Germinability of Cook Pine (Araucaria columnaris) Seeds Under Different Storage Conditions

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Up to 25 metric tonnes of seeds of Cook pine, Araucaria columnaris *(Forst. f.) Hook., are exported from Hawaii in abundant seed years. Excess seeds cannot be stored and used to fill orders in poor seed years because the seeds quickly lose their ability to germinate. The effects of storage temperature, seed moisture content, and nitrogen enrichment of storage containers on short-term and long-term seed germinability were studied in the laboratory. Results confirm the need for storage at near-freezing temperatures and seed moisture contents between 15 and 25%. Filling storage containers with nitrogen gas failed to prolong storage life of seeds and even air-filled storage containers failed to prolong storage of physiologically immature seeds. Tree Planters' Notes 39(3):17-25; 1988.*

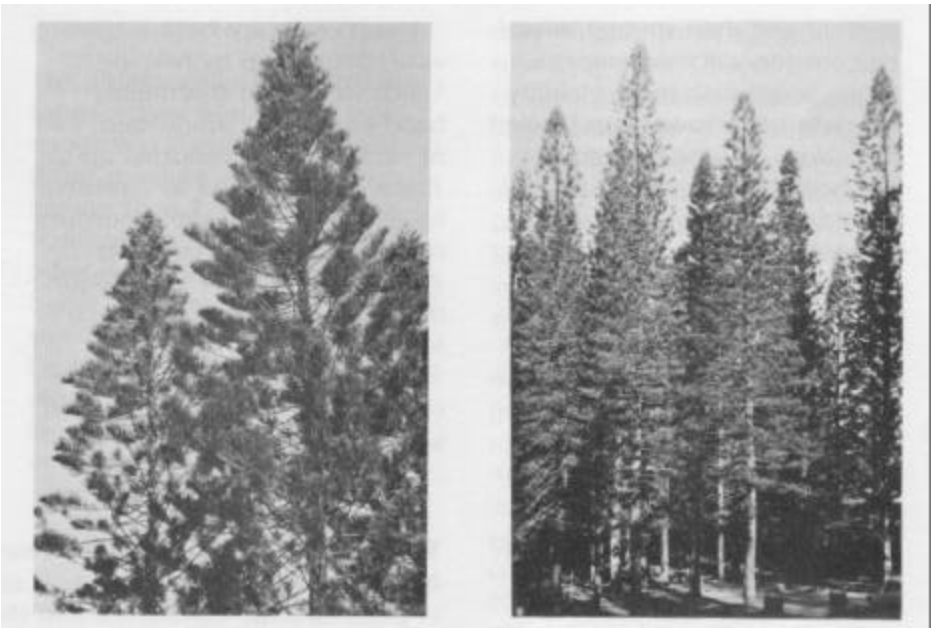

Figure 1—*A native of New Caledonia, Polynesia, and the Isle of Pines, Cook pine* (Araucaria columnaris) *is an evergreen coniferous tree that has been introduced to Hawaii and Florida. Although the tree is not a pine, its pine-tree-like shape and symmetry make it desirable as a Christmas tree and as an ornamental. In Hawaii, scattered stands of pure Cook pines make ideal recreation areas.*

Cook pine—*Araucaria columnaris* (Forst. f.) Hook.—is the most abundant Araucaria species in the Hawaiian Islands (fig. 1). It is commonly, but incorrectly, locally referred to as Norfolk Island pine (*A. heterophylla* [Salisb.] *Franco* [syn. *A. excelsa* (Lamb) R. Br.]), a species sparsely represented in the State.

Demand for *A. columnaris* seeds from Hawaii is great: up to 25 metric tonnes (wet weight basis) have been exported in abundant seed years, which usually occur every 4 or 5 years (9, 23). This quantity represents the harvest from about 130 hectares of public and private plantations statewide. Stands on Oahu alone yielded about 13 metric tonnes of seed in 1985 (6).

