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For immature loblolly (Pinus taeda L.) *and slash (*P. elliottii *Engelm.) pine cones collected over a range of dates and stored for, 3 to 5 weeks, seed yields were reduced but adequate, and germinability increased with increasing cone storage periods. If slightly reduced yields and viability are acceptable, loblolly and slash cone collections can begin 2 to 3 weeks before maturity if the cones are stored before processing. Longleaf (P.* palustris Mill.) *pine cones should be collected only when mature, as storage decreased germination of seeds from immature cones. Biochemical analyses to determine reducing sugar contents of seeds and x-rays to determine if the seedcoats are filled shore promise as seed maturity indices.*

Additional keywords: *Pinus palustris, P. taeda, P. elliottii,* germination, biochemical composition, cone specific gravity, cone storage.

The demand for southern pine seeds is increasing rapidly in response to escalated forest regeneration programs. Traditionally. cone collections are made within a 2- to 3-week period after specific gravity drops below 0.89, the normal index of maturity (Wakeley 1954) . Because of limited labor available for collecting cones, this period is too short to allow collection of enough cones to provide an adequate seed supply. If the period could be extended by 2 to 3 weeks, the number of seeds collected might easily be doubled.

An extended collection period is reportedly possible if slightly immature cones are stored prior to kilning (Bevege 1965, Waldrip 1970, McLemore 197 5) . However, maturity indices that reflect the ripeness of seeds as well as cones after storage have not been developed. The tests reported here were installed to determine maturation of southern pine cones and seeds collected on different dates and stored for various periods. X-rays and biochemical analyses were evaluated as possible indices of seed maturity.

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METHODS

Collection, Storage, and Processing

Cone and seed maturity were tested for loblolly (*Pinus taeda L.*), longleaf (*P. palustris* Mill.), and slash (*P. elliottii* Engelm.) pines from natural stands in the Palustris Experimental Forest in central Louisiana. For each of the three species, 24 cones were collected from each of four trees selected for their large seed crops. To provide a range of seed maturity, collections were made weekly from September 15 to October 13, 1969, for loblolly and longleaf, and from July 12 to September 22,1971, for slash pine. Since the earliest (September 15) collection of loblolly seed proved mature, collections for loblolly and longleaf were begun 5 weeks earlier the following year and were repeated biweekly (August 10 to October 17, 1970). The 24 cones from each tree were divided into four equal groups for tests of cone and seed maturity. One group was tested for specific gravity and moisture content immediately after collection, and the other three groups received one of three storage treatments to determine if the cones would ripen independently of the tree. The cones were stored in open paper bags in an unheated building for 1, 3, or 5 weeks prior to processing. As each of the storage treatments was completed, the cones were opened by drying for 3 days in a gas-fired kiln (38°C). Cone maturity was determined by seed yield. Casehardened cones were considered immature, and their seeds were obtained by opening the cones mechanically. Seeds from the 1- and 3-week storage treatments were immediately placed in a refrigerator (1°C) to slow development. At the end of the 5-week storage period seeds from all of the storage treatments were used for germination trials and biochemical analyses. After all of the seeds were cleaned, filled ones were separated from empties by liquid separation-loblolly in water, longleaf in n-pentane, and slash in 1:1 ethanol and water.

Germination tests.-Germination tests were made under standard laboratory conditions. Because loblolly seeds are dormant, they were stratified for 28 days at 1° C on a sand-peat medium. Longleaf and slash were left unstratified. One hundred seeds were sown in factorial combination for each tree, collection date, and storage treatment.

Biochemical analyses.-Because of species differences in size, 50 longleaf, 100 loblolly, and 75 slash pine seeds for each tree-treatment date combination were freeze-dried and stored at 1° C. They were then analyzed for lipids according to Ching's method (1963) , for reducing sugars by a modified Somogyi method (Nelson 1944), and for protein or insoluble nitrogen and total soluble sugars by methods reported previously (Barnett and Naylor 1970).

X-rays.-Before processing, the slash pine seeds to be used for the biochemical analyses were x-rayed to determine how much of the seed cavity was filled by the embryo and megagametophyte.

Analyses.-For each species, analyses of variance were conducted to determine differences in seed yields, germination, and biochemical properties (0.05 level of significance).

RESULTS AND DISCUSSION

Loblolly

Morphological characteristics and viability.-In both collection years, the specific gravity of loblolly cones averaged approximately 0.89 near October 1, when cone moisture contents were about 110 to 120 percent.

In 1969, the average number of seeds released per cone tended to increase with each subsequent collection date and with increasing lengths of cone storage (table 1). Cones from the early collections appeared to benefit most from a long storage period, as evidenced by improved yields with longer storage. Cones collected as early as September 15, when the specific gravity was 0.99, and stored for 5 weeks yielded 38 seeds per cone, which compared favorably with the 47-seedper-cone average obtained for the final collection after 1 week of storage, when specific gravity averaged 0.80. For cones collected on September 29, when specific gravity was 0.88, the average yield per cone after 1 week of storage was only one seed, but yields increased appreciably both after longer storage and with subsequent collections.

