developed similar seeding ratios to those treated with free seed. The success of seeds covered by mineral or organic materials, the appearance of new seedlings from cones in the second season, and the reduced seeding ratios on square scars compared to narrow slits are all consistent an important role for dehydration in seedling success. These results provide part of the basis of understanding required to design more consistently effective protocols for direct seeding of jack pine and black spruce from ground level using delimbing wastes. Preliminary experiments indicate that cone-bearing branches or tops could be used to produce similar (jack pine), or greater (black spruce) levels of stocking than that from heat-extracted seeds.

### **Management Guidelines**

1) Naked seed sown on scars should be covered with  $\geq 1.5$  cm of soil, or a lesser depth of woodchips/sawdust, in the spring or fall.

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- 2) Small scars reduce damage to the site and may increase seeding ratios.
- Cones should be manually collected or mechanically processed in the first season after felling.
- Mechanically prepared black spruce or jack pine cones should be commuted at low speeds (≤875 rpm) using large screen sizes that produce mostly intact cones.
- 5) Cones may be sown on scars in the spring, summer or fall.

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Vincent AB (1965) Black Spruce. A Review of its Silvics, Ecology and Silviculture. Queens Printer and Controller of Stationary. Ottawa, Canada. Cat. No. Fo 47-1100 Map 1. A cartoon depicting the location of the experimental sites. (A) The Iroquois Falls site. (B) The Shining Tree site.

Figure 1. The effect of screen size on the recovery of viable free seed ( $\bullet$ ) and viable seeds in within cones ( $\blacktriangle$ ) from 200 g batches of, (A) 1 year old black spruce black spruce cones, and (B)  $\ge$  1 year old jack pine cones, commutated in a Farm King hammer mill operated at 875 rotation per minute.

Figure 2. The effect of scarification on the 120 day seeding ratio of seeds and cones of black spruce at Iroquois falls and Shinning tree and jack pine at Shinning tree sown in June 1994. N = 24 scars or adjacent application spots on undisturbed soil with 250 jack pine, 300 black spruce seed or 5 cones per spot.

Figure 3. The effect of scar dimensions on the seeding ratio of 150 black spruce seed sown at Iroquois falls in October 1994 and measured in July 1995. N = 6 scars per treatment.

Figure 4. The natural variation in germination success of (A) 300 black spruce seed per scar sown at Iroquois Falls, and (B) 250 jack pine seed per scar sown at Shinning Tree. Seeds were sown in June 1994 on adjacent 25cm x 25cm scars spaced one meter apart on uniform terrain.

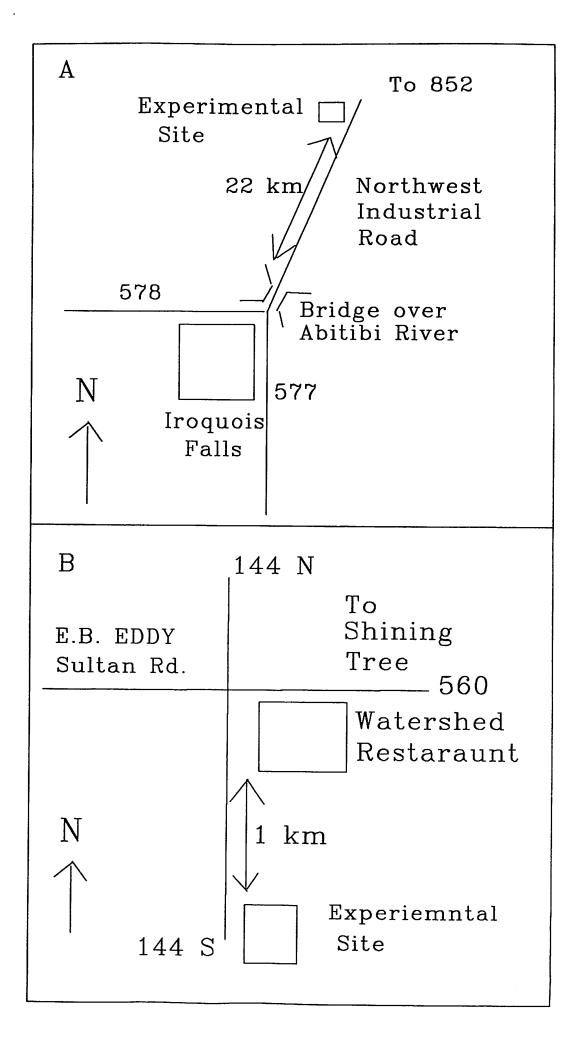
Figure 5. The effect of covering material depth on the seeding ratio of seeds sown in April 1995 with (A) 150 black spruce seeds per scar at Iroquois Falls, and (B) 125 jack pine seeds per scar at Shinning Tree. Wood chips of the indicated depth were spread over 25 cm x 25 cm scars. N=5 scars per treatment.

