Loblolly and shortleaf pine seed viability through 21 months of field storage: Can carry-over occur between seed crops?

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Abstract: To assess the potential for carry-over of loblolly pine (*Pinus taeda* L.) and shortleaf pine (*Pinus echinatu* Mill.) seeds from one year to the next, seed viability was determined by germination tests following field storage for up to 21 months. Treatments included seeds stored (i) in a freezer, *(ii)* on the forest floor, *(iii)* 1.8 m above the forest floor and exposed to precipitation, and (iv) 1.8 m above the forest floor and sheltered from precipitation. The germinative capacity of seeds storedin a freezer exceeded 95% for both loblolly and shortleaf pines after 7, 9, 19, and 21 months. Because seeds stored on the forest floor germinated naturally, laboratory germination after 7 months storage averaged less than 1% for both species. Loblolly pine seeds stored above the forest floor and exposed to precipitation had the highest germinative capacity (98% and 56% germination after 7 and 21 months, respectively). Relative ranking of viability retention for the remaining treatments was loblolly seeds sheltered from precipitation > shortleaf seeds sheltered from precipitation > shortleaf seeds exposed to precipitation.

Résumé : Après des périodes d'entreposage au champ allant jusqu'à 21 mois, la viabilité des graines de pin à encens (*Pinus taeda* L.) et de pin jaune (*Pinus echinata* Mill.) a été déterminée par des tests de germination dans le but d'évaluer la possibilité de conserver des graines d'une année à l'autre. Les traitements comprenaient des graines entreposees (i) au congelateur, *(ii)* sur le parterre forestier, (iii) à 1,8 m au-dessus du sol et exposées aux precipitations et (iv) à 1,8 m au-dessus du sol mais à l'abri des precipitations. La vigueur germinative des graines entreposees au congelateur a dépassé 95% pour les pins à encens et jaune apres 7, 9, 19 et 2 1 mois. Étant donné que les graines entreposees sur le parterre forestier ont germé naturellement, la germination en laboratoire apres 7 mois d'entreposage atteignait en moyenne moins de 1% chez les deux espèces. Les graines de pin à encens entreposees au-dessus du sol et exposées aux precipitations avaient conserve la vigueur germinative la plus élevée, soit respectivement 98 et 56% de germination apres 7 et 21 mois. Sur la base de la retention de la viabilité, le classement relatif des autres traitements Ctait graines de pin à encens à l'abri des precipitations > graines de pin jaune a l'abri des precipitations > graines de pin jaune exposées aux precipitations.
[Traduit par la Redaction]

Introduction

About two-thirds of pine stands in the southeastern United States originated from natural seedfall (USDA Forest Service 1988), and this method of regeneration continues to be important for perpetuating the species. Loblolly and shortleaf pines (*Pinus taeda* L. and *Pinus echinatu* Mill., respectively) are common associates throughout this geographic area and are the most important and widespread of the southern pines (Baker and Langdon 1990; Lawson 1990). For these two pine species, there have been persistent anecdotal reports of seedlings becoming established on forested sites following natural seed-crop failures (Smith and Bower 1961; Lawson 1990). These reports suggest that seeds from loblolly and shortleaf pines carry over through at least two winters before germination, but formal studies have failed to substantiate these field observations. For example, Little and Somes (1959), Wahlenberg (1960), and Barnett and McGilvray (199 1) concluded that few loblolly pine seeds remain viable in the forest floor through the second winter after dispersal. In all three investigations, environmental conditions were favorable to germination.

One might conclude from past research that loblolly and shortleaf pine seeds seldom if ever carry over more than one winter in field storage. However, in the present investigation, we hypothesized that pine seeds are stored at locations other than the forest floor, e.g., in retained old cones in crowns of living pines or on branches of harvested pines. These potential seed storage conditions might be unfavorable to seed germination while preserving seed viability. Our objectives in this investigation were *(i)* to monitor the viability of loblolly and shortleaf pine seeds stored in the field over a period of two growing seasons after their maturity and *(ii)* to compare the viability of pine seeds stored above ground with those stored on the forest floor. With the exception of storage on the forest floor, all other field storage conditions tested in this investigation were considered unfavorable to seed germination.

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Methods

Study area

Pine seeds were stored in the field over a period of 21 months in southeastern Arkansas, U.S.A., at 33°02′N, 91°56′W on the Crossett Experimental Forest. The storage location was below a mature loblolly pine canopy that shaded the forest floor. Some sidelight was present during early morning and late afternoon and sunflecks appeared intermittently. Precipitation averages 139 cm/year, with seasonal extremes being wet winters and dry autumns. Daily temperatures average 22°C during the growing season (March through September) and 11°C during the dormant season (October through February) (USDA 1979).

