Dispersal and viability of seeds from cones in tops of harvested loblolly pines

Michael G. Shelton and Michael D. Cain

Abstract: Seed supply is one of the most important determinants of successful natural regeneration. We conducted a study to determine the potential contribution of cones in the tops of harvested loblolly pines (*Pinus taeda* L.) to the stand's seed supply if trees were felled after seed maturation but before dispersal, Closed cones, collected in October 1996, were stored in wire cages with periodic removals over 2 years to determine the number and viability of extracted seeds. Storage sites were an opening in a seed-tree stand and a closed-canopy pine-hardwood stand in southeastern Arkansas. Of the initial 83 viable seeds/cone, 73% had dispersed in the opening and 63% in the closed stand by March 1997, which is considered the end of the normal dispersal period from standing trees. By October 1997, only 1 viable seed/cone remained in the opening and 5 viable seeds/cone in the closed stand, indicating rather complete dispersal or mortality of seeds by the first summer after harvest. Results indicate that cones in tops of trees cut during the 2-month period after seed maturation and before substantial dispersal can make an important contribution to the stand's seed supply, especially in reproduction cutting methods where most of the trees are harvested.

Résumé : La reserve de graines est l'un des plus importants déterminants pour assurer le succès de la regeneration naturelle. Nous avons réalisé une étude pour déterminer dans quelle mesure les cones presents dans la tête des pins à encens (*Pinus taeda* L.) récoltés contribuaient à la reserve de graines du peuplement si les arbres étaient coupes à l'automne, après la maturation des graines mais avant leur dispersion. Des cones fermés, ramassés en octobre 1996, ont été entreposés dans des cages de broche et Bchantillonnb périodiquement pendant deux ans pour determiner le nombre et la viabilité des graines extraites des cones. Les sites d'entreposage comprenaient une ouverture dans un peuplement semencier et un peuplement de pins et de feuillus avec un couvert ferme situés dans le sud-est de l'Arkansas. Des 83 graines viables par cône dénombrées au depart, 73% s'étaient dispersées dans l'ouverture et 63% dans le peuplement ferme en mars 1997, qui est considéré comme la fin de la période normale de dispersion des graines chez les arbres debout. En octobre 1997, seulement une graine viable par cône restait dans l'ouverture et cinq graines viables par cône dans le peuplement ferme, ce qui indiquait une dispersion plutôt complete ou la mortalité des graines lors du premier été après la coupe. Les résultats indiquent que les cones presents dans la t&e des arbres coupes durant la période de deux mois qui suit la maturation des graines, avant qu'une dispersion substantielle survienne, peuvent contribuer de facon importante à la reserve de graines du peuplement, particulièrement dans le cas des méthodes de coupe de régénération où la plupart des arbres sont coupes.

[Traduit par la Redaction]

Introduction

Because of lower establishment costs, natural regeneration is a viable option for loblolly pine (*Pinus taeda* L.) that appeals to many landowners, and about two-thirds of the pine stands in the southeastern United States originated from natural seedfall (USDA Forest Service 1988). Most natural regeneration methods rely on retained trees to produce an adequate seed supply for regeneration. However, trees that were felled during reproduction cutting can also potentially contribute to the seed supply if they were felled after seed maturation but before complete dispersal. Barnett (1976) reported that loblolly pine cones can yield viable seeds when collected in early September, although cones are not normally collected for seed extraction until early October in the South. Loblolly pine seed dispersal from standing trees is normally 50% complete by the second week in November and is 90% complete by the first of January (Cain and Shelton 1996). Trees felled in reproduction cutting during the 2-month period from early September to early November could potentially make a substantial contribution to the stand's seed supply by dispersing seeds from cones in felled tops. The contribution of seeds from tops of cut trees would likely be most important to regeneration success in reproduction methods where most of the stand's trees are harvested, such as the seed-tree method or small clearcuts. This phenomenon could also impact plantations by establishing high densities of nonplanted pines if no site preparation was conducted to remove tops.

Although seed dispersal from standing trees of southern pines has been the subject of numerous studies (Campbell 1967; Brender and McNab 1972; Cain and Shelton 1996; Shelton and Wittwer 1996), we are aware of no earlier investigation of seed dispersal from cones in tops of felled trees. Objectives of the study were (i) to determine the potential

Received May 25, 2000. Accepted October 10, 2000. Published on the NRC Research Press website on February 13, 2001.

