Distinguishing features of loblolly and shortleaf pine seeds: implications for monitoring seed production in mixed stands

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Abstract: Monitoring seed production in mixed loblolly pine – shortleaf pine (*Pinus taeda* L. and *Pinus echinata* Mill., respectively) stands may require identifying individual seeds by species. Although loblolly pine seeds are on average heavier and larger than those of shortleaf pine, there is considerable overlap in these properties for individual seeds. In this study the properties of six seed lots of each species from Arkansas and Louisiana were examined. Seed weight for loblolly pine averaged twice that of shortleaf pine, but seed length and width differed by only 13 and 27%, respectively. Seed-coat thickness was the most consistent difference observed between the two species: large shortleaf pine seeds had thinner seed coats than small loblolly seeds, but this property was slow and tedious to measure. By contrast, differences in seed-coat thickness were readily detected when conducting a cut test for seed soundness by subjectively assessing the force required to cut the seed. In a blind test, 12 evaluators estimated within ±10% of the known composition of 10-seed subsamples 86% of the time for the cut test compared with only 57% when using seed appearance alone; inexperienced evaluators were only slightly lower in accuracy than experienced ones. Use of the cut test as a subjective estimate of the force required to cut the seed appears to be reasonably accurate in distinguishing these two species for most purposes.

Résumé : Il pourrait être nécessaire d'identifier l'espèce de chaque graine pour faire le suivi de la production de graines dans les peuplements mixtes de pin à encens (*Pinus taeda* L.) et de pin jaune (*Pinus echinata* Mill.). Même si les graines du pin à encens sont en moyenne plus lourdes et plus grosses que celles du pin jaune, il y a beaucoup de chevauchement dans ces propriétés lorsqu'on considère les graines une à une. Les propriétés de six lots de graines de chaque espèce provenant de l'Arkansas et de la Louisianne ont été examinées dans le cadre de cette étude. Le poids des graines du pin à encens atteignait en moyenne le double de celui des graines du pin jaune, mais la longueur et la largeur des graines ne différaient seulement que de 13 et 27%, respectivement. L'épaisseur de l'enveloppe de la graine était la différence la plus constante observée entre les deux espèces : les grosses graines du pin jaune avaient une enveloppe plus mince que les petites graines du pin à encens, mais cette propriété était longue et fastidieuse à mesurer. Par contre, la différence dans l'épaisseur de l'enveloppe de la graine était facilement décelable en évaluant de façon subjective la force nécessaire pour couper la graine lorsqu'on faisait le test pour vérifier l'état de santé des graines. Dans un test aveugle, 12 évaluateurs ont estimé la composition de sous-échantillons de 10 graines à ±10% de la composition connue 86% du temps en les coupant, comparativement à seulement 57% en se fiant uniquement à l'apparence des graines. Les évaluateurs inexpérimentés avaient une précision seulement légèrement inférieure à celle des évaluateurs expérimentés. L'utilisation de ce test pour obtenir une estimation subjective de la force requise pour couper la graine semble fournir une précision raisonnable pour distinguer à toute fin pratique ces deux espèces.

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Introduction

Loblolly and shortleaf pines (*Pinus taeda* L. and *Pinus echinata* Mill., respectively) are common associates throughout most of the southern United States and are among the most important and widespread of the southern pines (Baker and Langdon 1990; Lawson 1990). These two species share many silvical characteristics and occur naturally in mixed stands. Experienced observers use needle length, number of needles per fascicle, bark features, and cone size to distinguish loblolly from shortleaf pine trees. However, these traits can often overlap because of both genetic and environmental variation. Seeds of loblolly and shortleaf pines are also similar in characteristics, both being dark, ridged, and angular. The most commonly mentioned distinction between seeds of the two species is that on average loblolly pine seeds are heavier and larger than those of shortleaf pine, but individual seeds of the two species overlap in physical properties (Wakeley 1954). The ability of resource managers to distinguish individual seeds of loblolly and shortleaf pines is important because (1) natural regeneration will be increasingly used especially on public lands, (2) information on shortleaf pine seed production is lacking for good sites, where mixed stands frequently occur (Wittwer and Shelton 1992), (3) the

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seedling / seed ratio for loblolly pine may be greater than that of shortleaf pine (Cain 1991; Shelton and Wittwer 1992), and (4) some landowners favor loblolly over shortleaf pine because of higher timber growth rates. Thus, we designed a study to compare the properties of loblolly from shortleaf pine seeds and to evaluate possible tests for distinguishing the two species.