Suppliers of *A. columnaris* seeds face two problems: rapid loss of viability during shipment and inability to store seeds for more than a few months without significant loss of viability. This loss is particularly disturbing because suppliers do not have reserves for filling orders in years of poor seed production.

These problems are common to many large-seeded tropical tree species (18, 24). Their seeds are shed in a moist condition and are short lived. Seeds can be successfully stored for only a few weeks or months and must be kept at relatively high temperatures and high seed-moisture contents. Seeds with these viable characteristics have been labeled *recalcitrant* (15).

In contrast, seed viability of most temperate zone trees species can be retained for years by first drying to about 5% moisture

content and then storage in sealed containers at low temperatures. Seeds with these viability characteristics have been labeled *orthodox. Araucaria columnaris* has been provisionally classified as intermediate in seed storage physiology (21).

The interaction of seed moisture content and storage temperature, including freezing, on longevity of *A. columnaris* seeds and the effect of storage under nitrogen gas were examined.

Materials and Methods

Seeds were supplied by Hurov Seeds from cones collected in Nuuanu Valley, Oahu, HI, in August 1972 and September 1973. In 1972, the cones were beginning to disintegrate, so cones and loose seeds were packed in polyethylene bags for shipment. In 1973, the cones were still closed, so each cone was wrapped separately in plastic wrap. Both seed lots were shipped to the USDA Forest Service's National Tree Seed Laboratory, Macon, GA, for testing.

Moisture content (wet weight basis) of both lots was estimated when seeds arrived 4 days later by using four 3-g subsamples ovendried for 16 hours at 105 °C and cooled for 2 hours over desiccant before final weighing (10). Fresh seed moisture contents were 27% in 1972 and 68% in 1973. There were about 1,235 (± 40 SD) seeds per kg fresh weight in 1973.

Intact cones for both lots were easily broken up by twisting. Seeds were then thoroughly hand mixed and subdivided. Part of each lot was ovendried up to 32 days at 22 °C and 40% relative humidity to the desired moisture contents. Moisture contents after drying were 21 and 7% for the 1972 seed lot and 26 and 6%, for the 1973 seed lot.

Seed groups at each moisture content were further subdivided and assigned at random to different storage treatments (table 1). There were not enough seeds supplied to test all possible treatment combinations.

Seeds were stored in 0.5-liter air-tight glass jars. The loosely packed seeds occupied about 65 to 80%, of each jar. Where used, nitrogen gas was slowly injected through a tube lowered to the bottom of each jar before sealing. An estimated 90% of the air was thus displaced by nitrogen gas.

Table 1—*Seeds of Cook pine* (Araucaria columnaris) *subjected to combinations of initial seed moisture content, storage period, composition of gas in storage containers, and storage temperature*

Initial moisture (%)	Storage period (mo)	Air				Nitrogen			
		24 °C	15 °C	3 °C	−7 °C	24 °C	15 °C	3 °C	−7 °C
1972 seed lot									
27	0	*							
	3	*	*	*			*	*	*
	6	*	*	*	*				
21	0								
	3	*	*	*					
	6	*	*	*	*				
7	0								
	3	*	*	*					
	6	*	*	*	*				
1973 seed lot									
68	0	*							
	6								
	12								
	24								
26	0	*							
	6			*	*			*	*
	12				*				*
	24				*				*
6	0	*							
	6				*				*
	12				*				*
	24				*				*

After seeds were stored, they were tested for germination in walk-in germination chambers programmed for 30 °C and 2,150 lux for 8 hours and 20 °C and darkness for 16 hours. Seeds were placed on crepe cellulose germination paper (Kimpak) moistened with 100 ml of tap water, fitted into shallow 12.7 by 17.8 cm plastic boxes, and covered. No additional water was added during the germination period. For each replication, 50 or 100 seeds were used. Each treatment combination was replicated 4 to 16 times.