M.G. Shelton' and M.D. Cain. USDA Forest Service, Southern Research Station, P.O. Box 3516, Monticello, AR 71656-3516, U.S.A.

'Corresponding author. e-mail: mshelton@fs.fed.us

 DOI: 10.1139/cjfr-31-2-357

for cones in the tops of felled loblolly pine trees to contribute to the stand's supply of viable seeds and (ii) to determine the possible fate of seeds dispersed during the growing season when cold, moist stratification that normally promotes germination would not occur.

Methods

Study area

The study was located on forest lands of the School of Forest Resources, University of Arkansas at Monticello. The study site is in the West Gulf Coastal Plain (33°37′N, 91°46′W). Elevation is 98 m with a rolling topography. The soil is a Sacul loam (clayey, mixed, thermic, Aquic Hapludult), which is a moderately well-drained upland soil with a site index of 24 m for loblolly pine at 50 years (USDA 1976). The growing season is about 240 days with seasonal extremes being wet winters and dry autumns. Annual precipitation averages 134 cm.

Two sites were located for cone storage. A closed-canopy site was located in a mature loblolly pine – hardwood stand. Basal area in trees >9 cm diameter at breast height (DBH) averaged 25.7 m^2/ha for pines and 6.4 m^2/ha for hardwoods; there were also 2.8 m^2/ha in trees 19 cm DBH. Light intensity at 1.37 m in height averaged 7% of full sunlight at noon during a clear summer day, and the canopy exerted 97% ground coverage. An open site was in a 20 × 20 m cleared area within a pine seed-tree stand with approximately 10 sawtimber-sized trees/ha. This area intermittently received shadows from adjacent trees during the winter months but was mostly in full sunlight during the summer. The open site was 0.4 km from the closed-canopy site.

Field procedures

In early October 1996, closed cones were collected from about 20 tops of recently harvested loblolly pines in a mature sawtimber stand in southeastern Arkansas. Immediately after collection, cones were transported to the study sites and placed in storage frames designed to simulate logging tops but also to provide protection of cones and seeds from predators. These frames were 0.5 m square and made of 1.3-cm mesh galvanized hardware cloth. The frames were held 0.5 m above the forest floor by legs constructed of 0.6 cm diameter steel. This arrangement allowed some movement of the frame, which we felt was representative of small branches in the top of a felled tree. Within each frame, there were nine vertical cylindrical containers constructed of 1.3-cm mesh hardware cloth; they were 8 cm in diameter and 10 cm tall. One cone was placed in each cylinder with the cone's apex pointed down. A top constructed of 0.35-cm mesh hardware cloth was used to cover each frame. There were 16 frames at each location, which provided for four removals from field storage with four replicates over a 2-year period. Removals were planned in early March and October of 1997 and 1998. Before seed extraction, cones were always removed from storage when closed; if required, cones were gently sprayed with water the night before removal to cause closure.

Based on results from the first phase of the study, additional cones were sampled from loblolly pine tops within logged areas about 1 km from the open study site. Logging was conducted from late September to early October 1998. One area was a mature loblolly-shortleaf pine (*Pinus echinata* Mill.) stand that was being thinned; the other area contained residual sawtimber-sized pines being removed from a regenerated shelterwood stand. Cones were sampled from tops in late February, early March, and mid-June of 1999 after rain events caused closure. There were eight nine-cone samples in February and June and four in March. In the first two samples, cones were collected representatively from all angular orientations within the tops of felled trees. During the June collection, cones were sampled by orientation classes: up (300–60°), side (60-120 and 240–300°), and down (120–240°). Angular orientation of cones was visually estimated when viewing the cone perpendicular to its long axis while still attached to the branch. A subsample of cones from the March collection was placed in the metal storage frames with four replicates each of up and down orientations in the open site only. Cones with an up orientation averaged 36" from vertical, while those with a down orientation averaged 154°. A subsample of cones from the June collections were also placed in the storage frames, but we retained their observed orientation class from the field. In this latter sample, two removals were planned with four replicates of each removal and orientation class. For cones of the side orientation class, the surface facing the sun was apparent from a color change. In June 1999, we also inventoried the orientation of cones on tops by making counts by orientation class on about 12 pine tops in each harvested area.