Field and laboratory procedures

Mature pine cones were collected in mid-October 1993 from a naturally established stand in southeastern Arkansas. Loblolly and shortleaf pine cones came from, respectively, four and three felled trees of sawlog size (>30 cm in diameter at 1.37 m above ground). Cones were pooled by species, and seeds were extracted following procedures described by Wakeley (1954). After hand dewinging, tilled seeds were separated from empty seeds and organic debris by floating in a water bath for 30 min (loblolly seeds) or 3 h (shortleaf seeds).

Storage treatments

Before storage, potentially viable seeds of both species were dried to 10% moisture (dry-weight basis). Seed storage packets were made by uniformly spacing seeds within two layers of cotton cheesecloth that were sandwiched between two pieces of fiberglass window screen and held in place by two pieces of 1.3-cm grid, galvanized hardware cloth. Each hardware-cloth packet measured 14 by 15 cm and permitted the isolation of 56 seeds. Exposed ends of the hardware cloth were folded 180" at the four comers to secure the seeds, cheesecloth, and fiberglass screen in between. These storage packets were devised to protect seeds from predation and to minimize cross contamination from potential pathogens that may have infected individual seeds. Cotton cheesecloth was used to temporarily retain moisture from rain events and thereby simulate natural seed storage in cones that wet during rain.

In early November 1993, 12 packets of loblolly pine seeds and 12 packets of shortleaf pine seeds were placed in each of the following laboratory or field storage conditions:

(1) Control. Seed packets were placed in a laboratory freezer at –18°C. When field packets were pulled for germination, three control packets for loblolly and three for shortleafpine were taken from freezer storage, and seeds were stratified and germinated along with those from field storage.

(2) Storage on forest floor litter. Seed packets were placed on a reconstructed forest floor consisting of pine and hardwood litter (28 Mg/ha) typical of natural loblolly-shortleaf pine stands (Switzer et al. 1979). Seed packets were covered with wire cages to protect them from disturbance by animals. Three packets from each species were pulled from field storage and stratified for germination tests in June and August 1994. By June 1995, remaining seeds in litter storage had rotted and were discarded after we conducted a cut test to confirm their condition.

(3) Storage above the forest floor and exposed to precipitation. Seed packets were suspended in a wire cage at a height of 1.8 m above ground and were exposed to natural weather conditions that occurred at the storage site.

(4) Storage above the forest floor and sheltered from precipitation. Seed packets were suspended at a height of 1.8 m above ground inside a National Weather Service instrument shelter. Seeds were exposed to ambient temperatures and humidities during the course of the study but were sheltered from precipitation and sunlight.

Duration of field storage was as follows: 7 months (until June 1,1994), 9 months (until August 15, 1994), 19 months (until June 1,1995), and 2 1 months (until August 15, 1995). These time intervals permitted an evaluation of seed viability through two growing seasons after maturity. After designated storage times, three packets containing 56 seeds each for loblolly and shortleafpine were removed from all storage locations, and seeds were stratified for 30 days on moist, sterile sand at 4°C. Following stratification, seeds were transferred to a germination room. Thirty-day germination tests were conducted on moist, sterile sand in accordance with published recommendations (Wakeley 1954). During germination, seeds were exposed to 10 h of full-spectrum fluorescent light and 14 h of darkness during each 24-h period. Temperature in the germination room was maintained at 21°C but increased to 24°C when the lights were on. Germination was considered complete when the seed coat had lifted from the sand.

Seeds that had germinated within selected packets during the first 7 months of field storage were segregated into a separate component for discussion. During germination counts, seeds with fungal growth were withdrawn from the sand flats to prevent contamination of residual seeds. These contaminated seeds were cut open to determine potential viability and were then discarded. After 30 days on germination flats, ungerminated seeds were cut open to confirm that they were filled.

In a peripheral investigation, potential cone storage of seeds was assessed in April 1996. From the branches of 10 harvested loblolly pines, we collected a subsample of 227 cones that matured during autumn 1995 and 129 cones that matured during autumn 1994. Felled pines were >50 cm DBH and >50 years old. Filled seeds were separated, stratified, and tested for germination as previously described.

