The effect of storage temperature and duration on northern red oak acorn viability and vigour

by Thomas L. Noland[1,*], Andrée E. Morneault[2], Daniel C. Dey[3] and Dave Deugo[4]

ABSTRACT

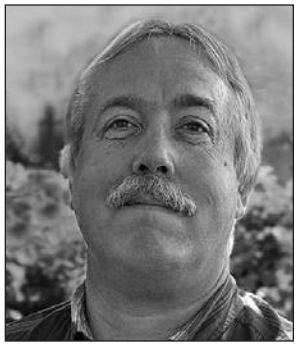
Three separate collections of Ontario sources of northern red oak (*Quercus rubra* L.) acorns were made to determine the effects of long-term cold storage at +2°C, -1°C, and -2°C on their viability and vigour. We measured acorn moisture content, percent germination during storage, speed of germination and total germination values, root regrowth of seeds that germinated in storage, incidence of fungal contamination, and condition of ungerminated acorns. Viability and vigour peaked six to 12 months after acorns were placed in storage, but decreased with continued storage. After 18 months in storage, ≥60% of the acorns germinated in four of the five seedlots tested and, after 30 months in storage, ≥53% of the acorns germinated in three of the five seedlots tested. Acorn viability was only minimally affected by storage temperature; however, since temperatures above -2°C allowed acorns to germinate during storage, the preferred long-term storage temperature was -2°C. Our results suggest that, assuming proper storage conditions, most red oak seedlots will maintain relatively high germination levels when stored at -2°C for 18 months. Seedlots with particularly high initial germination and vigour may be successfully stored for up to 30 months.

Keywords: germination, recalcitrant seed storage, seed storage temperature, seed storage longevity, *Quercus rubra*

RÉSUMÉ

Trois ensembles distincts de glands de chêne rouge nordique (*Quercus rubra* L.) en provenance de l'Ontario ont été rassemblés dans le but d'identifier les effets de l'entreposage au froid à long terme à +2°C, -1°C, and -2°C sur leur viabilité et leur vigueur. Nous avons mesuré le niveau d'humidité des glands, le pourcentage de germination au cours de l'entreposage, la vitesse de germination et les valeurs de germination complète, de reprise de la croissance des racines des semences qui avaient germées au cours de l'entreposage, de l'incidence de la contamination fongique et l'état des glands n'ayant pas germé. La viabilité et la vigueur ont atteint une valeur maximale de six à douze mois après l'entreposage des glands, mais ont diminué en fonction du temps d'entreposage. Après 18 mois d'entreposage, ≥60% des glands avaient germé dans quatre des cinq lots de semences testés et après 30 mois d'entreposage, ≥53% des glands avaient germés dans trois des cinq lots de semences étudiées. La viabilité des glands a été peu affectée par la température d'entreposage; cependant, compte tenu que les températures supérieures à -2°C ont permis aux glands de germer au cours de l'entreposage, la température d'entreposage à long terme préférée a été -2°C. Nos résultats suggèrent que, sous des conditions appropriées d'entreposage, la plupart des lots de semences de chêne rouge maintiendront de forts taux de germination lorsqu'entreposés à -2°C pour une période de 18 mois. Les lots de semences ayant initialement un fort taux de germination et une grande vigueur pourraient être entreposés avec succès pour une période allant jusqu'à 30 mois.

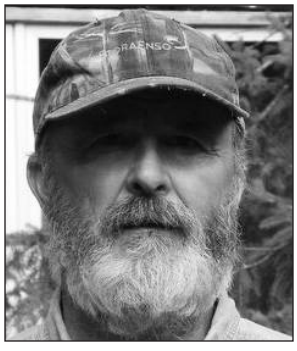
Mots clés : germination, entreposage des semences récalcitrantes, température d'entreposage des semences, longévité des semences entreposées, *Quercus rubra*



Thomas L. Noland

Andrée Morneault

Daniel C. Dey



Dave Deugo

[1]Ontario Forest Research Institute, Ontario Ministry of Natural Resources, 1235 Queen St. East, Sault Ste. Marie, Ontario P6A 2E5.
[2]Southern Science and Information Section, Ontario Ministry of Natural Resources, 3301 Trout Lake Rd., North Bay, Onatraio P1A 4L7.
[3]USDA Forest Service Northern Research Station, 202 ABNR Bldg., University of Missouri-Columbia Columbia, MO 65211-7260 USA.
[4]Formerly Ontario Ministry of Natural Resources, Rural Route #2, 1350 High Falls Rd., Bracebridge, Ontario P1L 1W9 Canada; retired. Current address: Rural Route #5, Purbrook Rd., Bracebridge, Ontario P1L 1X3.
* Corresponding author. E-mail: tom.noland@ontario.ca

Introduction

Northern red oak (*Quercus rubra* L.) is an ecologically and economically important tree species throughout its range in North America, including in the Great Lakes–St. Lawrence and Carolinian forests of Ontario. Regeneration of red oak without fire or other practices to reduce competition from other tree species is often difficult (Lorimer 1993). Regenerating oak forests to sustain current levels of oak stocking is a worldwide problem (Watt 1919, Thadami and Ashton 1995, Li and Ma 2003, Götmark *et al.* 2005, Pulido and Díaz 2005, Zavaleta *et al.* 2007). For some time now, the Ontario Ministry of Natural Resources (MNR) has been investigating ways to improve oak regeneration in the province (Deugo *et al.* 2006).

