Progress Notes

Aussi disponible en français

No. 94, February 1979

Preliminary trials of radio-tagging black spruce seed with Manganese-54 for seed fate studies

by A.M. Martell[1] and W.F. Merritt[2]

Abstract
Field trials were conducted to determine the fate of black spruce (*Picea mariana*) seeds placed on upland black spruce clearcuts in northern Ontario. The seeds were radio-tagged by adsorbing five microcuries of Manganese-54 onto ion exchange resin beads and then glueing the beads to the seeds with epoxy. The seeds were recovered with the use of a Geiger–Muller survey meter 13–48 weeks after placement. Only 5% of the seeds germinated, about 3–4% were destroyed by rodents in summer, and 3–4% were destroyed by birds, primarily in the fall.

Introduction
The use of a radio-active tag to follow the fate of conifer seeds in the field was first suggested by Lawrence and Rediske (1959). A suitable radio-isotope for such studies should be a strong gamma emitter, in order to penetrate the soil, and a low alpha and beta emitter, so that it will not be phytotoxic. It should also have a sufficiently long half-life to follow the fate of the seeds for up to one year. Scandium-46 has been frequently used (Black 1969; Lawrence and Rediske 1959, 1962; Quink, Abbott, and Mellen 1970; Radvanyi 1966, 1970*a*, 1971), but its half-life is only 84 days. Radvanyi (1970*a*, 1971) used Zinc-65 because of its 244-day half-life, and Mathies *et al.* (1972) and Radvanyi (1968, 1970*a*, 1970*b*, 1971, 1972) used Cobalt-60 because of its 5.26-year half-life, but both of those isotopes are strong beta emitters and may be phytotoxic to seeds.

We chose Manganese-54 because it emits only gamma radiation, and therefore is unlikely to be phytotoxic, and because it has a 303-day half-life. Although the gamma emission energy of ^{54}Mn (0.83 MeV) is less than that of the other isotopes that have been used in seed fate studies (1.12–1.17 MeV) we felt that it would be sufficient for detection in the field.

Methods
Other researchers have radio-tagged conifer seeds by soaking them in solutions of the chosen isotope. That technique presents two problems. Even with subsequent coating of the seeds with latex, the isotope has frequently leached into the soil (Lawrence and Rediske 1959; Quink *et al.* 1970; Radvanyi 1971, 1972). Also, small mammals have been reported to detect seeds by olfaction (Howard and Cole 1967). Therefore, it would not be desirable to coat the seeds with latex. We chose to adsorb the ^{54}Mn onto Dowex-50 ion exchange resin beads, and then to glue the beads onto the seeds in order to overcome the leaching problem and to minimize alterations to the seed coat and, therefore, to the natural odour of the seed.

Dowex 50W-X10 20-50 mesh cation exchange resin (H^+ form) was sieved to select the larger beads. We obtained manganese-54 carrier-free in 0.5 M HCl from the supplier. The adsorption step was carried out on batches of 10 or 20 beads which were added to 3 ml of 1 M HCl in a 15-ml glass centrifuge tube. For a 10-bead batch, about 60 μCi of ^{54}Mn solution was pipetted into the tube and heated in a boiling water bath for 20 minutes. The beads were handled in the solution using a transfer pipette whose bore was selected to be slightly larger than the diameter of the beads. Manipulation of the beads was viewed with a 4-power visor magnifier. After labelling, we transferred the beads to another 15 ml centrifuge tube using the transfer pipette and washed them twice with distilled water. They were then air-dried and stored in a shielded vial. Sequential batch labelling was carried out in the original solution with serial addition of beads and tracer. The beads were capable of holding an average of 5 μCi of ^{54}Mn per bead.

The beads were glued to the cotyledon end (blunt end) of the seed with 3-hr epoxy. "Silicone Seal", Dow Latex 512-R, 5-min epoxy, and "Krazy Glue" were also tested as stickers, but 3-hr epoxy and "Silicone Seal" were easiest to apply and gave the most satisfactory bond. As "Silicone Seal" formed a rough bond and tended to pick up dirt, we used 3-hr epoxy. Each seed was picked up with forceps touched to a mound of fresh epoxy, and then touched to a bead. The seed with attached bead was then placed on a sheet of plexiglass to dry.

To test whether the 3-hr epoxy would be toxic to the seeds, we attached non-radioactive beads to seeds and tested for germination one month and 10 months later. For each test, four replicates of 100 seeds each were germinated in the laboratory at 21°C for 14 days (Fraser 1970).