Methods

The woods-run seeds tested in this study came from six seed lots for each species. Each seed lot represented collections from a minimum of six trees. Three loblolly and shortleaf pine seed lots were collected and processed from mixed stands in southeastern Arkansas by the authors in 1993 and 1994. Three of the shortleaf pine seed lots were collected in the Ouachita Mountains of west central Arkansas in 1967, 1969, and 1980 by USDA Forest Service personnel. One loblolly pine seed lot was collected and processed by the authors from a pure stand located in north central Louisiana in 1994. Another loblolly pine seed lot came from southern Arkansas in 1980 and was provided by Georgia Pacific Corporation. The remaining loblolly pine seed lot was collected in central Louisiana in 1972 and was provided by USDA Forest Service personnel. All seeds, except those collected in 1994, had been in cold storage since collection. Although most seed lots had been collected using the considerable care required for research purposes, we inspected subsamples from each seed lot regarding purity and performed a cut test on any questionable seeds (i.e., loblolly seeds in the shortleaf seed lots and vice versa). In no circumstance did the authors feel that there was cross-contamination. Mean seed weight was determined for each seed lot, and the overall mean for each species was within ±2 mg of the regional means reported by Wakeley (1954). It was thus decided to combine the individual seed lots by species. A combined seed lot was composed of the same number of seeds from each individual seed lot.

The combined seed lots were prepared for testing by soaking in deionized water for 24 h. Seeds that floated were discarded. After soaking, one group of seeds was air dried for 48 h, while another group was stored in moist paper towels for the same time period. The moisture content of a subsample of the air-dried and wet seed lots was determined by oven-drying subsamples (24 h at 105°C); the air-dried seeds averaged 12.2 and 10.9% (dry-weight basis) for loblolly and shortleaf pine, respectively ($P = 0.07$), while wet seeds averaged 24.6 and 30.4%, respectively ($P = 0.002$).

Physical characteristics of the tested seeds were determined by measuring a number of randomly selected individual seeds from the air-dried seed lots. Weight of 200 individual seeds for each species was determined to 1 mg. Seed length and width were determined on 100 seeds for each species to 0.01 mm under 20× magnification. Half of the seeds measured for length and width were cut perpendicular to their long axis, and the thickness of the seed coat was determined by averaging measurements for the two sides along the narrow axis of the cut face of each seed. The weight of the seed coat was determined for 25 seeds of each species by cutting the seeds, removing the endosperm and embryo, and then weighing the seed coat. Specific gravity was determined as follows: (1) obtaining volume by water displacement of five 50-seed samples for loblolly pine and five 100-seed samples for shortleaf from the wet seed lot and (2) weighing seeds after removal of surface moisture and after oven-drying. The force required to cut seeds was determined for a 100-seed sample from the air-dried and wet seed lots of each species. Using a sharp, single-edged razor blade, a steadily increasing force was applied downward to seeds placed on a platform balance (0–10 kg capacity) and recording the registered value to the nearest 0.01 kg. Seeds were cut perpendicular to their long axis, replacing razor blades after about 100 seeds.

The accuracy of distinguishing loblolly and shortleaf pine seeds was determined on seed subsamples of known composition. Factors evaluated were (1) the type of evaluation (size test versus cut test), (2) the experience level of evaluators (inexperienced versus experienced), and (3) the moisture status of seeds (dry versus wet).

In the size test, seeds were separated by species based on appearance alone with size being the principal factor and based on common knowledge that loblolly seeds are usually larger than those of shortleaf pine. The cut test is routinely conducted in seed testing to estimate potential viability (Bonner 1974). Seeds are cut using a sharp single-edged razor blade and the contents are examined; seeds with full, firm, undamaged, and healthy tissues are judged to be potentially viable.

There were seven experienced evaluators who had performed the cut test for 1 or more years on seeds from loblolly – shortleaf pine stands; they were either foresters or forestry technicians. The inexperienced evaluators had never conducted a cut test; one was a forester and four were junior-level forestry students.

Subsamples of 10 seeds of known species composition were created by randomly assigning the subsample to either wet or dry moisture content and then selecting the number of shortleaf pine seeds (from 0 to 10) with the remainder being loblolly pine seeds. Each evaluator tested 20 of the 10-seed subsamples using both the size and cut tests. In most cases, evaluators tested a different group of 20 subsamples for each type of test. For inexperienced evaluators, differences between loblolly and shortleaf pine seeds were briefly explained. All evaluators were allowed to study and cut a known group of loblolly and shortleaf pine seeds before taking the scored tests.

The accuracy of evaluations was determined by regressing the estimated number of shortleaf pine seeds in each 10-seed subsample with the known number and entering type of test, experience level, and moisture content as indicator variables. Regression coefficients and associated statistics were determined using linear regression (SAS Institute Inc.1989). A t test was used to separate mean differences in the properties of loblolly and shortleaf pine seeds.