A germinated seed was one whose radicle had emerged enough that the seed coat lifted off the germination paper. Abnormal germination included conditions described in seed testing rules (10). At the end of the 4-week test period, seeds were x-rayed to determine the number of full ungerminated seeds.

The results were analyzed by untransformed and arcsin $\sqrt{x}$ transformed data. Treatment effects and interactions could not be properly assessed by usual statistical methods, in part because the assumption of equal variances among means was invalid, even when germination data were first transformed using arcsin $\sqrt{x}$. Instead, I compared treatment means by using the approximate *t* statistic (5) computed as follows:

$$t = (\bar{X}_1 - \bar{X}_2)/[s_1^2/n_1) - (s_1^2/n_2)]^{0.5}$$

The difference between means for each pair of different treatments was considered significant if the absolute value of *t* was greater than *T*, where *T* is the Bonferroni critical value for the number of pairwise comparisons being considered with an overall significance level of at most 0.05. Degrees of freedom for selecting the Bonferroni *T* were estimated by using Satterthwaite's approximation (13),

$$df = (s_1^2/n_2 + s_2^2/n_2)^2 / \{s_1^4/[n_1(n_1-1)] + s_2^4/[n_2(n_2-1)]\}$$

Graphic and tabular summaries and statistical tests are based on untransformed data. I chose to present untransformed data because they are readily interpretable and because results of all statistical comparisons were the same as obtained using arcsin $\sqrt{x}$ transformed data.

Results and Discussion

Germination of the 1972 lot of *A. columnaris* seeds averaged 78% (± 2.6 SE) on receipt at the National Tree Seed Laboratory. Significantly lower germination was achieved by the 1973 seed lot (61% ± 1.1 SE). Lower germination may have been due to seed immaturity because cones in 1973 had not begun to break apart as had cones collected in 1972. The 1973 seed lot had a much higher fresh moisture content than the 1972 seed lot—68 to 27%. Furthermore, immature seeds collected in 1976 showed higher moisture contents (47%) and lower germination rates (41%) than mature seeds collected a few weeks later in the season (40% moisture and 70% germination).

Another indicator of immaturity of the 1973 seed lot was the large increase in number of empty seeds over time (table 2). After just 6 months' storage, there were significantly more empty seeds than initially. This contrasts with results for the 1972 seed lot, which showed no appreciable increase in empty seeds after 6 months' storage (table 2).

Two other factors could have contributed to lower germination for seed collected in 1973: climatic effects on viability and intraspecific variability, but neither was evaluated.

Storage temperature. Clearly, *A. columnaris* seeds did not retain viability when stored at room temperature (table 3). Even storage at 15 °C did not prevent germinability decreasing from 78% (fresh seeds) to 40% in 3 months. Loss of germinability during warm storage was accentuated for seeds with high moisture content (fig. 2). After 6 months' storage, such seeds collected in 1972 and stored at 24 or 15 °C did not germinate.

Table 2—*Average number of empty* Araucaria columnaris *seeds per 100 sown as determined by x-ray examination after germination tests, by seed moisture content and storage period*

Initial seed moisture (%)	Storage period (mo)	Average no. of empty seeds /100	
		1972	1973
26–27	0	15.8 a ± 1.6	2.25 a ± 0.85
	6	13.8 a ± 2.6	35.84 b ± 2.50
6–7	0	—	1.50 a ± 1.50
	6	14.3 a ± 3.1	33.76 b ± 3.24

Within column means followed by the same letter do not differ significantly ($P > 0.05$). Values are means ± standard errors.

Table 3—*Average percent germination in seed lots of* Araucaria columnaris *harvested in 1972 and 1973 and stored at different temperatures for 3 and 6 months*

Storage temperature (°C)	Average percent seed germination		
	1972		1973
	3 months	6 months	6 months
− 7	—	59.1 a ± 3.4	8.4 a ± 1.3
3	61.8 a ± 3.3	50.2 a ± 2.0	23.8 b ± 2.6
15	39.5 b ± 3.0	13.5 b ± 3.6	—
24	8.8 c ± 4.0	7.1 b ± 3.4	—

Within column means followed by the same letter do not differ significantly ($P > 0.05$). Values are means ± standard errors.