Several factors restrict red oak regeneration from seed, whether natural or assisted. Northern red oak (NRO) produces an abundant seed crop only every three to five years, referred to as mast seed years (Auchmoody *et al.* 1993, Sork *et al.* 1993). Also, viable acorns do not persist in the forest seed bank because a combination of rodent predation, insect infestation, and fungal contamination of acorns prevents them from surviving beyond the first spring after dispersal (Winston 1957). Insect infestation and fungal contamination can also reduce acorn vigour and viability (Bonner and Vozzo 1987, Gribko *et al.* 2002, Lombardo and McCarthy 2009) and therefore limit the storage life of acorns. As a result of all of these factors, a regular annual supply of fresh acorns is not available for regeneration. In addition, because NRO acorns are recalcitrant, meaning they do not survive drying or freezing and must be kept moist to remain viable, long-term storage is difficult (Korstian 1927, Olson 1974, Bonner and Vozzo 1987). Acorns will die when stored below 32% moisture content and storage for longer than one year at or below -5°C is also lethal (Farmer 1975). Furthermore, acorns can rapidly lose viability when stored at 1°C to 3°C for more than one year (Creasey *et al.* 1992), during which time significant numbers will often germinate (Suszka and Tylkowski 1982). Such pregerminated acorns are difficult to plant in nurseries. Fungal contamination (Winston 1957, Washington 2003) and weevil infestation (Winston 1957) may also limit the viable storage life of NRO acorns. These long-term storage issues limit the annual supply of acorns required to grow red oak seedlings in nurseries or sow seeds in harvested areas requiring regeneration.

Maintaining NRO acorn viability in long-term storage without significant numbers germinating is a major challenge, although in one study germination during storage was minimal at -1°C and prevented altogether at -3°C (Suszka and Tylkowski 1982). In addition, NRO acorns retained most of their initial viability for 18 months, following which viability rapidly declined (Suszka and Tylkowski 1982, Suszka *et al.* 1996). However, these trials were performed using Polish seed sources and, to our knowledge, these approaches have not been extensively tested with North American seed sources. If successful, these techniques may make it possible to extend the NRO acorn supply for up to two years after an abundant seed year.

The main objective of our initial study was to determine whether red oak acorns collected in Ontario could be stored below freezing for extended periods and retain their viability without germinating in storage. A secondary objective was to assess the effect of above- versus below-freezing storage temperatures on seed viability. In a subsequent trial, further objectives were to determine (1) whether seed source influences the ability of acorns to tolerate storage, i.e., maintain germination capacity, (2) whether acorns that germinated during storage continued root development during germination tests, (3) the effect of storage temperature on the incidence of fungal infection in stored seed, and (4) reasons for germination failure of stored acorns.

Methods

We conducted three separate trials using red oak acorns collected from different locations during mast seed years and stored under slightly different conditions so each trial is described separately below. In all three trials, initial germination percentages of red oak seed were compared with germination percentages achieved after storage for various periods under temperatures slightly below and slightly above freezing.

Storage trial 1 (ST1)

A bumper crop of NRO acorns prompted Dr. Dan Dey, formerly with MNR, to collect acorns from the ground below 10 trees in the Sault Ste. Marie, Ontario, area during the fall shedding period in October 1993. Acorns were divided into two equal lots and placed in 5-mil (0.005″ thick or 0.127 mm) polyethylene bags for storage at +2°C (walk-in cold storage room) and -1°C (refrigerator). Bonner (1993) recommended 4-mil to 10-mil thick polyethylene bags for acorn storage because they retain moisture but allow adequate air exchange for the physiologically active acorns.

To break their dormancy, fresh NRO acorns require a 30- to 45-day stratification period at +1 to +5°C (Olson 1974). For the initial germination test, about 500 acorns were placed into a 5-mil plastic bag and given a 45-day moist stratification period at +2°C beginning December 23, 1993. Following the stratification period, acorns were removed and subjected to a 10-minute float test in water (Gribko and Jones 1995) and floating acorns were discarded. Moisture content was determined for a sample of 25 acorns after oven-drying at 103°C for 18 hours.