Results and discussion

Seed physical properties

The mean loblolly pine seed weighed over twice as much as that of shortleaf pine, but the difference in length and width of the two species was only 13 and 27%, respectively (Table 1). Species differences were statistically significant ($P \leq 0.0001$). Within each species, seed weight was more variable than dimensions. For example, coefficient of variation for weight averaged 25% for the two species, compared with 12% for length and 14% for width. Mean properties of the seeds used in this study were similar to regional averages reported for each species by Wakeley (1954). The fact that seed weight and dimensions differ in magnitude reflects a difference in specific gravity between the two species, with shortleaf pine seeds being lower than that of loblolly. For the seed lots tested in this study, specific gravity averaged 1.26 and 1.13 for loblolly and shortleaf pine seeds, respectively, on a wet-weight basis ($P = 0.02$), and comparable values on a dry-weight basis averaged 0.98 and 0.86, respectively ($P = 0.01$). The observed difference in specific gravity raises the possibility

Table 1. Physical properties of individual seeds used in this study.

Species	Mean*	Coefficient of variation	Min.	Max.
		Weight (mg)		
Loblolly pine	23.4	23.0	13	39
Shortleaf pine	11.4	27.0	6	20
		Length (mm)		
Loblolly pine	5.3	12.3	4.0	7.1
Shortleaf pine	4.6	12.0	3.3	5.6
		Width (mm)		
Loblolly pine	3.7	14.4	2.8	5.0
Shortleaf pine	2.7	14.4	2.0	3.7
		Seed-coat thickness (mm)		
Loblolly pine	0.26	16.7	0.18	0.34
Shortleaf pine	0.12	16.0	0.10	0.16
		Force required to cut when dry (kg)		
Loblolly pine	2.18	19.9	1.06	3.20
Shortleaf pine	0.52	23.9	0.31	0.82
		Force required to cut when wet (kg)		
Loblolly pine	1.61	23.3	0.85	2.90
Shortleaf pine	0.42	28.5	0.16	0.82

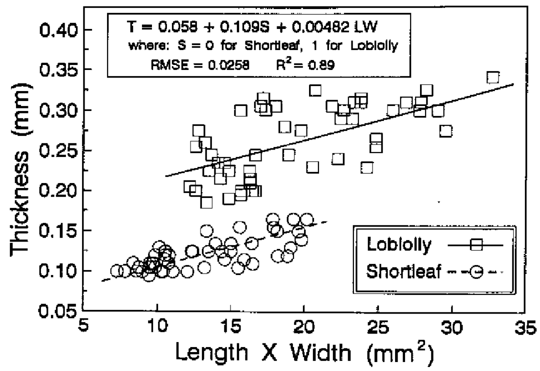
*Each mean is based on 200 seeds for weight; 100 seeds for length, width, and force; and 50 seeds for seed-coat thickness. Species means differ for each parameter at $P = 0.0001$.

of using a float test for separating loblolly and shortleaf pine seeds when mixed together. However, we feel that the potential of using a float test is low because (1) seed moisture content influences whether a seed floats or sinks, (2) void loblolly pine seeds would float together with both sound and void shortleaf pine seeds initially, and (3) this test is difficult to apply to small seed lots.

Although the mean seed properties significantly differed between the two species, considerable overlap occurred in the weight and size of individual seed (Table 1). Both species had seeds that weighed between 13 and 20 mg. For the seed lots tested here, 28% of the shortleaf pine seeds and 33% of the loblolly pine seeds fell within this common range for seed weight, and a similar degree of overlap was observed for length and width. This overlap makes distinguishing individual seed based on weight and size difficult between species when they are mixed together: small loblolly pine seeds can be mistaken for large shortleaf pine seeds and vice versa.

Thickness of the seed coat was the most consistent difference observed between the seeds of loblolly and shortleaf pines (Table 1 and Fig. 1). Seed-coat thickness increased slightly with seed length and width, but values for large shortleaf pine seeds were consistently below that of small loblolly pine seeds. Seed-coat thickness for loblolly pine averaged about twice that of shortleaf pine (0.26 versus 0.12 mm, respectively), and there was no overlap in the

Fig. 1. Relationship of seed-coat thickness (T) and the product of seed length (L) and width (W) for loblolly and shortleaf pine seeds.



ranges of the two species. The seed coat accounted for a higher percentage of the total seed weight for loblolly pine than for shortleaf pine (58 versus 41%, respectively; $P = 0.0001$); this difference was also noted by Barnett (1976).