The adverse effect of warm storage on germinability of seeds with high moisture content has been attributed to respiratory heating or microbial proliferation or both (4, 7). Heat may reach lethal levels and storage microorganisms may partially or wholly decay seeds in storage. Even if such seeds are not destroyed, saprophytic fungi may proliferate so rapidly when seeds are removed from storage and placed in conditions favorable for germination that they decay before germinating. Decay during and after storage was evident in this study. Half of the seeds stored at 24 °C and at moisture contents of 27 and 21% decayed before 3 months elapsed. Mold was a serious problem during germination for some hydrated seeds stored at 24 and 15 °C. At lower temperatures, neither problem was apparent.

Seeds must be stored at temperatures just above or possibly just below freezing, so as to minimize loss of germinability (fig. 2). However, even cold storage failed to prevent statistically significant losses of germinability for most treatments. The only cold storage treatments that prevented significant loss of viability were a) 3 months' storage at 3 °C and with seed moisture of either 27 or 21 % and b) 6 months' storage at -7 °C and with seed moisture of either 27 or 21% (fig. 2).

The data from the 2 collection years differ regarding the effect of below-freezing temperature on seed storage life. Seeds collected in 1972 and stored at -7 °C for 6 months germinated as well as those stored at 3°C (table 3). Both cold storage treatments resulted in significantly greater germination than the two warmer storage treatments. In contrast, seeds collected in 1973 and stored at -7 °C for 6 months germinated more poorly than seeds stored at 3 °C (table 3).

The beneficial effect of low-temperature storage on prolonging viability of recalcitrant *Araucaria* seeds has been observed by others (3, 14, 19). Akamine (1, 2) reported that 7 °C was the optimum storage temperature for *A. excelsa* (syn. *A. heterophylla*), provided seeds are kept under 60 to 75% relative humidity. Slightly lower temperatures

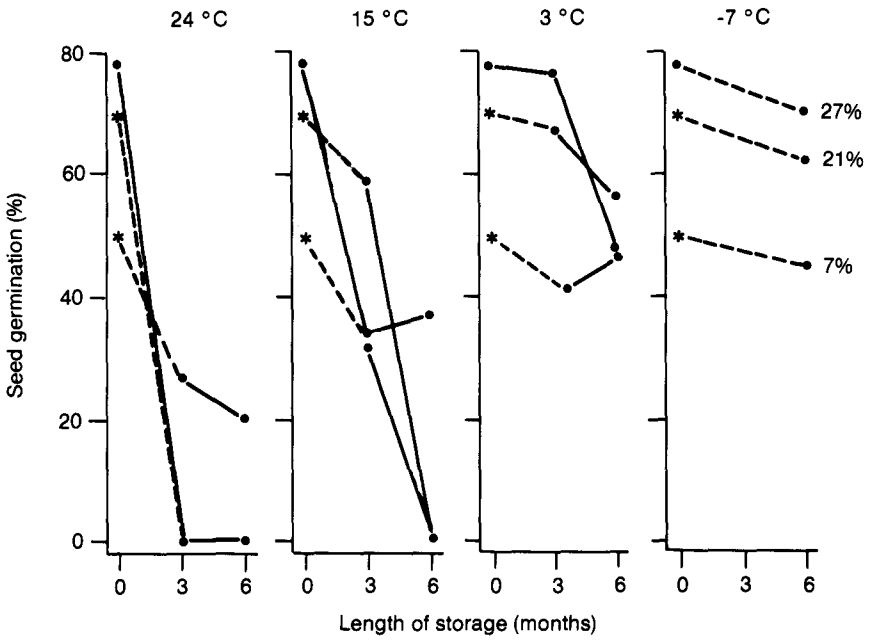

Figure 2—*Percentage germination of the 1972 lot of* Araucaria columnaris *seeds before and after storage at 24, 15, 3, and −7 °C and at seed moisture contents of 27, 21, and 7 percent (fresh weight basis). Asterisks (*) denote estimated percentage germination. Broken lines connect estimated and measured values. Vertical bars are standard errors of the means.*