All remaining stratified acorns were soaked at room temperature (22°C) for 24 hours in aerated, deionized (DI) water. After soaking, 25 more acorns were removed to determine their post-soak moisture content and dry weight (as described above). Of the remaining acorns, 400 were put into plastic germination boxes (50 acorns per box) that contained moist sand. Four replications each consisting of two germination boxes of 50 seeds were then placed in a controlled environment chamber with the following conditions: 75% humidity, 8-hour, 30°C day (08:00–16:00); and 16-hour, 20°C night (16:00–08:00). Germination was defined as radicle protrusion of 1 cm or more plus geotropic curvature.

Although an official germination test (ISTA 1996) lasts 28 days, we counted germinants for 60 days or until we had two consecutive count days with no new germinants. Germinants were counted twice a week. Acorns for additional tests were selected in advance and subsequent germination tests were completed similarly for each storage temperature, except 200 seeds were used (50 per replication) with no additional stratification treatment. Acorns that germinated during storage were tracked separately. Additional germination tests were conducted after 6, 12, 18, 30, 42, and 54 months. Because of insufficient seed survival at +2°C, the final germination test (after

54 months of storage) was conducted only with seed stored at -1°C. Insufficient seed numbers prevented moisture content determination for this germination test.

Storage trial 2 (ST2)

In the second trial, acorns were collected from the ground during the fall shedding period at two sites in Widdifield Twp (UTM 621100/5132100 and 620150/5132950) and one site in Phelps Twp (UTM 645540/5137200), both in central Ontario, on September 17 and 18, 2004. Prior to shipping to the Ontario Forest Research Institute (OFRI) for processing, acorns were stored for about seven weeks in plastic bags in an outdoor storage shed. After arrival at OFRI in October, acorns from the three locations were bulked together, separated into two equal lots and stored at +2°C and -2°C in large 5-mil polyethylene bags. Germination tests were conducted as described for ST1 after 0, 6, 12, 18, 24, and 30 months of storage. During germination tests, we recorded the number of acorns removed due to heavy fungal infection.

Storage trial 3 (ST3)

In the third trial, acorns were collected from the ground during the fall shedding period from three separate locations in central Ontario: Seedlot 1 – Phelps Township (17T 0645591 5137425) collected September 27 and October 3, 2008; Seedlot 2 – Almonte (N 45 18.335 W 76 21.593 UTM 18 T 0392882-5018219) collected October 7-9, 2008; and Seedlot 3 – Muskoka Lakes Township (Port Carling and Milford Bay areas, Monck and Medora wards) collected October 20, 2008. Although acorns should only be float-tested for a few minutes to identify the non-viable acorns (which float; Bonner and Vozzo 1987), seedlot 1 was floated for about 60 hours before shipping and only sinkers were shipped, as suggested by Gribko and Jones (1995). Such a long soak is not standard operational practice in Ontario. A longer (16- to 24-hour) soak for a float test is only recommended if acorns are collected from a dry site or during a dry autumn (Bonner and Vozzo 1987). Seedlot 2 was floated for one minute before shipping and only sinkers kept. All seedlots were shipped to OFRI via courier. Within two days of receipt, approximately equal amounts of each seedlot were placed into two separate 5-mil polyethylene bags and placed in the +2°C and -2°C walk-in coolers for long-term storage. Seedlot 3 was floated at OFRI for 10 minutes just before germination testing, and only sinkers were kept. The acorns were surface dried before being returned to long-term storage. Seedlots 2 and 3 contained only about 2000 acorns each, a sufficient amount for conducting four germination plus moisture content tests.

The initial germination test for all three seedlots began after 45 days of stratification at +2°C. For the initial germination test, 400 acorns (100 per replication) from each seedlot were germinated in the fall of 2008. For all germination tests, any acorns chosen that had germinated in storage were recorded and placed in germination boxes, with additional radicle growth during the germination test period recorded to note the ability to continue developing into viable seedlings. Moisture content was determined before and after a 24-hour soak in DI water as described in ST1; similarly, germination test conditions were as described in ST1. Subsequent germination tests using 200 seeds (50 per replication) were conducted after 12, 24, and 30 months of storage at +2°C and -2°C. During these tests we recorded the number of acorns removed due to heavy fungal infection. All acorns that failed to germinate during the germination test of ST3 were cut in half and visually examined to determine their condition. Acorns with no visual evidence of tissue discoloration or damage were included in the "healthy" category, acorns with cotyledon and/or embryo discoloration were included in the "infected by fungi", and acorns with insects present or tissue damage and holes in the pericarp were included in the "damaged by insects" category.