We conducted the field trials on a 1975–76 clearcut 2 km south of Manitouwadge, Ontario (49°07′N, 85°50′W), and on a 1972–73 clearcut 21 km northeast of Manitouwadge. Before cutting, both sites had been mature, upland black spruce (*Picea mariana*) – feather moss (*Pleurozium schreberi*) forest. We established on each site a series of plots each with 100 flagged points at 2-m spacing. Adjacent plots were at least 20 m apart. A single radio-tagged black spruce seed was placed within 25 cm of each flag, and a coloured plastic tooth-pick was inserted nearby to mark the approximate location. We used a hand-seeder to place 5–10 untreated black spruce seeds on top of each radio-tagged seed in order to simulate an operational seed spot. Because of mechanical damage to the epoxy bond during placement, some plots contained only 96 or 97 radio-tagged seeds. Radio-tagged seeds placed on the 1972–73 clearcut in October 1976 (2 plots) and May 1977 (2 plots) were recovered in September 1977 after 48 weeks and 15 weeks respectively. Radio-

[1]CWS, Great Lakes Forest Research Centre, Sault Ste. Marie, Ont. P6A 5M7. Present address: CWS, 204 Range Road, Whitehorse, Yukon Territory Y1A 4Y4.
[2]Environmental Research Branch, Atomic Energy of Canada, Ltd., Chalk River, Ont. K0J 1J0.

tagged seeds placed on the 1975–76 clearcut in October 1976 (3 plots) were recovered in May 1977 (1 plot) and August 1977 (2 plots) after 30 weeks and 46 weeks respectively; and seeds placed on the 1975–76 clearcut in May 1977 (3 plots) were recovered in August 1977 after 13 weeks.

In order to recover the radio-tagged seeds, we scanned the marked spots with a Geiger-Muller survey meter (Texas Nuclear 2650 Series). A side-window probe was used for a general search within a meter radius of the flagged point, and an end-window probe (3 cm in diameter) to locate the exact seed spot. The radio-tagged seed and the surrounding soil were collected with a garden trowel and placed in a cardboard container. We then air-dried the contents of the container and examined them in the laboratory under a 1.5X magnifying lamp for the radio-tagged seed or bead.

We determined the fate of seeds with a bead still attached by examination under a dissecting microscope. Seeds without external sign of change were cut open. The seed was classed as unchanged if the endosperm was whole, as dessicated if the endosperm had noticeably shrunk, and as mouldy if fungal hyphae were present. A seed was classed as germinated if the seed coat was open and entire. Seeds with damaged seed coats were classed as destroyed by animals. The damage was assigned to rodents if the seed coat was cut open longitudinally, to birds if the seed coat was cracked horizontally, and to insects if the seed coat was perforated by a neat hole. The seed fate classes are similar to those described by Lawrence and Rediske (1962) and Radvanyi (1966, 1971).

Results and discussion

In the germination trials in the laboratory, 395 of 400 beaded seeds germinated after one month of storage, and 399 of 400 beaded seeds germinated after 10 months of storage. In both trials all 400 non-beaded, control seeds germinated. The 3-hr epoxy was not toxic to the seeds.

The recovery rate was 67–82% except for the fall trial on the 1972–73 clearcut, where only 36% of the radio-tags were recovered (Table 1). Three factors affected the recovery rate: birds, chipmunks, and germination. Birds will often consume entire black spruce seeds (personal observation) and could, therefore, remove radio-tagged seeds from the site. Chipmunks will cache seeds, often at some distance from the spot where they collect them, and could likewise remove radio-tagged seeds from the site. Least chipmunks (*Eutamias minimus*) were present on the 1972–73 clearcut, but not on the 1975–76 clearcut. Migrating Lapland Longspurs (*Calcarius lapponicus*) and Horned Larks (*Eremophila alpestris*) appeared to be more abundant on the 1972–73 clearcut than on the 1975–76 clearcut in the fall. We believe that the activities of birds and least chipmunks contributed to the low recovery rate for the fall trial on the 1972–73 clearcut.

At the time of recovery in August and September all seed spots were examined for seedlings. Radio-tags were found at only 19% of the stocked plots, significantly fewer than for non-stocked plots (72%) (X^2 = 48.72, df = 1, P<0.001). We suggest that the seed coats were lifted above the ground surface on the cotyledons of the growing seedlings, and were then dispersed by wind and/or water. That would, however, account for only a small proportion of the missing beads.

We ran into two unexpected problems with the radio-tags: a high proportion of the beads disintegrated, and a high proportion of the beads that did not disintegrate became detached from the seeds (Table 1). The data indicate that more disintegration and detachment of the beads occurred in summer than in winter, and more occurred on the 1972–73 clearcut than on the 1975–76 clearcut. Because four nights of heavy frost occurred on the study area in early June 1977, we suspect that repeated freezing might be the problem. Laboratory tests of repeated freezing and thawing of dry beaded seeds prior to the field trials showed no change to either the bead or the bond. However, when we soaked the beads in water and then subjected them to repeated freezing and thawing, we found that they initially cracked and then disintegrated, producing results similar to those observed in the field. The 1972–73 clearcut had more vegetation and moss cover than the 1975–76 clearcut because of the length of time since cutting, and consequently had moister soil conditions. That may explain why the rate of disintegration and detachment was higher on the 1972–73 clearcut than on the 1975–76 clearcut.

