Fungi on Eastern White Pine Seeds With High Moisture Content Can Survive Ultralow Temperatures

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Inoculated fungi-Fusarium sporotrichoides Sherb and Mucor hiemalis Wehmer-on eastern white pine (Pinus strobus L.) seeds with high moisture content survived well after 35 days of storage at -18, - 80, -145, and -196 °C. This shows that storing tree seeds at ultralow temperatures will not eliminate fungal pathogens. Tree Planters' Notes 40(4) :34-36; 1989.

There have been suggestions recently that tree seeds should be stored at subfreezing temperatures to maintain germinability (15). Cryopreservation of seed germplasm at or near the temperature of liquid nitrogen has the potential for reducing deterioration of seed to such a low level that, essentially, seeds could be preserved forever (14). Mittal and Wang (7, 8) have reported on the prevalence and pathogenicity of fungi on eastern white pine seeds.

Whether or not the seeds become infected while they are still in cones on trees, during collection of cones, or during the extraction, processing, and storage of seeds, harmful fungi must be eradicated before sowing. Storing fungal cultures with some cryopreservatives (to avoid nutritional changes that may show up as unwanted morphological or physiological characters) in liquid nitrogen for long periods has been demonstrated and the procedure is in use at a few type culture collections (2-5). However, the effects of ultralow temperatures on the survival of fungi on tree seeds have never been reported. A study of these effects was conducted on eastern white pine (*Pinus strobus* L.) as part of an investigation evaluating treatments to decontaminate infected seeds.

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Materials and Methods

Cones of eastern white pine were collected from 8 trees in Algonquin Park, Ontario (lat. 45° 53'30" N.; long. 70°42'30" W.; elevation 200 m) in the first week of September 1985. Seeds were extracted after the cones were air-dried in a cone shed for 6 weeks. Before they were inoculated with fungi, seeds were dipped in 1% sodium hypochlorite for 10 minutes, then rinsed three times successively in distilled water ("decontamination"). They were then soaked in distilled water for 16 hours to assist establishment of inoculated fungus in seeds. About 800 seeds were rolled individually over fresh cultures of both *Fusarium sporotrichoides* Sherb. and *Mucor hiemalis* Wehmer (160 seeds each in five 9-cm-diameter petri dishes containing 8-day-old culture on potato-dextrose-agar). Another lot of 800 seeds that were not decontaminated were soaked in distilled water, stored without fungal inoculations, and tested as controls.

The seeds were kept overnight at room temperature in the petri dishes, then 200 seeds from each treatment were placed in cryotubes for storage at -18, -80, -145, and -196 °C. Fifty seeds of each treatment from each temperature regime were removed at 3, 7, 18, and 35 days. Five seeds from each sample were placed in each of 10 petri dishes (9 cm in diameter) containing potato-dextrose-agar, and colonies of fungi were identified and counted by light microscopy.

Similarly, a lot of 400 seeds was inoculated with *M. hiemalis* only, and groups of 50 seeds each were then stored at -18, -80, -145, and -196 °C and subsequently sampled. For determination of moisture contents, duplicate samples of 50 seeds each from these treatments was determined after 3, 7, 18, and 35 days by oven-drying for 16 hours at 105 °C (6).

The storage systems used for the various temperature regimes were: a deep-freeze room for -18 °C, the Cryostar chest freezer (Queue Systems) for -80 °C, the TA-90 cryo-container (Minnesota Valley Engineering Cryo-biological Systems) for -145 °C (vapor phase of liquid nitrogen), and the Omega EM-32 cryo-container (Minnesota Valley Engineering Cryo-biological Systems) for -196 °C (liquid nitrogen).

Results and Discussion

Nearly 100% of the inoculated seed sampled after 3, 7, 18, and 35 days at all four temperatures yielded both *F. sporotrichoides* and *M. hiemalis*, indicating that the inoculum on the seeds survived under ultralow temperatures. Other fungi such as *Altemaria, Penicilhum, and Trichoderma* spp. were present on uninoculated seeds after most of the cold treatments. The ability of fungi to withstand ultralow temperatures eliminates the possibility that low-temperature treatments could be used to eradicate these pathogenic fungi from seeds. Results also indicate that some fungi persist on seeds for at least 35 days.

Within the last two decades, the successful preservation of living cells and organisms has been achieved through cryogenics. Fungal cultures have been stored in cryopreservative agents such as 10% (v/v) glycerol and 5% (v/v) dimethyl sulfoxide. Revival of fungi is reported to be most successful when cultures are cooled slowly (3, 9, 11). At these low temperatures, fungal metabolism is suppressed, and if the initial shock of freezing is survived, the cultures remain viable indefinitely (1, 5, 10, 12, 13). However, in the present case the fungi survived on seeds without use of any cryoprotective agent and also without slow cooling, indicating considerable tolerance by *F. sporotrichoides* and *M. hiemalis* to the initial shocks of freezing.

Moisture content of seeds was originally 5 to 6% and increased to about 26% after 16 hours of soaking in water. Moisture contents of seeds inoculated by *M. hiemalis*, as well as those of uninoculated seeds, which ranged from 22 to 26% after storage, showed little change after storage at -18, -80, -145, and -196 °C. It should be noted that our findings might have been compounded by the high moisture content of the inoculated seeds, which could favor fungal survival and adversely affect seed germination. Stanwood (14) has reported that seed moisture content is probably the most critical factor relating to successful cryopreservation, and, hence, an optimum seed moisture content must be attained. Further work is needed to determine the survival and pathogenicity of fungi on dry seeds following their storage in ultralow temperatures.

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