(2 to 4 °C) have also been recommended for moist *A. excelsa* seeds (12) cited in King and Roberts (11). Tompsett (22) cautiously suggested that long-term storage (>18 months) of A. columnaris seeds might be achieved at -18 °C if seed moisture content was first lowered to 7%.

Seed moisture content. Data for the 1972 seed lot indicated that the effect of seed moisture content on germination depends on storage temperature (fig. 2). Seeds stored at 24 or 15 °C for 6 months germinated best if first dried to 7% moisture. Seeds stored at 3 °C for 6 months germinated equally well at all three moisture contents. Seeds stored at -7 °C for 6 months germinated best if they were not dried.

Although germination of dried but unstored seeds was not determined for the 1972 seed lot, I suspected that ovendrying reduced viability (see estimated initial values in figure 2). Test results for the 1973 seed lot (fig. 3) showed a decline of seed viability as a result of ovendrying. Germination of fresh, undried seeds at 68% moisture content averaged 62%. Drying seeds to 26 and 6% moisture reduced germination to 50 and 11 %, respectively. The latter germination percent was significantly lower than the other two percentages. Either high temperature or desiccation or both contributed to the loss of viability.

Ignoring for the moment the effect of heating, the length of drying time alone could account for loss of germination ability. It took 28 days of drying at 22 °C followed by 4 additional days at 32 °C for the 1973 seed lot to reach 6% moisture content. It took 10 days of drying at 22 °C and constant 40% relative humidity for seeds to reach 26% moisture content. Akamine (1) found that *A. excelsa* seeds exposed to ambient temperature (21 to 27 °C) lost about half of their germinability in 1 month, regardless of relative humidity (ambient to constant 90%). Havel (8) reported an even greater loss for *A. hunsteinii* after 1 month's exposure to ambient temperatures (13 to 35 °C) and relative humidities in New Guinea.