Experimental design and data analysis

Analysis of variance was conducted for a completely randomized, split plot in time and space. A split-plot design was used because each storage method and each time interval were singular. All factors were considered fixed. Individual storage packets containing 56 seeds each were used as replications. Placement of seeds on forest litter resulted in zero germination after 9 months of field storage; consequently that treatment was deleted from the analysis. Germination percent was analyzed following arcsine square-root transformation, but only nontransformed percentages are reported. Differences in storage time and storage methods were isolated by the Ryan-Einot-Gabriel-Welsch multiple-range test (REGWQ) at a = 0.05 (SAS Institute Inc. 1989).

Results and discussion

For both species, control seeds stored in a freezer had germinative capacities that ranged from a low of 96% for loblolly pine seeds after 21 months storage to a high of 99% for both loblolly and shortleaf pine seeds after 9 months storage (Fig. 1). Within species, there were no significant differences ($P > 0.05$) in germinative capacity among the various durations of freezer storage. This was important to show that reductions in seed germinative capacity were mainly attributable to the method of field storage and the date of removal from field storage rather than initial seed quality.

Species x storage, species x time, and storage x time interactions were statistically significant (Table 1). The significant ($P = 0.0001$) species x storage interaction was attributed to the fact that in field storage, loblolly pine seeds germinated best after exposure to precipitation, whereas shortleaf pine seeds germinated best after being sheltered from precipitation. This anomaly is partially explained by the propensity of shortleaf pine seeds to germinate in packets exposed to precipitation, i.e., 43% germination during 7 months of storage as compared with 0% germination for loblolly seeds. After rain showers, there was apparently sufficient moisture retained by the cotton cheesecloth in storage packets to promote germination of many shortleaf pine seeds during the first 7 months of field storage. Barnett (1976) also found that shortleaf pine seeds are less dormant than loblolly pine seeds.

There was a consistent decline in seed germination for each species as time in storage increased (Fig. 1), but the magnitude

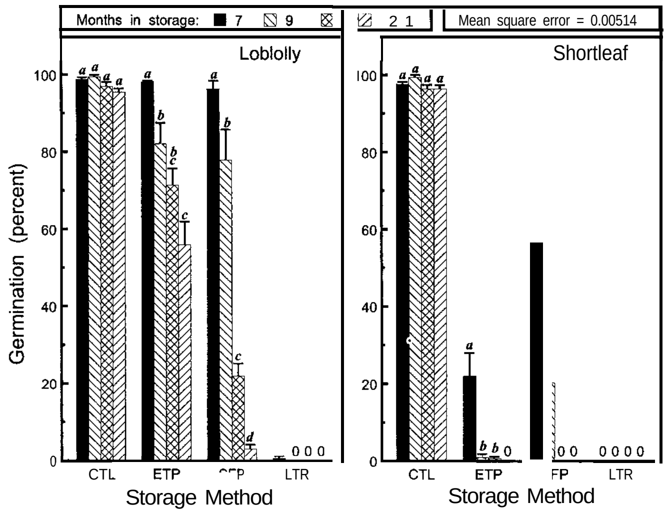
Table 1. Analysis of variance for the germination of loblolly and shortleaf pine seeds through 2 1 months of field storage.

		Seed germination*	
Source of variation+	df	Mean square	$P > F$
SP	1	3.6277	0.0001
Error I, Rep x Sp	4	0.0065	
Stor	2	5.5991	0.000 1
Sp x Stor	2	1.2455	0.0001
Error II, Rep x Sp x Stor	8	0.0116	
Time	3	1.1624	0.0001
Error III, Rep x Time	6	0.0092	
Sp x Time	3	0.0629	0.0272
Error IV, Rep x Sp x Time	6	0.0099	
Stor x Time	6	0.2939	0.000 1
Sp x Stor x Time	6	0.0125	0.0586
Error V, Rep x Sp x Stor x Time	22	0.005 1	

*Litter storage resulted in no germination after 7 months and was therefore dropped as a factor in this analysis. Of the remaining observations, two were dropped as outliers because of excessive contamination or mislabeling.

†Sp, species; Rep, replicate; Stor, storage.

Fig. 1. Effect of storage time (+SE) on germination of loblolly and shortleaf pine seeds by method of storage. Bars coded with the same letters within storage method and species are not significantly different according to REGWQ grouping at a = 0.05. Storage methods: CTL, control; ETP, exposed to precipitation; SFP, sheltered from precipitation; LTR, litter. Litter storage resulted in no germination after 7 months and was therefore dropped as a factor in statistical analysis.



of decline was greater for shortleaf pine seeds than for loblolly pine seeds, which resulted in the species x time interaction (P = 0.0272) (Table 1). The longer the seeds were exposed to changing abiotic and biotic factors (i.e., moisture, temperature, and microorganisms), the greater the degree of deterioration, and shortleaf pine seeds deteriorated faster than loblolly pine seeds. The rapid deterioration of shortleaf pine seeds may be attributed to their thinner seed coat as compared with loblolly pine (Shelton and Cain 1996).