Although effective in distinguishing the two species, measuring seed-coat thickness is a slow and tedious determination, rendering it impractical for the large number of seeds that are normally collected when monitoring seed production. However, the difference in seed-coat thickness between the two species was very apparent when conducting a cut test on mixed seed lots of the two species, i.e., loblolly pine seeds required about four times more force to cut than those of shortleaf pine (Table 1). The average force needed to cut a loblolly pine seed was 2.18 kg when air dried and 1.61 kg when wet, and comparable averages for shortleaf pine were 0.52 and 0.42 kg, respectively (moisture content was significant at $P = 0.0001$ for both species). In addition, no overlap occurred between species in the range of force required to cut seeds. Measuring the force required to cut seed appears to be a very accurate way to distinguish these two species, but this procedure requires a suitable platform balance and increases the time required to process seeds. Thus, an evaluation of accuracy is needed for distinguishing seeds of loblolly and shortleaf pines based on the subjective determination of the force required to cut seeds.

Subjective tests for distinguishing species

Effects of the variables tested in this study (test type, evaluator experience, and seed moisture content) on the accuracy of estimating the composition of seed subsamples are shown in Table 2. Results showed clear differences between the type of test and the experience level of evaluators, but the difference between the moisture status of seeds was not significant ($P = 0.31$).

The cut test was considerably more accurate in estimating the known species composition than the size test. Evaluators were within ±10% of known composition 86% of the time for the cut test compared with 57% for the size test. Experienced evaluators were only slightly more accurate than inexperienced ones when using the cut test;

Table 2. Regression coefficients and associated statistics for the relationship of estimated and known number of shortleaf pine seed in 10-seed samples with a variable species composition.

Variable*	Coefficient	SE	*P*
Intercept	2.393	0.157	0.0001
Known number	0.713	0.021	0.0001
Type of test	−0.395	0.121	0.0012
Experience level	−0.353	0.123	0.0042
Moisture status	0.127	0.124	0.3078

*The equation is $E = b_0 + b_1K + b_2T + b_3\,\mathrm{EX} + b_4M$, where E is the estimated number of shortleaf pine seeds, K is a known number of shortleaf pine seeds, T is the type of test (0 = size test and 1 = cut test), EX is the experience level (0 = inexperienced and 1 = experienced), and M is the moisture content (0 = dry and 1 = wet). The R^2 was 0.73, and root mean square error (RMSE) was 1.32.

experienced evaluators were within ±10% of known composition 87% of the time compared with 85% for inexperienced evaluators. However, inexperienced evaluators overestimated the percentage of shortleaf pine seeds to a greater extent than experienced ones.

The regression equations relating the estimated and known number of shortleaf pine seeds also provide an indication of the accuracy of species determination (Table 3). If the test results had been perfect, regression equations would have an intercept of zero and a slope of one, and the coefficient of determination would have a value of one. However, all intercepts were significantly different from zero at $P \leq 0.05$, and all slopes were significantly different from one at $P \leq 0.05$. These results indicate a significant difference between the estimated and known composition of the 10-seed samples. The greater variability of the size test was reflected in its lower values for coefficient of determination, which were 0.61 and 0.53 for inexperienced and experienced evaluators, respectively, compared with values of 0.85 and 0.91 for the cut test, respectively.

The overall mean composition indicated a slight tendency to overestimate the number of shortleaf pine seeds. The greatest overestimation was for the inexperienced evaluators using the size test; they missed the overall mean shortleaf pine composition by 11 percentage points (a known mean of 52% for shortleaf pine versus an estimated mean of 63%). Overall, the best results were for the experienced evaluators using the cut test; they only overestimated the mean shortleaf pine composition by 4 percentage points (a mean known composition of 52% versus 56% estimated). Inexperienced evaluators were only slightly less accurate for the cut test, and for this group, only 5 percentage points separated the mean known and estimated shortleaf pine composition (a known composition of 50% versus 55% estimated).

Using a subjective evaluation of the force required to cut seeds appeared to be a rapid and relatively accurate way to distinguish the seeds of the two species. Although results indicated that cut-test accuracy was not affected by seed moisture content, variation in seed moisture content will narrow the difference in force required to cut seeds of the two species; therefore, air-dried seeds are recommended for testing. Results of the cut test for species distinction might be improved by initially sorting seeds into potential groups based on seed size, and then using the cut test to make further refinements. It is also possible to increase accuracy by conducting the cut test on a platform balance so that the force required to cut the seeds can be measured rather than estimated (air-dried seeds cutting with a force of less than 0.85 kg are judged to be shortleaf pine).

Table 3. Regression coefficients and associated statistics for the relationship between the estimated and known percentage of shortleaf pine seed in 10-seed subsamples with a variable species composition.

Type of test	Experience level of evaluators	Regression coefficient* b_0	b_1	RMSE	r^2
Size test	Inexperienced	3.22	0.602	1.42	0.61
Size test	Experienced	2.77	0.573	1.59	0.53
Cut test	Inexperienced	1.49	0.812	0.98	0.85
Cut test	Experienced	0.87	0.900	0.85	0.91

*The equation is $E = b_0 + b_1K$, where E is the estimated number of shortleaf pine seeds and K is a known number. RMSE, root mean square error.

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