Desiccation, not heat, was probably the time-dependent

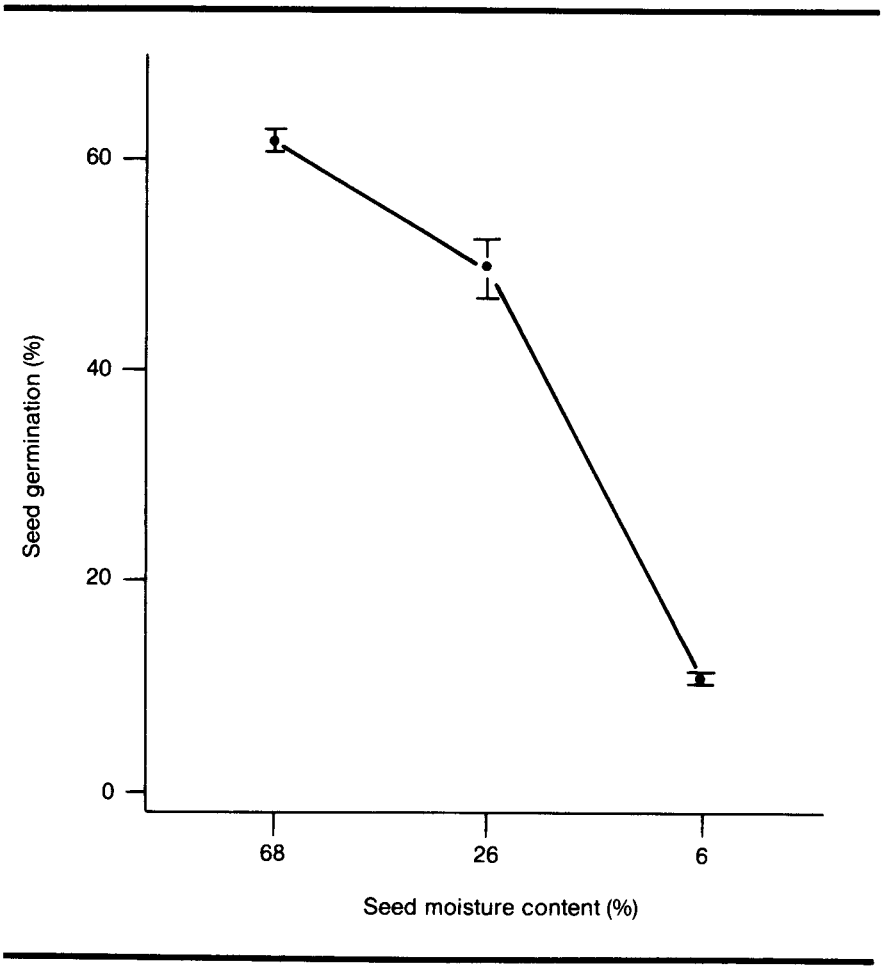

Figure 3—*Percentage germination of the 1973 seed lot before storage as a function of seed moisture content. Vertical bars are standard errors of the means.*

factor contributing most to loss of seed viability during ovendrying. Tompsett (21) reported that initial germination of A. columnaris seeds dried to 7% moisture content was about 30% or half the value achieved by fresh seeds at 36% moisture content.

However, he found that seeds dried to 22, 15, and 12% moisture content germinated nearly as well as fresh seeds. In contrast, fresh seeds of *A. hunsteinii*, a recalcitrant seed species, could be dried from 53 to 32% moisture content with no loss of germination ability (19). Below 32% moisture, germination declined until at 14% moisture seeds failed to germinate.

Immaturity of the 1973 seeds may have also contributed to the loss of germination ability upon desiccation. Harrington (7) noted that immature seeds in general have a shorter life-span than do mature seeds and are likely to lose viability upon drying.

The elevated temperature need to bring seeds from the 1973 seed lot down to 7% moisture could have been another factor leading to the significant loss of germinability. Roberts (16) suggested that if seeds are dried by heating, the higher the initial seed moisture content the greater their probability of damage-a suggestion supported by results of the current test, in that, unstored 1972 seeds dried from 27 to 7% moisture had estimated germination above 45%, whereas, unstored 1973 seeds dried from 68 to 6% had germination of only 10%.

Nitrogen enrichment of storage jars. For undried seeds from the 1972 seed lot, storage in a nitrogen-enriched environment at above freezing temperatures adversely affected germinability. After 3 months' storage at 3 and 15 °C, germination was about 20% lower in high-nitrogen (low-oxygen) atmospheres than in air (fig. 4). This result agrees with the observation by Roberts (17) that, in general,

oxygen benefits seeds stored at high moisture contents and adversely affects seeds stored at low moisture contents.

Similar results were reported by Tompsett (20), who found that germination of recalcitrant, hydrated *A. hunsteinii* seeds decreased as oxygen concentration was reduced from 21 to 0%. He suggested that lower seed respiration accompanying lower oxygen concentrations may inhibit repair of cellular damage and thereby reduce seed longevity.

Three-month germination of frozen (-7 °C) seeds stored in a high-nitrogen environment was not significantly different from that of undried, unstored seeds (fig. 4)-80 and 78%, respectively. Although 3-month data for frozen seeds in air were not available because of the limited seed supply, germination probably would not have been significantly different from that for frozen seeds in a high-nitrogen environment or for undried, unstored seeds. I suspect this would have been the case because all biological processes, including respiration and degradation processes, would be slower in frozen seeds. So reduced oxygen concentration would have less effect than it would on unfrozen seeds.

