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Effects of Extended Prechilling on Laboratory Germination and Fungal Infection in Seeds of White Spruce and Eastern White Pine

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Prechilling treatments of up to 15 weeks did not show any detrimental effect on laboratory germination of Picea glauca *(Moench) Voss and* Pinus strobus *L. seed. Infection was generally greater on germinants of treated seed than on those of untreated seed, but fungal infection of germinants did not increase with the duration of prechilling treatment. Differences in infection between treated and untreated seed could be due to the spread of infection among seed during the moist prechilling period.* Tree Planters' Notes 38(4) : 6-9; 1987.

White spruce (*Picea glauca* (Moench) Voss) and eastern white pine (*Pinus strobus* L.) are among the top 10 tree species used for reforestation in Canada. National forest seed surveys (8) show that 5.3 billion viable seeds of white spruce and 79.2 million seeds of eastern white pine were used for reforestation during 1982-83.

The prechilling (moist cold stratification) of dormant seed of many tree species has beneficial effects on seed germination (1-3, 7, 19). However, when sowing schedules are disrupted by unexpected changes in weather or breakdowns of seeding equipment, etc., it is sometimes inevitable that prechilling treatment of seeds is extended beyond the 21 and 28 days recommended for spruce and pine, respectively. Such extensions frequently cause anxiety about the potential loss of seed germinability and increased damage by disease organisms.

The presence and pathogenicity of fungi on conifer seed has been demonstrated by Gibson (5), Prisyazhnyuk (13), Shea (14), Uroševic (15), Kozlowska (9), Neergaard (12), Mittal (10), and others. Some seed-borne fungi have the ability to develop and spread during moist cold conditions and cause germination failure (4). The present investigation was conducted to determine the effect of long-term prechilling on fungal development and the germination of seed as part of a comprehensive laboratory and greenhouse examination of seed-borne fungi of white spruce and eastern white pine.

Materials and Methods

White pine cones were collected by climbing 8 trees in Algonquin Park, ON (lat. 45° 53'30" N., long. 77°42'30" W., 200 m elevation) during the first week of September 1985. Similarly, a bulk cone collection of white spruce was made from 11 felled trees in the Rocky Mountain/ Clearwater Forest (lat. 52°14' N., long. 115°18' W., 1160 m elevation), Alberta on August 30, 1985. Seed were extracted and cleaned after the cones had been air drying for 6 weeks.

Samples of about 8,000 similar-sized (by sieving) seed were taken from the total obtained for each species. Four replicates of 100 seed each were x-rayed to determine the percentage of filled, empty, or underdeveloped seeds. Four similar replicates were tested for germination and fungal infection without prechilling to serve as control.

The remaining seed of each species were spread between two layers of moist Kimpak® in separate clear "Petawawa germination boxes" *(18),* and placed in a cold room (2 to 4°C) for the prechilling treatment.

Four 100-seed replicates were taken out after 2, 3, 4, 6, 8, 10, 12, 14, and 15 weeks of prechilling. Germination tests were conducted on moist Kimpak in clear Petawawa germination boxes, in G-30 Conviron germinators (Controlled Environments, Winnipeg, MB) under 30 °C with light (18 µE/m^2/s) for 16 hours and 20 °C in darkness for 8 hours (ISTA 1985).

Counts of germination and the incidence of mold on seed and the developing germinant were made every 3 days during a 21-day test period for white spruce and a 28-day period for white pine. Emergence of the radicle from the seedcoat was considered the criterion for germination. All seeds ungerminated at the end of the test period were cut to determine the number that were fresh, dead, or empty.

Seed moisture content after each prechilling period was determined on a dry-weight basis, by ovendrying two 100-seed samples at 105 °C for 16 hours.

Results and Discussion

X-radiographs of the white spruce and eastern white pine revealed that almost 100% of the seeds were filled and fully developed.

In both species, the major part of the increase in seed moisture content occurred in the first 2 weeks of prechilling, reaching 45% in the pine and 36% in the spruce; by the 14th week of prechilling the moisture content in pine and spruce had increased by an additional 13 and 15%, respectively (table 1).

Long-term prechilling did not have any detrimental effect on germination of either white spruce or white pine (table 1). Hellum (6) reported reduced germination of white spruce seeds from southwestern Alberta given prechilling treatments as compared to nonchilled seeds. He added that the total nursery germination in three seedlots of

Table 1—*Percentage moisture content; germination at 3, 6, and 9 days; total germination; and total fungal infection of eastern white pine and white spruce after 2 to 15 weeks of prechilling*