Statistical analyses

Germination value (GV), a measure of the speed and completeness of germination that is commonly used to estimate seedlot vigour, was calculated according to the formula by Czabator (1962). All data were tested for normal distribution (Sharpiro-Wilk test) and for equal variance using SigmaStat 3.0. Since both raw and transformed germination data sometimes failed at least one of these tests, the effect of length of storage, storage temperature, and seedlot (only for ST3) on percent germination and germination value was analyzed using Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks using SigmaStat 3.0. In ST3, only seedlots 2 and 3 were compared; seedlot 1 was excluded because of heavy fungal contamination of all acorns. For data analyzed by the Kruskal-Wallis ANOVA, significant differences ($P \leq 0.05$) for all pairwise comparisons were assessed using Dunn's method. All data passing the normal distribution and equal variance tests were analyzed using a general linear model for the ANOVA and all multiple pairwise comparisons were analyzed for significant differences ($P \leq 0.05$) using the Holm-Sidak method. Single pairwise comparisons were analyzed using a simple t-test. Pearson product moment correlation analysis of GV and storage duration was performed using SigmaStat 3.0.

Results

Moisture content

Prior to storage, acorn moisture content of the five individual oak seedlots ranged from a low of 37.5% for seedlot 3 of ST3 to a high of 46.7% for seedlot 1 of ST3 with the other three seedlots having a moisture content of 39% to 40%. Seedlots gained moisture with increasing time in storage (data not shown) and the overall average pre-soak moisture content by storage trial ranged from 42% to nearly 45% (Table 1). The increase in seed moisture content after a 24-hour aerated soak varied from 2.5% to 4.3%, but averaged slightly above 3% overall.

Table 1. Average pre-soak moisture content of red oak acorns during storage for each of the storage trials

Average	Moisture content (%)					
	Trial 1		Trial 2		Trial 3	
	+2°C	-1°C	+2°C	-2°C	+2°C	-2°C
By temperature treatment	42.9	42.5	44.8	42.4	42.3	42.0
Overall	42.7		43.7		42.2	

Effects of storage on acorn germination

On average, 22% of acorns stored at +2°C germinated during the storage period over all three trials (Table 2). In contrast, only a small percentage germinated when stored at -1°C and none when stored at -2°C. Thus, about 10 times more seeds germinated when stored at +2°C than when stored below freezing. Seedlot appeared to affect the proportion of seeds germinating in storage at +2°C, ranging from a low of 1% to a high of 44%. Germination during storage at +2°C increased with increasing storage duration in ST1 and ST3, but not ST2 (data not shown).

In ST3, only in seedlot 2 did most of the pregerminated seed continue developing roots during the germination test after 2.0 and 2.5 years of storage at 2°C (Table 3). For example, only 1% of the seeds that germinated in storage continued growing roots during the germination test of seedlot 1 while none did in that of seedlot 3. However, this only had a sizable effect on germination percentage in seedlot 1, where it dropped from a total germination of 51% to an actual germination of 1%.

Fungal contamination, heavy enough to require acorn removal from germination tests, increased with time in storage (Table 4). However, other than for seedlot 1 in ST3, no acorns had to be removed due to fungal contamination until they had been in storage for 24 months or longer. After that, the proportions of acorns removed during the germination tests depended more on the seedlot than the storage temperature. After 30 months in storage, heavy fungal contamination meant that more of the acorns stored at +2°C had to be removed from germination tests in ST2 than those stored at -2°C, but the opposite was true in ST3. For seedlot 1 of ST3, 100% of the acorns were removed due to fungal contamination during germination tests regardless of storage temperature or duration.

Germination

In ST1, initial germination percent was very high, close to 100%, and regardless of storage temperature remained high for 30 months (Fig. 1). After 42 months of storage, germination percent remained at 80% for acorns stored at -1°C but decreased to about 40% for acorns stored at +2°C, indicating that seed viability was decreasing gradually at the lower temperature and more rapidly at the higher temperature. Acorns in ST2 had a lower initial germination percent (88%) and germination percent dropped to 65% after 18 months of storage at +2°C and to 51% after 24 months storage at -2°C (Fig. 1). After 30 months, germination tests indicated that acorn viability was less than 50% regardless of storage temperature.

In ST3, initial germination was about 65% for seedlots 1 and 2 and 82% for seedlot 3 (Fig. 2). After 12 months, acorns from seedlots 2 and 3 stored at both temperatures showed significantly ($P \leq 0.05$) increased germination percentages, with means of 94% and 96%, respectively. For seedlot 1, after 12 months in storage germination decreased to 43% for acorns stored +2°C and to 0% for acorns stored at -2°C. This drastic and rapid decrease was probably related to the high level of fungal contamination after 12 or more months in storage, which necessitated removing acorns during germination tests (Table 4). Although germination declined in seedlots 2 and 3 after 30 months of storage, germination was at least 53% for acorns stored at -2°C and 69% for acorns stored at +2°C.

In the initial germination test in ST3, most of the acorns that failed to germinate appeared healthy based on visual inspection (Fig. 3). However, after 12 months in storage, over 90% of the ungerminated acorns exhibited fungal infection and about 7% suffered from insect damage.