Additional testing of below-freezing temperature in combination with high nitrogen concentration was done using

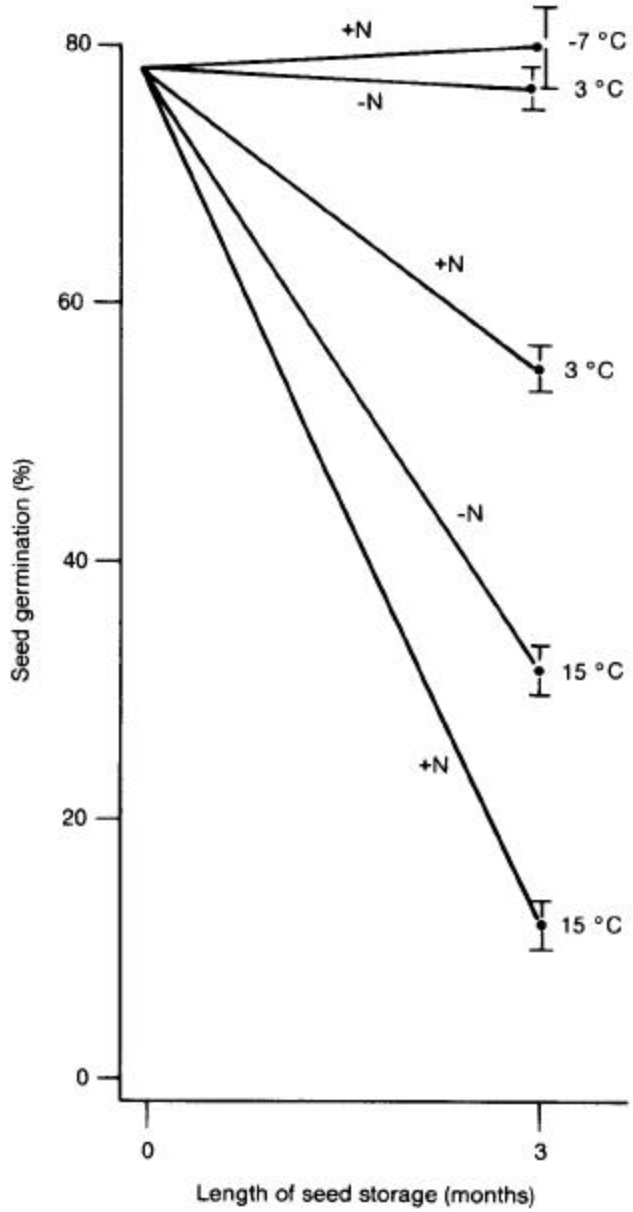

Figure 4—*Percentage germination of the 1972 seed lot after 3 months of storage in jars with (+ N) and without (− N) nitrogen gas and at −7, 3, and 15 °C. All seeds were initially at 26% moisture content. Vertical bars are standard errors of the means.*

the 1973 seed lot. In contrast to results with the 1972 seed lot, adding nitrogen to storage containers did not prolong germinability of frozen seeds from the 1973 seed lot (fig. 5).

Summary

The implicit objective of this research and that of Akamine (1, 2) and Tompsett (22) was to provide seed suppliers and nursery workers with short-term and long-term storage criteria for maintaining high seed viability. These experiments did not fully satisfy this objective. The best germination achieved after short-term storage (<1 year) was 70% in the current study. The best germination achieved after long-term storage was 20% (22). These relatively low germination values are probably unacceptable to seed suppliers.

Integrating the results of this study with those of Tompsett (22), yields several general recommendations:

1. Store seeds in air-tight containers at temperatures near or below freezing. Controlled desiccation of seeds before storage may be desirable.
2. Dry seeds quickly, but not at oven temperatures above about 24 °C. Seed moisture content should be about 15 to 25% when storage is done at temperatures near freezing.