Prechilling treatment	Moisture content	Germination			Total germination*	Total fungal infection
		3 d	6 d	9 d		
Eastern white pine						
0 (control)	4.4	0	4.5	33.75	95.0	3.0
2 wk	45.1	0	6.75	48.0	100.0	30.5
3 wk	47.9	0	14.5	77.0	99.7	36.0
4 wk	45.3	0	19.0	74.25	99.5	45.5
6 wk	50.7	0	17.25	69.0	93.7	6.0
8 wk	47.8	0	89.25	91.25	98.0	36.0
10 wk	51.9	0	53.25	75.75	99.5	14.75
12 wk	56.7	0	39.75	67.0	98.7	13.25
14 wk	58.2	0	43.5	73.0	99.7	34.24
15 wk	53.1	0	41.75	69.75	90.5	14.25
White spruce						
0 (control)	5.1	0	62.75	86.5	97.0	11.5
2 wk	36.4	0	90.0	95.5	100.0	19.75
3 wk	38.3	0	93.0	94.25	99.7	31.75
4 wk	40.3	0	90.0	95.25	99.2	21.25
6 wk	42.7	0	92.75	93.25	98.7	16.0
8 wk	43.2	0	89.25	91.25	98.0	18.75
10 wk	46.5	21.75	93.25	94.5	100.0	12.5
12 wk	46.1	15.0	94.25	95.75	99.0	18.5
14 wk	51.3	66.5	90.5	92.75	99.7	35.0
15 wk	49.0	29.75	90.0	90.0	100.0	23.0

All values are means of four 100-seed replicates except those for moisture content of eastern white pine, which are means of two 100-seed replicates.

*Total germination for eastern white pine was measured at 28 days and for white spruce at 21 days.

white spruce gradually decreased with the duration of prechilling treatment up to 115 days.

Our results, however, did not show any decline in germinability even after 15 weeks of prechilling. For white pine our results show a decline in germination of 8% after 15 weeks of prechill. This may be the maximum period tolerated before the seed start to lose germinability. Additional tests are necessary to verify this decline.

The pine and spruce seeds used in this study did not exhibit strong dormancy characteristics, and prechilling improved total germination measured at 28 days by only 5 and 3%, respectively. A more obvious, and potentially beneficial, effect of prechilling was the more rapid germination seen in both species. As the prechilling period increased to 8 weeks, the percentage of germination of pine seeds measured after 6 days increased to a maximum. In the case of spruce, a 2-week prechilling treatment increased germination at 6 days by about 87% over the unchilled control, and after prechilling for 10 to 15 weeks, many spruce seeds germinated after only 3 days.

In most cases, a higher mold incidence was found after prechilling (table 1). However, the duration of the prechilling treatment did not appear to increase or decrease the incidence of fungal infection of the germinant. This was probably due to factors conducive to fungal spread, such as the high moisture content of prechilled seeds and the extended light duration, high humidity, and temperature in germinators during testing. The variations in percentage of mold incidence on germinants of both pine and spruce were due to development and spread of fungi in pockets on seed during prechilling.

Fungal infections did not reduce the germination of seed but seedling quality was much affected. Infection could be detected on the germinants of white spruce after 13 days from sowing, and of white pine after 21 days from sowing and the number of infected germinants greatly increased during the following week. The infection occurred mainly on the cotyledons as they emerged from the seedcoat. Moldy seeds and infected seedlings were not removed from germination boxes because international rules for seed testing (7) require that seed germination cannot be considered normal until all essential structures are visible, i.e., a complete seedling with seedcoat shed. Infected seedlings in the germination boxes could have facilitated the spread of infection.

Mittal (10) reported that the fungi associated with seeds become important when they are active. Mittal and Wang (11) reported that three species of seed borne fungi in white spruce can arrest seedling emergence, eventually leading to the death of seedlings. In this study, the late development of fungi on developing seedlings may be attributed to the time taken by the fungi to become activated on the seedcoat and then to move onto cotyledons where tissue decay began.

Because germination tests are conducted with only water being available to support growth, it is possible that fungal development is attenuated by poor nourishment of the germinant following rapid development and probable exhaustion of stored food within the endosperm. It also seems reasonable that the poor nutrient status of large germinants would make them less able to fend off infection. However, if that was the case, the risks of severe damage under field conditions are less because a nutrient source would be available to the developing seedlings.

The higher infection percentage in the germinants raised from prechilled seed than the nonchilled seed might be due to faster germination in the former, exposing cotyledons earlier and for a longer period to fungi. It may be possible to alleviate the fungal problems in laboratory tests by removing the germinants as soon as they reach seedling vigor class 2 stage, just before shedding the seedcoat (16, 17), or by removing the lids of the germination boxes after 7 to 14 days from sowing.

These results are from laboratory tests, with growing conditions that are probably more suitable for fungi than are the nursery or greenhouse conditions. However, possibilities for severe damage from seed-borne fungi on densely seeded beds under closed, moist environments cannot be overlooked. Surface sterilization of seeds before prechilling could be a good preventive measure.

Conclusions

From the results of this study, we can conclude that:

1. The rate of germination of white spruce and eastern white pine seed was increased with the prechilling and the maximum germination reached after 3 and 8 weeks of prechilling for white spruce and eastern white pine, respectively, thus supporting earlier reports.
2. Prechilling for up to 15 weeks does not have any adverse effect on white spruce seed germination, although eastern white pine seed germination began to decrease at the end of this time.
3. The incidence of fungal infection during laboratory germination tests increased with extended prechilling, probably because of the high moisture during prechilling and the testing conditions. This had no apparent detrimental effect on seed germination.

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