As time in storage increased, germination was constant for control seed lots but declined for exposed and sheltered seed lots stored in the field (Fig. 1). This change in direction of response contributed to the significant (P = 0.0001) storage x time interaction (Table 1).

Loblolly pine was the only species to yield viable seeds after storage on litter. However, the germinative capacity of litter-stored loblolly seeds averaged only 0.6% and that occurred only in the 7-month storage treatment (Fig. 1). In assessing seeds at 7 months of litter storage, we found that radicles had emerged from 82% of loblolly pine seeds and from 96% of shortleaf pine seeds, but all had died in the storage packets because seedling emergence was prohibited by the fiberglass screen. Results from litter storage are consistent with those of Little and Somes (1959), Wahlenberg (1960), and Barnett and McGilvray (199 1) because placement of seeds on the forest floor favors germination.

With stratification, the germinative energy of loblolly and shortleaf pine seeds tended to peak between 10 and 15 days after the germination tests began, regardless of the storage method. The germinative capacity of loblolly pine seeds averaged 51 percentage points higher than that of shortleaf pine seeds across all time and field-storage conditions (Fig. 1). Overall, field storage was best for loblolly pine seeds exposed to precipitation. In that treatment, the germinative capacity of loblolly pine seeds averaged 98% after 7 months in storage but declined to 56% after 2 1 months in storage.

Germinative capacity of loblolly pine seeds sheltered from precipitation was similar to that of seeds exposed to precipitation during the first 7 to 9 months of field storage (Fig. 1). However, germinative capacity of loblolly pine seeds dropped precipitously after 19 and 2 1 months of sheltered storage. We first attributed this germination difference between exposed versus sheltered storage to temperature variation, but subsequent temperature measurements within and outside the shelter indicated a maximum differential of only 1 °C. We next considered moisture variation. To obtain information regarding fluctuations in seed moisture content in sheltered versus exposed seed, we monitored the weight of four test lots of loblolly and shortleaf pine seeds from mid-August to mid-September 1995. The moisture content (dry-weight basis) of seeds that were exposed to rainfall ranged from 9 to 30%, whereas that of the sheltered seeds ranged from 8 to 15%. During this 1 -month evaluation, seed moisture content generally did not fall below the recommended range of 9 to 12% for long-term seed storage (Wakeley 1954).

A more likely cause of reduced seed viability in storage became apparent during germination tests. We noticed different types of fungal growth on seeds that were exposed to precipitation as compared with seeds sheltered from precipitation. These fungi developed around seeds during the 30-day germination tests, and the most severely colonized seeds were cut open and assessed as nonviable. Since the color and morphology of fungi developing on sheltered seeds differed from that on exposed seeds, we concluded that different fungi were present. We propose that the different seed storage methods provided distinctly different environmental conditions, which may have favored the establishment of different microorganisms in and on the seeds (Mason and Van Arsdell978). Because control seeds appeared to be generally free of fungal growth during the 30-day germination tests, we surmise that fungal colonization increased during 7 to 2 1 months of field storage.

Conclusions

Based on results of this study, seeds of loblolly and shortleaf pines that mature in one year may carry over a second year before germination. However, it is highly unlikely that seeds disseminated in autumn through winter of one seed year will carry over a second year if deposited on the forest floor, especially if environmental conditions favor germination. It is more likely that biennial carry-over of seeds from these two species occurs within old cones. Under conditions tested in this 2 1 -month storage study, loblolly pine seeds appeared to have greater potential for carry-over than shortleaf pine seeds because most shortleaf pine seeds tended to germinate or deteriorate in storage while loblolly pine seeds remained dormant and viable.

Since cones are retained on loblolly and shortleaf pine branches for several years after they produce viable seeds, we propose that cone retention of seeds may occur on branches of live trees or cut trees if harvesting takes place in late summer at the time of seed maturity. To substantiate our hypothesis, loblolly pine seeds were collected in April 1996 from 1994 and 1995 cones and tested for germination; this was 14 and 2 months after the end of normal dispersal of the respective seed crops. The 1995 cones yielded 1 viable seed per 7 cones, and the 1994 cones yielded 1 viable seed per 26 cones. Although field carry-over of loblolly and shortleaf pine seeds is an intriguing phenomenon that has not previously been confirmed through definitive research, the process probably has little operational application for natural regeneration.

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