Figure 6. A comparison of jack pine seeds covered by simulated transported materials (wood chips), uncovered seed and seeds within cones. Scars were  $25 \text{ cm} \times 25 \text{ cm}$ . Plots were established in June 1994 at Shinning Tree and measured in (A) October 1994, and (B) October 1995. N=12 scars per treatment with 250 seeds or 5 cones per scar.

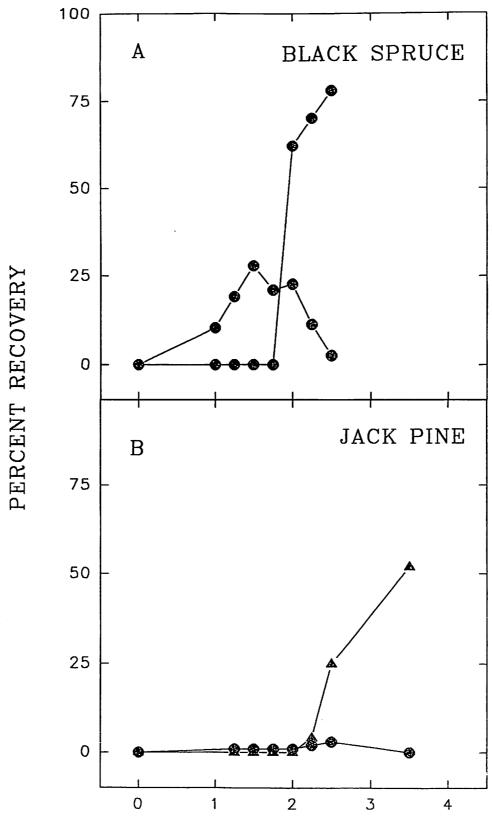
Figure 7. A comparison of black spruce seeds covered by simulated transported materials (wood chips), uncovered seed and seeds within cones. (A), Plots were established in June 1994 at Shinning Tree and measured in October 1994. (B), Plots were established at Iroquois Falls in June 1994 and measured in October 1994 or established in May 1995 and measured in July 1995. The plots were established at Shinning tree in June 1994 and measured in October 1995. The similar result of each of these trials were averaged together. N=6 scars per treatment with 150 or 300 seeds per scar.

Figure 8. The effect of the nature of covering material on the seeding ratio of jack pine sown at Iroquois Falls in July 1995 and measured in October 1995. Seeds were covered with a 1.5 cm layer of the indicated material. N = 8 scars per treatment with 250 seeds per scar.

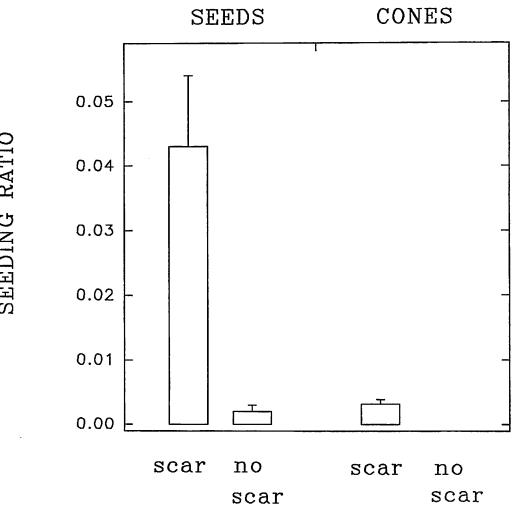
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SCREEN SIZE, cm