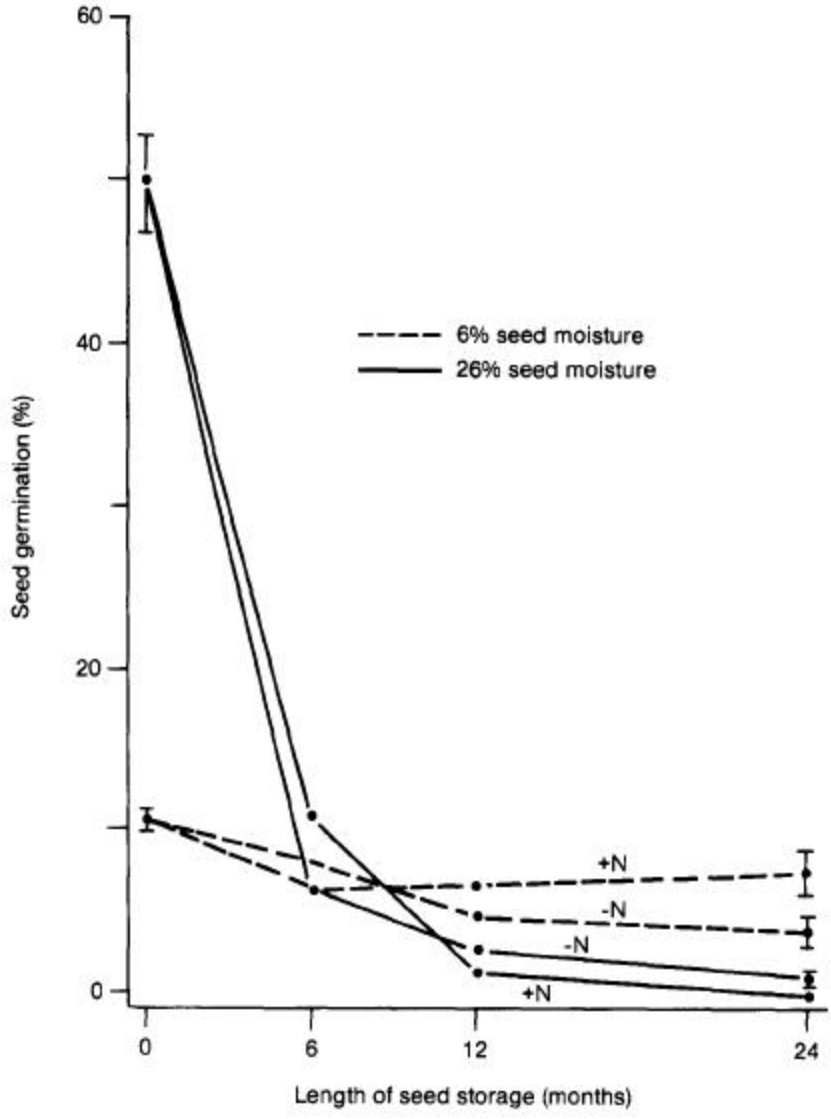

Figure 5—*Percentage germination of the 1973 seed lot after 6, 12, and 24 months of storage as a function of initial seed moisture content and with (+ N) and without (– N) nitrogen gas added to storage containers. Vertical bars are standard errors of the means.*

3. Do not store seeds in nitrogen gas—especially if storage temperature is above freezing.

Adherence to these recommendations does not guarantee long-term maintenance of high germinability. Success at prolonging viability of *A. columnaris* seeds appears quite variable. Part of the variability may be attributed to collection of physiologically immature seeds, as was the case in this study. Collectors should harvest only fully ripened seeds, a condition indicated by disintegration of cones on the trees and relatively low seed moisture contents (<40%).

Suppliers should also be aware that successful storage is only half of their problem; delivering viable seeds to buyers is the other half. Rapid deterioration after removal from storage and during shipment and germination is likely (3). Studies addressing the effect of shipping methods on germinability of stored Araucaria seeds remain to be done.

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