When the germination data for both storage temperatures in each trial were combined, germination values (GV) peaked after six to 12 months of storage (Table 5). Statistical analyses indicated that neither storage temperature nor seedlot in ST3 significantly affected GV. The acorns in ST1 maintained a relatively higher GV for a longer time in storage than did those in ST2 and ST3. In general, germination values decreased with time in storage, resulting in significant negative correlations between GV and months in storage (Table 6). The coefficient of correlation (r) values were stronger and more negative in ST2 and ST3 when the correlation excluded the GV from the initial germination and began with the peak GV values at six or 12 months.

Table 2. Effect of storage temperature on red oak acorn germination while in storage

	Germination (%)			
Storage trial: storage temperature low, high	Low temperature mean[a]	Low temperature high[b]	High temperature mean	High temperature high
ST1[c]: -1°C, +2°C	8	12	44	66
ST2: -2°C, +2°C	0	0	28	37
ST3 Lot 2: -2°C, +2°C	0	0	15	25
ST3 Lot 3: -2°C, +2°C	0	0	1	2
Overall average	2	3	22	32

[a] Mean denotes average of all germination tests for the entire seedlot.

[b] High denotes the highest value for a single germination test.

[c] ST1 = Storage trial 1, ST2 = Storage trial 2, ST3 = Storage trial 3.

Table 3. Germination performance of red oak acorns that germinated in +2°C storage in storage trial 3

	Acorns (%)			
Seedlot storage temperature and duration	Germination total[a]	Germination in storage	Root growth continued during germination test	Germination actual[b]
Lot1: +2°C, 2.0 years	51	50	1	1
Lot2: +2°C, 2.0 years	92	25	98	91
Lot2: +2°C, 2.5 years	78	25	64	69
Lot3: +2°C, 2.5 years	69	2	0	67

[a] Pregerminated seed plus seed that germinated during germination test.

[b] Total germination minus pregerminated seed that failed to grow new roots during germination test.

Table 4. Percent of red oak acorns removed from germination tests due to fungal contamination in storage trials 2 (ST2) and 3 (ST3)

Months in storage	Storage trial/temperature							
	ST2		ST3 seedlot 1		ST3 seedlot 2		ST3 seedlot 3	
	+2°C	-2°C	+2°C	-2°C	+2°C	-2°C	+2°C	-2°C
0	0	0	0.5	n/a	0	n/a	0	n/a
6	0	0	n/a	n/a	n/a	n/a	n/a	n/a
12	0	0	100.0	100.0	0	0	0	0
18	0	0	n/a	n/a	n/a	n/a	n/a	n/a
24	24.5	24.0	100.0	100.0	0	0	0.5	0.5
30	60.0	33.0	n/a	n/a	3.0	9.5	6.5	20.0

[a] n/a = not applicable

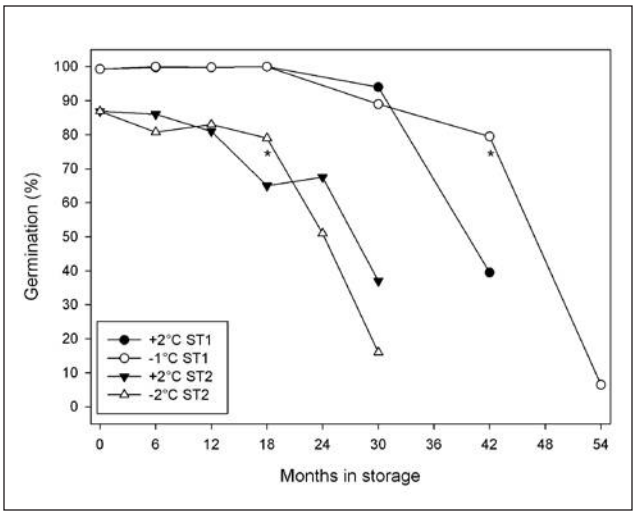


Fig. 1. Effect of storage temperature and storage duration on germination of northern red oak acorns in trials 1 (ST1) and 2 (ST2). * Indicates storage temperature resulted in significantly different ($P \leq 0.05$) percent germination of a seedlot pair.

Discussion

Oak is a species that produces recalcitrant acorns that die when their moisture content drops below 30% to 35% (Farmer 1975, Pritchard 1991, Connor 2004); therefore, initial moisture content of red oak acorns is critical to their survival in storage (Korstian 1927, Olson 1974). Goodman *et al.* (2005) reported that NRO acorn desiccation tolerance varied significantly with family and concluded that genetic variability affected the degree to which acorns tolerated desiccation. The critical minimum moisture content for maximum red oak acorn survival in cold storage has been estimated as 30% to 32% (Bonner 1973; Farmer 1975) although Suszka and Tylkowski (1982) suggested it may be as high as 35%. All seedlots in this study were at or above an average of 37.5% moisture content before storage, indicating that few to none of the acorns tested were likely below the critical moisture content affecting viability during long-term storage. As well, acorn moisture content in this study increased with time in storage, a trend also noted by Suszka and Tylkowski (1982).