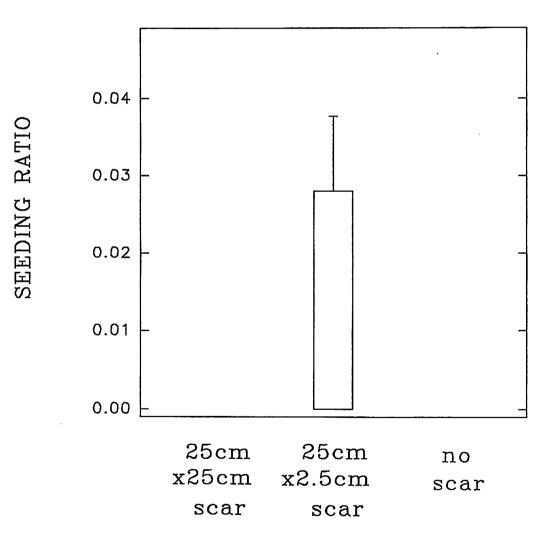


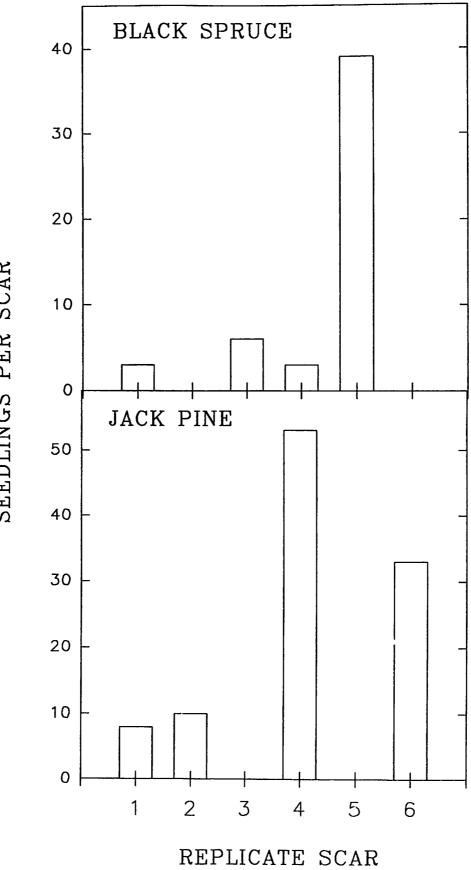
SEEDING RATIO

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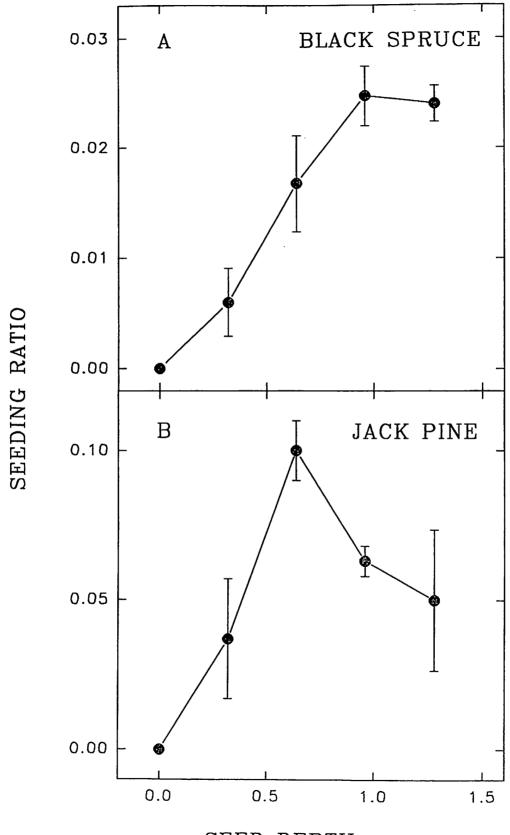