To determine the possible fate of seeds dispersed later than normal, we deposited packets of seeds on the soil surface in closed-canopy and open locations bimonthly beginning in April 1999 and continuing through October 1999. Seeds came from cones collected in March 1999. After hand dewinging, filled seeds were separated from void seeds and debris by floating in a water bath for 30 min and collecting the sinking (filled) seeds. Packets were made by uniformly spacing 56 seeds between two pieces of fiber glass window screen that were held in place by two pieces of 1.3-cm mesh hardware cloth while in field storage. The packets were intended to protect seeds from predation and to isolate seeds for reduced contamination. Each packet measured 14 × 15 cm. There were 10 packets for each of the four placements; four packets were placed in a prepared seedbed at the open and closed-canopy locations, and two packets were used for germination tests at that time. Packets were stored in a National Weather Service instrument shelter located in the open site until placed in prepared seedbeds. Bimonthly, packets were removed from the shelter and placed on the an exposed mineral soil surface, then finely ground surface soil from the area was sprinkled on packets until the seeds were lightly covered. Packets were periodically inspected after heavy rains, and soil was added as needed to keep the seeds lightly covered. Each seedbed area contained four packets representing a placement and was completely enclosed within 1.3-cm mesh hardware cloth to prevent predation.

To determine the natural pattern of opening and closing of cones in different locations, we randomly selected two groups of cones that matured in 1998 and placed them in the storage frames used in this study. The percent openness of each cone was visually assessed at approximately daily intervals from mid-May through June 1999. To determine cone temperature, a thermometer was inserted into a hole drilled in the base of a test cone and read after stabilization. Readings were also taken of the air temperature in a standard National Weather Service instrument shelter in the opening.

Laboratory procedures

After the specified period of field storage in wire frames, nine cones representing each replicate were allowed to air dry in cloth bags for several days until open. Seeds were then extracted by vigorously tumbling cones in a 20-L plastic bucket. Cones were then lightly heated (33°C) in a forced-draft oven for 24 h, and a second extraction was made. This process was repeated one additional time. About 90% of the seeds were obtained from the first extraction. Seeds were dewinged by hand. After counting, a germination test was conducted by using a subsample of seeds randomly drawn from each replicate. When ample seeds existed, the subsample was either two cups of 50 seeds each or one cup of 75 seeds. As the number of seeds declined below 75 per replicate, all seeds were used in the germination tests.

Test seeds were placed on moist, sterile sand in 10 × 10 cm plastic cups and stratified for 30 days at 4°C. The 30-day germina-

Fig. 1. Number and viability of seeds observed over a 2-year period during field storage of loblolly pine cones in an opening and a closed-canopy stand in southeastern Arkansas. Error bars are SE.

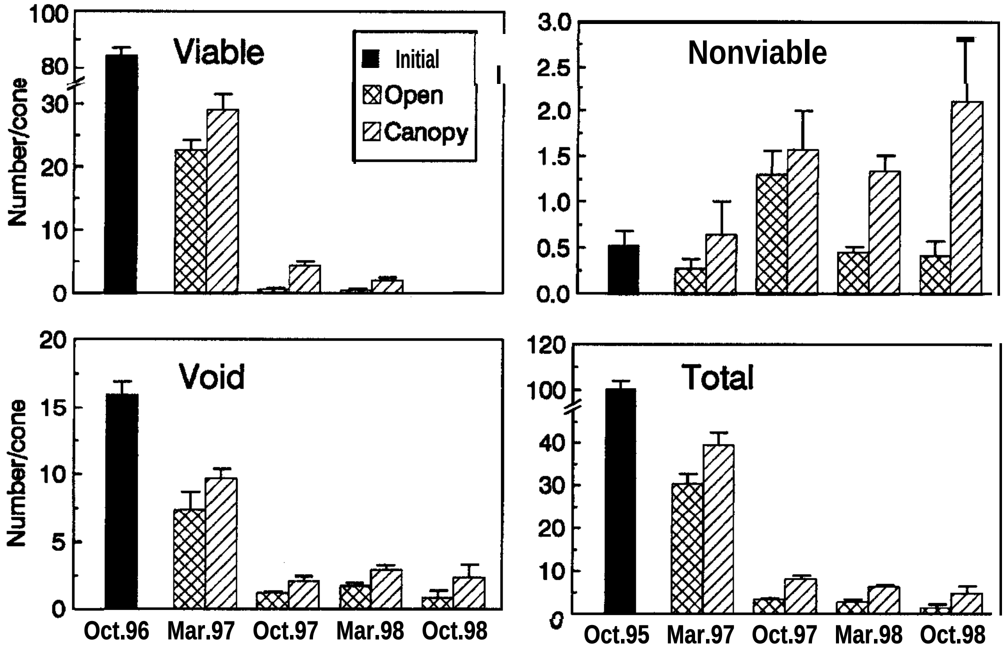


Table 1. Analysis of variance for the number of seeds retained in loblolly pine cones located in an opening and closed stand over a 2-year period by the type of germination exhibited.