The collection and handling of the acorns differed in the three storage trials of our study. For example, acorns for ST1 were put into cold storage at OFRI immediately after collection.

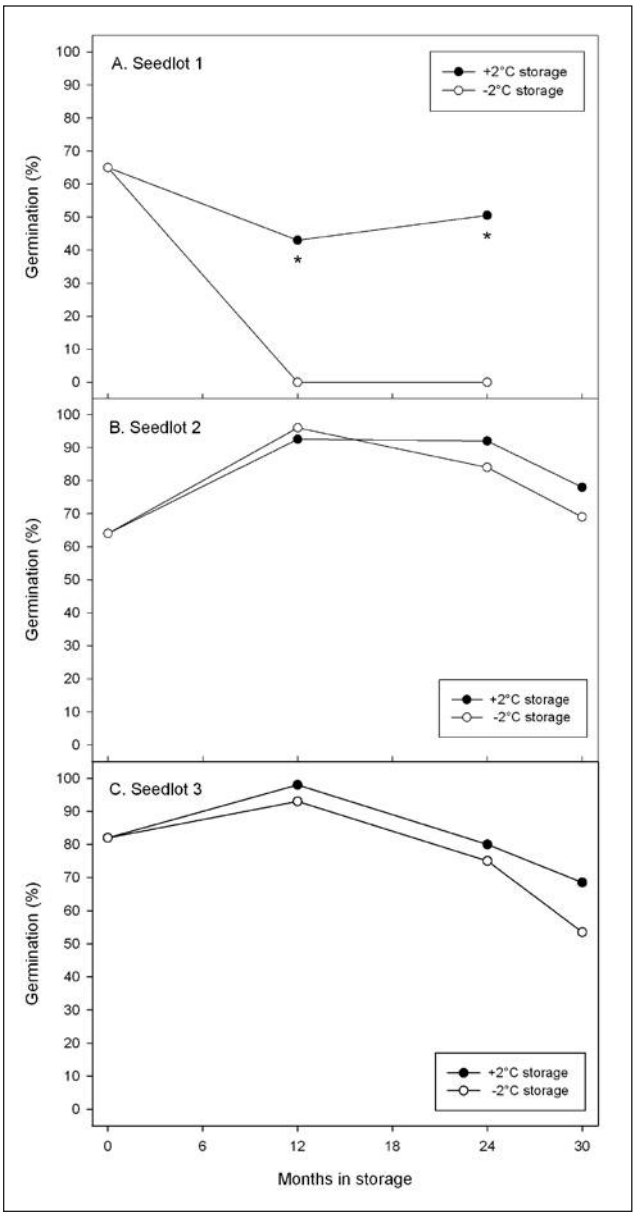


Fig. 2. Effect of storage temperature and storage duration on germination of three seedlots of northern red oak acorns in trial 3. * Indicates storage temperature resulted in significantly different ($P \leq 0.05$) percent germination of a seedlot pair.

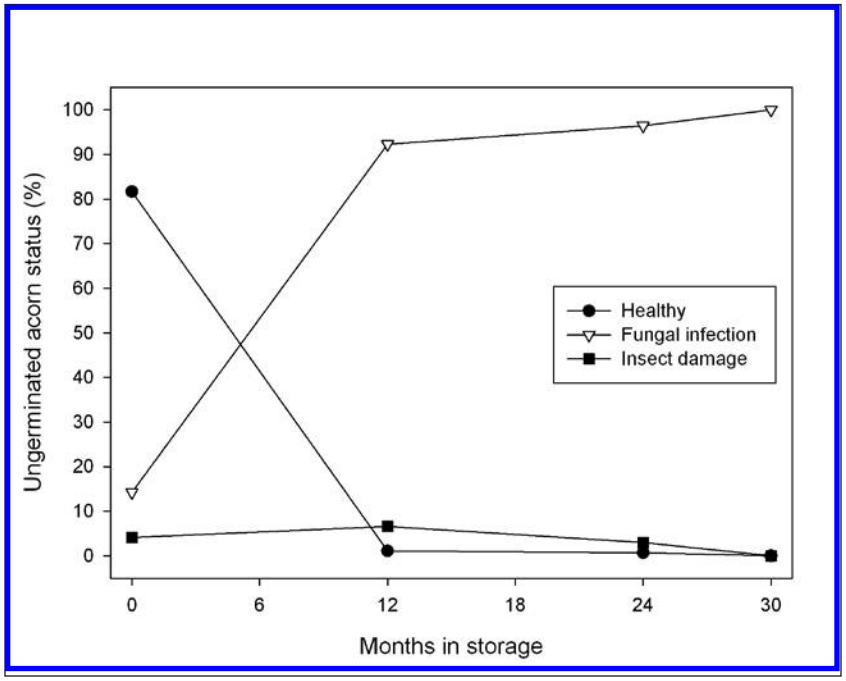


Fig. 3. Condition of northern red oak acorns that failed to germinate during germination tests of seedlots 2 and 3 in storage trial 3 by time in storage.