SEEDLINGS PER SCAR

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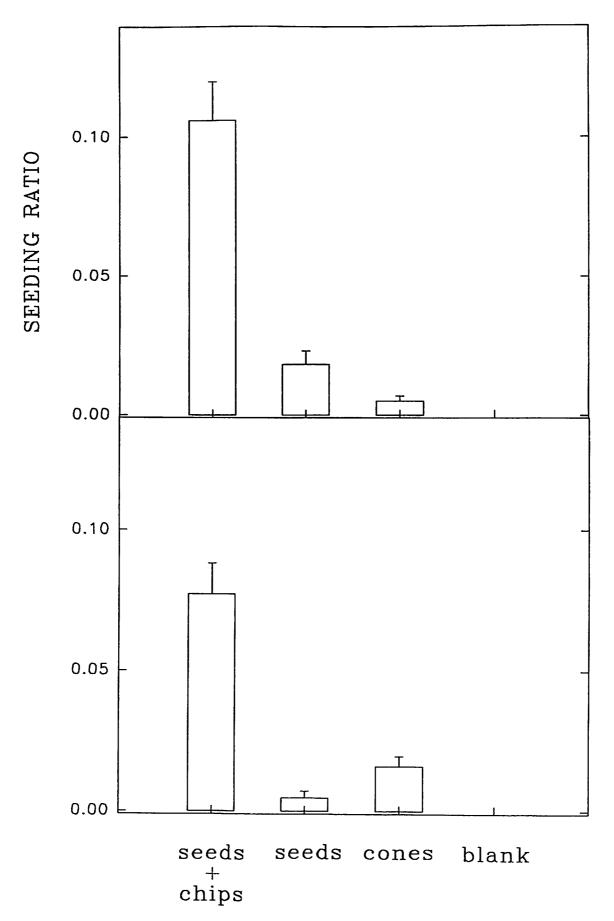
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SEED DEPTH, cm

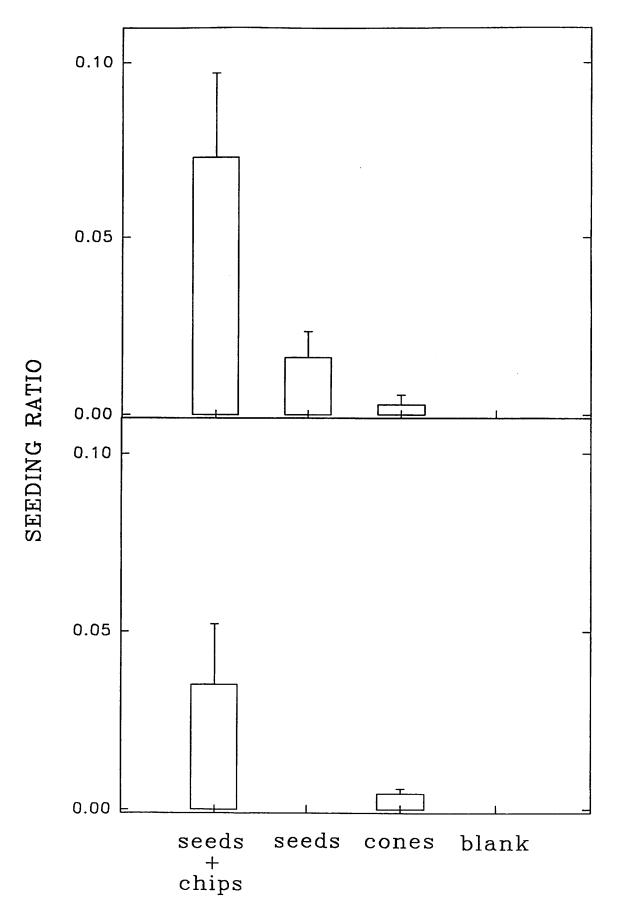
### JACK PINE



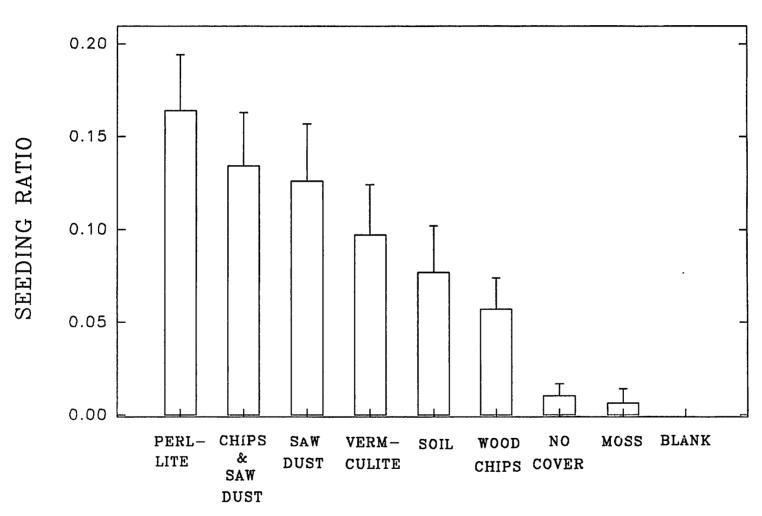
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BLACK SPRUCE



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JACK PINE