		Viable seeds		Nonviable seeds		Void seeds		Total seeds	
Source of variation	df	MSE	$P > F$	MSE	$P > F$	MSE	$P > F$	MSE	$P > F$
Location (L)	1	4.142	0.0003	1.177	0.0200	1.532	0.0066	6.732	0.0008
Error I, Replicate (R) × L	6	0.077		0.119		0.093		0.174	
Time (T)	3	35.417	0.0001	0.533	0.0194	5.304	0.000 1	31.279	0.0001
Error II, R × T	9	0.097		0.096		0.124		0.203	
L × T	3	0.339	0.0201	0.190	0.5029	0.028	0.9279	0.035	0.9094
Error III, R × L × T	9	0.062		0.225		0.186		0.444	

Note: The data were square-root transformed prior to calculation of MSE.

tion test was conducted with 10 h of full-spectrum fluorescent light and 14 h of dark in accordance with published guidelines (Wakeley 1954). Temperature in the germination room was maintained at 21°C. A seed was considered to have germinated normally when the seed coat lifted off the sand. Abnormal germination was based on guidelines described by Wakeley (1954). Seeds with fungi were removed immediately to reduce contamination; a cut test was conducted to determine if seeds were full or void (Bonner 1974). At the end of each 30-day germination test, a cut test was conducted on all ungerminated seeds; full seeds were classified as being decayed or potentially sound. A seed that germinated normally within 30 days was considered viable; any full seed that did not germinate normally was considered nonviable.

In February 2000, all packets were removed from field storage, opened, and inspected to count the number of previously germinated seeds based on remnants of a radicle or a split seed coat. A germination test was conducted on all ungerminated seeds as previously described, except that stratification was reduced to 15 days.

Statistical analysis

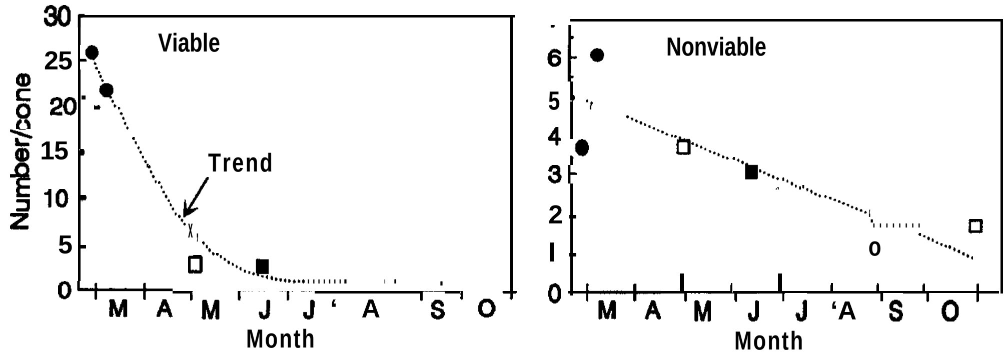
The homogeneity of treatment variances was tested by Bartlett's test (Steel and Torrie 1980). When the hypothesis of homogeneity of variance was rejected at a ≤ 0.05, data were square-root transformed, which provided homogeneity. Analysis of variance was conducted for a completely randomized, split plot in time and space. A split-plot design was used, because each storage location and each time interval was singular. All factors were considered fixed. Replicates were considered the seeds extracted from the samples of nine cones or from the 56-seed storage packets. Significance was accepted at a ≤ 0.05.