Table 5. Germination values in northern red oak acorn storage trial germination tests (mean ± std. err). Means within a column followed by the same letter do not differ significantly ($P \leq 0.05$)

Months in storage	Trial 1	Trial 2	Trial 3 (seedlots 2+3)
0	48.87±0.98a	10.64±0.46ab	1.76±0.68c
6	84.07±7.42a	41.61±0.88a	n/aa
12	35.59±5.03ab	17.27±2.62ab	41.89±6.23a
18	31.51±4.43ab	6.46±0.17bc	n/a
24	n/a	1.35±0.12bc	6.69±0.69b
30	7.27±1.68b	0.45±0.08c	3.70±0.42bc
42	8.76±1.29b		

[a] n/a = not applicable

Table 6. Correlation coefficients for time in storage vs. germination values (Pearson *r*, *P* value)

Months in storage	Trial 1	Trial 2	Trial 3 (seedlots 2+3)
0	-0.77, < 0.0001	-0.57, < 0.0001	-0.33, < 0.015
6	-0.78, < 0.0001	-0.71, < 0.0001	n/a
12	-0.78, < 0.0001	-0.83, < 0.0001	-0.84, < 0.0001

[a] n/a = not applicable

On the other hand, acorns for ST2 and ST3 were placed in temporary storage under varying conditions, such as the ST2 acorns being stored for seven weeks in an unheated shed before being sent to OFRI and placed in cold storage. These variations in conditions just after collection and before long-term storage likely affected the viability, vigour, and storability of the acorns used in this experiment. For example, ST2 showed high rates of fungal infection during the germination test after 30 months of storage and this may have been related to the seven weeks of storage at variable temperatures. However, these handling and collection differences are representative of the variation in operational collection practices applied to acorn seedlots in Ontario.

In our storage trials, some proportion of acorns always germinated during storage at +2°C (Table 2). During above-freezing cold storage, germination as high as 50% was noted in acorns of two southern red oak species (Bonner 1973). Suszka and Tylkowski (1982) found that up to 34% of NRO acorns germinated during +1°C storage. They also reported that no acorns stored at -3°C germinated and few acorns in two of five seedlots stored at -1°C germinated. Our results were similar, with no acorns germinating when stored at -2°C, an average of 8% germinating during storage at -1°C, and over 22% germinating when stored at +2°C. In ST3, except for seedlot 1, most of the pregerminated acorns remained viable and resumed root growth during the germination test (Table 4), which also coincides with results reported by Suszka and Tylkowski (1982). However, as noted by these authors, pregerminated acorns are impossible to machine-plant in nurseries and even planting them by hand can be difficult. Therefore, even though pregerminated acorns have been found to produce acceptable seedlings (Bonner 1982), preventing germination by storing acorns at -2°C to -3°C is operationally preferable.

Virtually all NRO acorns that fall to the ground are contaminated with various fungi and this contamination may even promote germination by softening the seedcoat (Washington 2003). However, depending on site moisture regime, fungal contamination can have varying effects on acorn germination. Only about 1% of acorns that fell on a river floodplain germinated the following spring (Winston 1957) whereas 62% to over 90% germinated on dry upland sites (Washington 2003). In our trial, visible fungal contamination of acorns increased with length of time in storage but, except for seedlot 1 of ST3, was not significant until after 24 months of storage (Table 4). The 100% fungal contamination of seedlot 1 in ST3 was probably related to its unconventional 60-hour soak just after collection. The soak was not aerated and may have resulted in anaerobic conditions that stressed and weakened the acorns, which may have predisposed them to fungal infection. Alternatively, the long soak may have spread fungal spores from a few particularly heavily contaminated acorns to the other acorns. In addition, these ground-collected acorns may have been contaminated with particularly virulent and/or unusually large numbers of fungal spores from the soil. Our results suggest that periods longer than six to 12 months in storage appear to result in a gradual decrease in seed viability and vigour that increases acorn

susceptibility to fungal infection. This result suggests that fungal contamination is an important factor that can limit the storage life of NRO acorns.

Insect predation can cause significant damage and death of NRO acorns leading to significant reduction of germination and reduced vigour of seedlings produced from infected acorns (Gibson 1982, Lombardo and McCarthy 2009). In an extensive multi-year survey in the United States and Canada, Gibson (1982) reported an overall average of just over 52% of all acorns were insect-infested. However, the results were quite variable and ranged from zero to 100% infestation depending on location and year. Insect predation had a minor effect on germination in our storage trial (Fig. 3), likely because most insect-infected acorns were removed during water flotation (Gribko and Jones 1995).