The disintegration and detachment of beads resulted in only a small number of them remaining attached to the seeds for an evaluation of seed fate. Because of the small sample size and because there were no marked differences in seed fate between the clearcuts, we combined the clearcuts in the analysis (Table 2). While most (80%) of the radio-tagged seeds were unchanged over winter, about half (52–58%) dessicated over summer. Mouldy seeds were most obvious in spring, and many of those seeds may have dessicated over summer. By spring, 5% of the fall-placed radio-tagged seeds had germinated, and by fall an average of 5% of the combined fall- and spring-placed, radio-tagged seeds had germinated. At the time of the fall recovery, 7.7% of the seed spots were stocked, indicating a germination rate not significantly different from that for radio-tagged seeds (X^2 = 1.53, df = 1, 0.5>P>0.1).

Rodents, primarily deer mice (*Peromyscus maniculatus*), destroyed 3–4% of the radio-tagged seeds during the summer, and birds destroyed 3–4% of the radio-tagged seeds, primarily in the fall. Only one radio-tagged seed was found destroyed by insects and no radio-tagged seeds were found destroyed by shrews. The total loss to birds and rodents may have been higher if, as discussed previously, chipmunks and/or migrating birds removed many seeds from the plots.

Seed predation by small mammals and birds may show great variation between years; differences of from about two-fold (Black 1969; Lawrence and Rediske 1959, 1962) to almost six-fold (Radvanyi 1970*a*, 1970*b*) have been reported. Radvanyi's values for destruction by mice alone ranged from 6% to 36%. If the predation rates we observed were minimal, then seed predation may reach serious proportions at least in some years. If, however, the rates we observed were, at least, "average", then seed predation does not appear to be a serious hindrance to the regeneration of newly harvested, upland, black spruce clearcuts.

Conclusions and recommendations

The use of Manganese-54 adsorbed onto ion exchange resin beads, which were then glued to seeds, allowed us to follow the fate of those seeds in the field for up to 48 weeks. There was no leaching of the isotope and there was minimal alteration to the seeds. We feel that the problem of disintegration and detachment of the beads can be easily overcome by coating them in epoxy or plastic to make them waterproof. The technique is relatively inexpensive and should be useful in a variety of field studies.

Acknowledgements

We thank the Ontario Paper Company and the Ontario Ministry of Natural Resources for their co-operation, and A. Macaulay, H. Pearce, D. Tracy, and S. Wheeler for their assistance with the study. J.E. Bryant, J.F. Carreiro, and J.W. Fraser offered useful comments on the manuscript.

References

Black, H.C. 1969. Fate of sown or naturally seeded coniferous seeds. Pages 42–51 *in* H.C. Black, ed. Wildlife and reforestation in the Pacific Northwest. Sch. For., Ore. State Univ., Corvallis.

Fraser, J.W. 1970. Cardinal temperatures for germination of six provenances of black spruce seed. Can. Dep. Fish. For., Can. For. Serv., Inf. Rep. PS-X-23. 11 pp.

Howard, W.E.; Cole, R.E. 1967. Olfaction in seed detection by deer mice. J. Mammal. 48:147–150.

Lawrence, W.H.; Rediske, J.H. 1959. Radio-tracer technique for determining the fate of broadcast Douglas-fir seed. Soc. Am. For. Proc. 1959:99–101.

Lawrence, W.H.; Rediske, J.H. 1962. Fate of sown Douglas-fir seed. For. Sci. 8:210–218.

Mathies, J.B.; Dunaway, P.B.; Schneider, G.; Auerbach, S.I. 1972. Annual consumption of cesium-137 and cobalt-60 labeled pine seeds by small mammals in an oak-hickory forest. U.S. At. Energy Comm., Oak Ridge Natl. Lab., Environ. Sci. Div., Contract No. W-7405-eng-26. 232 pp.

Quink, T.F.; Abbott, H.G.; Mellen, W.J. 1970. Locating tree seed caches of small mammals with a radioisotope. For. Sci. 16:147–148.

Radvanyi, A. 1966. Destruction of radio-tagged seeds of white spruce by small mammals during summer months. For. Sci. 12:307–315.

Radvanyi, A. 1968. Influence of small mammals on spring and winter placement of radio-tagged white spruce seeds in western Alberta. Unpubl. Rep. Can. Dep. Indian Aff. North. Dev., Can. Wildl. Serv., Edmonton, Alta. 35 pp.

Radvanyi, A. 1970*a*. Small mammals and regeneration of white spruce forests in western Alberta. Ecology 51:1102–1105.

Radvanyi, A. 1970*b*. Influence of small mammals on endrin and R-55 treated seeds of white spruce in western Alberta: preliminary field trials. Unpubl. Rep., Can. Dep. Indian Aff. North. Dev., Can. Wildl. Serv., Edmonton, Alta. 34 pp.

Radvanyi, A. 1971. Lodgepole pine seed depredation by small mammals in western Alberta. For. Sci. 17:213–217.

Radvanyi, A. 1972. Further testing and application of the R-55/graphite coating treatment for seeds. Unpubl. Rep. Environ. Can., Can. Wildl. Serv., Edmonton, Alta. 42 pp.