Results and discussion

Seed dispersal

The loblolly pine cones contained 83 viable seeds/cone in October 1996 when they were fully mature (Fig. 1). This number is typical of years with good seed crops (Wakeley 1954). By March 1997, viable seeds in the cones had declined by 73% in the opening and 65% in the closed stand, and the difference between areas was significant (Table 1). Seed dispersal from standing loblolly pines is normally considered complete by the end of February (Cain and Shelton 1996). The viable seeds were apparently dispersed, because there was no corresponding increase in the number of nonviable seeds, which averaged 0.5 seed/cone both initially and in March 1997. The viable seeds present in March (23 seeds/ cone in the opening and 29 seeds/cone in the closed stand)

Fig. 2. Number and viability of seeds from March to October 1999 in cones from tops of loblolly pine trees felled during operational logging in southeastern Arkansas in late September and early October 1998. The solid symbols are means from cones collected from the logging sites, while the open symbols are means from cones stored in the opening test site. The circles are means from cones sampled representatively from all orientations. The squares are means from cones stored by different orientation classes and weighted by the observed orientation classes at the logging sites.



were virtually all dispersed during the first growing season, because few remained in October 1997 (less than 1 seed/cone in the opening and 5 seeds/cone in the closed stand). Most of the viable seeds were apparently dispersed, because nonviable seeds increased only by 1 seed/cone over this period. Few viable seeds remained in the cones by March 1998 and virtually none the following October. The increase in nonviable seeds accounted for the decline in viable seeds over this period. Thus, the few remaining viable seeds from cones in March 1998 probably died over the second growing season rather than being dispersed. Void seeds showed a similar decline over time as with viable seeds. Our results suggest that void seeds were retained to a greater degree than full seeds (viable and nonviable); void seeds represented 15% of the total seeds present in October 1996 but increased to an average of 55% in October 1998.

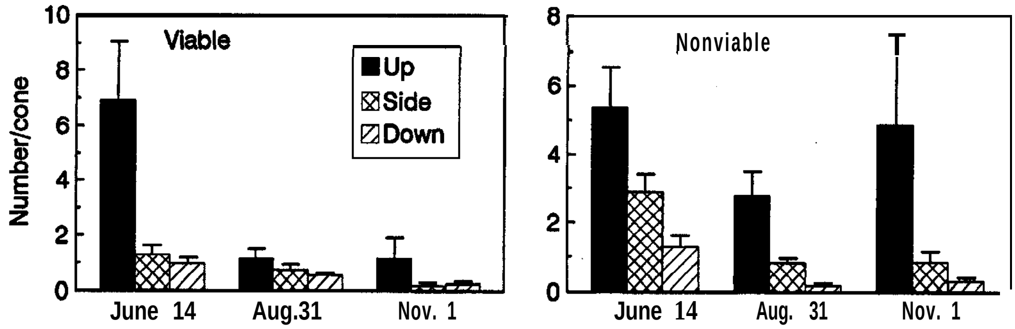
The cause of seeds being nonviable changed over the storage period; initially, abnormal germination accounted for 33%, and potentially sound but ungerminated seeds were 67%. However, in March 1997 and thereafter, most of nonviable seeds had decayed (94%), and very little was attributable to abnormal germination (4%) or potentially sound but ungerminated (2%). Most nonviable seeds apparently died during the storage period and decayed during the warm, moist germination tests.

The difference in dispersal pattern in the closed stand and open locations can be explained by differences in environmental factors, such as wind, temperature, dew, frosts, and humidity. These factors affect the drying of cones and thus the rate and degree of a cone's opening and closing. These factors also affect the environmental stresses that seeds are subjected to within cones. In May and June 1999, we observed that cones in the opening took an average of 3 days to fully open following a substantial rain (≈2.5 cm), while those in the closed stand took 6 days. Cones in the opening were noticed to slightly close during nights with heavy dew but were observed to fully reopen by midmorning of the following day. Dew did not visually affect the cone's closure beneath the closed canopy. During midday, the temperature of cones in the opening averaged 7.5°C higher than air temperature (i.e., in a weather instrument shelter in the opening), while those in the closed stand were 2.2°C below the opening's air temperature. The harsher environment of the opening compared with the closed stand resulted in a more rapid decline in seed viability during the first summer of storage. In March 1997, 98% of the full seeds germinated from both the opening and closed stand. By the following October, only 36% of the full seeds germinated from the opening compared with 74% from the closed stand ($P < 0.01$). During 1998, differences between the opening and closed stand in germination were not significant ($P > 0.05$); values averaged 41% in March and 27% in October. Cain and Shelton (1997) reported a similar decline in viability for loblolly pine seeds under field storage. There may be little operational significance of the slower decline in seed viability in the closed-canopy stand, as loblolly pine seedlings do not survive for long under such conditions. However, there may be microsites within an opening, such as in the shelter of tops or coarse woody debris, that might provide similar levels of protection.