In our trials, storage temperature did not have much influence on germination, except for seedlot 1 in ST3 (Fig. 1 and Fig. 2). As indicated by its low germination percentage and GV value of 9.5 after 12 months of storage, the vigour of this seedlot was quite low and virtually all of the acorns stored at +2°C that germinated did so during storage. Taking into account the number of pregerminated acorns that continued root growth during the germination test, the 1% effective actual germination of the acorns stored at +2°C for 24 months was virtually identical to the 0% germination for the -2°C acorns from this seedlot. Suszka and Tylkowski (1982) also reported that +1°C, -1°C, and -3°C storage temperatures had minimal differential effects on germination of NRO acorns after various periods of storage up to 4.5 years.

Storage duration significantly influenced germination in our study. For example, more acorns of seedlots 2 and 3 from ST3 germinated after 12 months in storage than in the initial germination tests (Fig. 2). About 80% of the ungerminated seed from the initial germination test of these two seedlots appeared healthy with no signs of decay or insect damage (Fig. 3). This suggests that the seed required a longer cold stratification period to break dormancy. Although the recommended cold stratification period is 45 days (Olson 1974, Creasy and Myland 1992), the optimal cold stratification period to completely break NRO acorn dormancy could be as long as 70 to 84 days (Suszka and Tylkowski 1982). Alternatively, these acorns may have been somewhat immature and required a few months of after-ripening to attain their maximum germination potential (Brown 1939).

Beyond six to 12 months of storage, acorn viability and germinant vigour gradually decreased with increasing time in storage. However, for four of the five seedlots, germination remained at 60% or higher for 18 to 30 months. In fact, three of the five seedlots maintained 53% or higher germination at all storage temperatures for 30 months. In contrast, after 29 months of cold storage all of the red oak seedlots tested by Suszka and Tylkowski (1982) had germination percentages of 47% or lower, with most in the 0% to 30% range. After two years of storage at +3°C, Farmer (1975) reported that 60% of the acorns in the NRO seedlot with the highest initial moisture content (55%) germinated, while 18%, 0%, and 0% germinated in seedlots that had initial moisture contents of 45%, 35%, and 25%, respectively.

After 30 months of storage, red oak seedlot vigour, as measured by GV, was reduced and varied among seedlots (Table 5). The ST1 seedlot had a GV of 7.27, almost double that of ST3 seedlots 2 and 3 mean GV of 3.7, and was much higher than the GV of 0.45 of the ST2 seedlot. These differences are likely related to the initial seed quality of these seedlots. As a measure of all aspects of germination performance including vigour, GV and other single index methods have their limitations (McNair *et al*. 2012), but the use of GV has been validated via testing with several pine seedlots over multiple years. This testing included a comparison of GV results to nursery emergence performance (Bonner 1986) and indicated that a reduction in seed vigour affected nursery emergence more than it did laboratory germination. For example, Suszka and Tylkowski (1982) reported lab germination of NRO seedlots averaged only about 1.4% higher than nursery emergence after one winter of cold storage, but after 29 months of cold storage lab germination was 12% higher than nursery emergence for the same seedlots.

The key goal of long-term NRO acorn storage is to maintain seed viability and prevent germination in storage. Maintaining acorn vigour during storage is also important since it influences subsequent nursery performance. Our results suggest that an acorn float test lasting 60 hours may reduce the vigour of a seedlot as well as the length of time it can be stored and should be avoided. In addition, we found that storage at -2°C in 5-mil plastic bags appears to be a reliable method of maintaining NRO acorn viability for extended periods while preventing germination during storage. Using this method, most northern NRO seedlots could be stored for at least 18 months or two winters (Suszka and Tylkowski 1982), possibly even as long as three winters, and still be used for nursery seedling production. In our trial, after 18 months of storage four of five seedlots tested would have probably produced a good yield of nursery seedlings. Even if actual emergence were on average 12% lower than lab germination (as per Suszka and Tylkowski 1982), the 90% average germination of the ST1 seedlot and 73% average germination of seedlot 2 of ST3 after 30 months of storage would likely produce at least one seedling per two acorns sown in the nursery.

After 30 months of storage, the resulting seedlings may not have as high a growth rate as seedlings produced from fresher seed. Suszka and Tylkowski (1982) reported that seedlings produced from acorns stored for five winters (54 months) grew to an average height of 6.4 cm compared with seedling heights of 18 cm to 20 cm for acorns stored for two winters (18 months). However, the influence of acorn storage duration on NRO seedling growth rates needs more testing before the effects can be quantified.

Summary

For most northern red oak seedlots, viability and vigour sufficient to produce a good crop of seedlings in the nursery after two winters of storage can be achieved by storing acorns at -2°C in a sealed 5-mil plastic bag. Seedlots with particularly high initial germination percentages and vigour may be stored at -2°C for as long as three winters and still provide a reasonable rate (≥50%) of emergence two and a half years after the acorns mature. This storage practice will help to bridge the gap between abundant seed crop years.

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