Field validation

To determine if the results in our protected storage frames were similar to that found in the field, we conducted additional cone sampling in nearby loblolly pine stands that had been operationally logged from late September to early October 1998. The initial base of viable seeds was not known but should have been similar to that in 1996, because both years produced bumper seed crops (≈2 500 000 sound seeds/ha). The number of viable seeds in early March 1999 were very similar to that found in our 1996 study (Fig. 2). The night before the early March collection, a severe rain and wind storm broke the crowns or collapsed about 20 loblolly pine trees in the stand that was being sampled for cones in logging tops. We collected current-year cones from these trees to confirm the difference in dispersal pattern between standing trees and tops. There were 1.3 viable seeds/cone from the previously standing trees compared with 21.8 seeds/cone from the tops ($P < 0.01$). The different seed dispersal pattern from cones of standing trees versus tops of felled trees undoubtedly reflects agitation and drying by the wind, which would affect cone openness. Data of Cain and Shelton (1997) suggest that a few loblolly pine seeds may be held so tightly within cones that they may not be dispersed under normal circumstances.

Fig. 3. Number and viability of seeds in cones with different orientations from loblolly pine trees felled during operational logging in southeastern Arkansas in late September and early October 1998. In June 1999, cones were collected at the logging site. Cones had been stored in the opening test site since the June sampling for the other sampling dates (also in 1999). Error bars are SE.

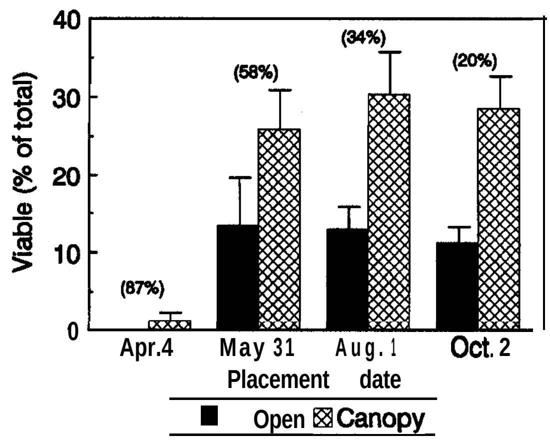


To more accurately time the dispersal of seeds during the spring and summer, we placed some of the March 1999 cones in storage frames (open site only) and included an orientation with the cone apex pointed up in addition to the down orientation used in the 1996 study. In early May 1999, cones with down orientation had 1.7 viable seeds/cone compared with 4.2 for the up orientation (P = 0.05). So, most of the viable seeds had apparently dispersed in March and April, because there was no apparent increase in nonviable seeds. To confirm this, another field sampling was conducted in mid-June 1999; however, we kept the cone samples separated by angle-orientation class as they occurred on the tops to investigate the effects of orientation on seed retention. An inventory of tops indicated that most of the cones had a side orientation (47%) with equal amounts in up (27%) and down (26%) orientations. In June 1999, cones with an up orientation had about 7 viable seeds/cone, while those pointed down or on the side had about 1 seed/cone (P = 0.005) (Fig. 3). Seeds from cones with up orientation were mostly dispersed later during the summer, because only about 1 viable seed/cone remained in November 1999, and there was no substantial increase in seeds with failed germination. As with the 1996 study, virtually all viable seeds had disappeared from cones by autumn, 1 year after maturing.

Seed fate

Our results indicate that, in a bumper seed year, over 20 viable seeds/cone could potentially be dispersed from tops after early March of their first year if loblolly pines are felled after seed maturation but before dispersal. Seeds dispersed outside of the normal pattern exhibited from standing trees would not receive the cool, moist stratification that promotes germination. To determine the possible fate of these seeds, seeds extracted from the March 1999 cone collection were placed into packets and were stored in a weather instrument shelter (open site only) awaiting periodic placement in prepared seedbeds. At the time of placement, their viability declined linearly during the summer, averaging 87% for the placement on April 1 and 20% on October 2 ($P \leq 0.01$). These results agree well with the decline in viability over the summer that was observed in the cones sampled in 1999, where the viability of full seeds decreased from 78% in early March to 2 1% in early November in the opening. So we feel that these seeds accurately represented seeds in cones that potentially could be dispersed.

Fig. 4. Percentage of viable seeds from packets stored in a weather instrument shelter located in the opening site until placed on mineral soil seedbeds at the indicated dates and locations during 1999. Packets were removed for germination testing in February 2000. The number in parentheses above each bar cluster is Percentage of viable seeds from a subset of packets that were tested for germination at the time of placement. Error bars are SE.



Virtually all the seeds of the April placement either germinated or died. When inspected in February 2000, 76% of the seeds from the April 1999 placement had remnants of radicles or had split seed coats; only 1% of the total seeds were still viable from the closed stand and 0% from the opening (Fig. 4). Subsequent placements during the summer and early autumn indicated potential carry-over of viable seeds to the next growing season of 1 1–13% in the opening and 24–28% under the closed canopy (P = 0.01). The higher potential carry-over rates of pine seeds under the closed canopy probably reflected a less harsh environment than in the opening.

Conclusions

From the standpoint of natural regeneration, the importance of seeds dispersed from cones on felled tops depends on the proportion of the stand that is cut when compared with that retained, thus, the potential is greatest in seed-tree stands and in small clearcuts. The contribution of seeds from tops to the stand's seed supply will also undoubtedly be influenced by when harvesting occurs, the specific methods employed, and the product classes removed. Results of our study showed that cones in tops from an early autumn harvests could potentially disperse up to 75% of their viable seeds in time for germination to occur during the spring; thus, the potential contribution of tops to the stand's seed supply is large. In addition, these seeds are probably dispersed close to the tops, where regeneration is difficult to obtain because the seedling-to-seed ratio is low (Grano 1949; Shelton and Murphy 1999). The contribution of seed-bearing cones in tops of felled trees is probably more important for regeneration during average seed crops than in good seed crops. Dispersal of seeds from cones in tops of felled trees appears to be facilitated by exposure to sunlight, which promotes drying and the cone's opening, but seed dispersal from cones in tops is prolonged when compared with that of standing trees. Up to one-third of the seeds dispersed from cones during the summer could potentially carry over to the following growing season.

References

Barnett, J.P. 1976. Cone and seed maturation of southern pines. USDA For. Serv. Res. Pap. SO-122.

Bonner, ET. 1974. Seed testing. In Seeds of woody plants in the United States. *Coordinated by* C.S. Schopmeyer. U.S. Dep. Agric. Agric. Handb. 450. pp. 136-152.

Brender, E.V., and McNab, W.H. 1972. Loblolly pine seed production in the lower Piedmont under various harvesting methods. J. For. 70: 345-349.

Cain, M.D., and Shelton, M.G. 1996. Considerations for natural regeneration of loblolly and shortleaf pines. The Consultant, **41**(3): 20–23.

Cain, M.D., and Shelton, M.G. 1997. Loblolly and shortleaf pine seed viability through 2 1 months of field storage: can carry-over occur between seed crops? Can. J. For. Res. 27: 1901-1904.

Campbell, T.E. 1967. Loblolly-shortleaf pine seedfall in Louisiana. J. For. 65: 894-895.

Grano, C.X. 1949. Is litter a barrier to the initial establishment of shot-deaf and loblolly pine reproduction? J. For. 47: 544-548.

Shelton, M.G., and Murphy, P.A. 1999. Disturbance from the initial harvest implementing uneven-aged silviculture in a pins hardwood stand in southwestern Mississippi. *In* Proceedings of the tenth biennial southern silvicultural research conference. *Edited by* J.D. Haywood. USDA For. Serv. Gen. Tech. Rep. SRS30. pp. 397–401.

Shelton, M.G., and Wittwer, R.F. 1996. Seed production in natural shortleaf pine stands in the Guachita and Ozark Mountains. South. J. Appl. For. 20: 74-80.

Steel, R.G.D., and Torrie, J.H. 1980. Principles and procedures of statistics: a biometrical approach. McGraw-Hill Book Co., New York.

USDA Forest Service. 1988. The South's fourth forest: alternatives for the future. USDA For. Serv. For. Resour. Rep. 24.

U.S. Department of Agriculture (USDA). 1976. Soil survey of Drew County, Arkansas. USDA Soil Conservation Service, and Arkansas Agriculture Experiment Station, Washington, D.C.

Wakeley, P.C. 1954. Planting the southern pines. U.S. Dep. Agric. Agric. Monogr. 18.