The fact that germination was not affected by either date of collection or storage treatment shows that collections were not begun early enough to allow observations of seed development. It is obvious that seed maturity of loblolly seeds occurs several weeks before cones are ripe enough to open fully: These results were confirmed by the 1970 loblolly tests, in which collections commenced 5 weeks earlier than in 1969 and storage was omitted. In 1970, seed yields were nil through September 21 but were satisfactory thereafter. Germination was 58 percent for the seeds collected on September 7, 79 percent for September 21, and 96 percent for October 5. No viable seeds were obtained from the August collections.

Loblolly pine cone collections normally begin about October 1, when specific gravity-the conventional index of maturity-is about 0.89. Results in this study indicate that collections can commence 2 to 3 weeks earlier if some reductions in yield and viability are acceptable and if the cones are stored 3 to 5 weeks before processing.

Biochemical analyses.-Although there were significant differences among individual trees in contents of total sugars, lipids, and protein-nitrogen, there were no differences caused by collection date or storage treatments (table 1). The only notable biochemical changes measured were levels of reducing sugars, which dropped steadily with

Table 1.--Characteristics of loblolly pine cones and seeds after various collection dates and storage periods

Collection date (1969) and storage period (weeks)	Cone specific gravity	Cone moisture content	Seed yield per cone	Germi-nation	Reducing sugars	Total sugars	Lipids	Insoluble nitrogen
		Percent	*Number*			- - - - - - - - *Percent* - - - - - - - -		
September 15	0.99	129.3	...	...	0.53	2.4	16.7	2.4
1			0	96	.04	1.6	17.0	2.5
3			14	98	.03	1.7	17.2	2.2
5			38	98	.02	1.5	16.0	2.6
Average			17	97	.16	1.8	16.7	2.4
September 22	.93	119.9	...	...	.40	1.8	17.8	2.8
1			22	95	.05	1.5	17.4	2.2
3			27	97	.03	1.5	18.2	2.3
5			28	98	.03	1.6	17.4	2.4
Average			26	97	.13	1.6	17.7	2.4
September 29	.88	112.1	...	...	.14	1.7	18.3	2.4
1			1	98	.03	2.5	18.1	1.7
3			40	98	.02	2.8	18.3	2.3
5			65	98	.03	2.8	19.3	2.7
Average			35	98	.06	2.4	18.5	2.3
October 6	.82	103.6	...	...	.O6	3.1	19.3	1.7
1			35	98	.02	3.0	19.1	1.6
3			45	98	.04	2.6	17.7	1.6
5			38	98	.02	2.3	18.0	2.2
Average			39	98	.04	2.8	18.5	1.8
October 13	.80	84.3	...	...	.02	2.7	18.3	2.2
1			47	99	.01	2.8	18.7	2.2
3			52	99	.02	3.3	18.7	2.2
5			44	99	.02	2.8	18.2	2.3
Average			48	99	.02	2.9	18.5	2.2

each successive collection date. The percentage of reducing sugars after cone storage was very low regardless of the date of collection, an indication that reducing sugars were converted to other products during the first week of storage. Reducing sugar content of seeds may therefore indicate cone maturity. In this study, cone maturity (specific gravity of 0.88) occurred when the reducing sugar content of the seeds was 0.14 percent.

The extension of the collection period in 1970 resulted in a much greater range of values in all the constituents measured; however, at a given date, all levels compared closely with those obtained the previous year.

Longleaf

Morphological characteristics and viability.-Differences between cone collections made in 1969 and 1970 showed that longleaf cones sometimes ripen considerably later than loblolly. Longleaf cones collected in 1969 reached maturity as indicated by a specific gravity of 0.89 on September 29; but in 1970 ripeness did not occur until October 17. Differences in individual trees may explain some of the variation. Cone moisture contents of longleaf varied slightly from that of loblolly; specific gravity was 0.89 when moisture content was about 140 percent.

Like loblolly, yields of longleaf seeds increased both from early to late collections and with increasing cone storage periods (table 2) . Unlike loblolly, late collections of longleaf showed increased yields after storage, even when specific gravities were as low as 0.80. The highest yields were obtained with cones stored 3 to 5 weeks when initial specific gravities were 0.89 or below. Yields for cones collected earliest (September 15) and held for 5 weeks were only about half those obtained from the later collections.

Viability increased with each successive collection date; however, germination of the seeds from immature cones decreased with the length of cone storage; only seeds from the last collection retained high viability over the 5-week storage period.

These results indicate that longleaf cones should be collected only when mature; however, a storage period longer than 8 weeks would diminish seed storability (McLemore 1961).

The 1970 tests confirmed that cones collected and processed before specific gravity is less than 0.90 fail to open and release seed. Seeds were obtained only from the final cone collection (October 19); germination was 93 percent.

Biochemical analyses.-Percentages of reducing sugars for unstored cones in the 1969 collections ranged from 0.64 to 0.19 from the early to the late collection (table 2) . Except for the latest (October 13) collection, the values for each collection date dropped markedly during

Table 2.--Characteristics of longleaf pine cones and seeds after various collection dates and storage periods

Collection date (1969) and storage period (weeks)	Cone specific gravity	Cone moisture content	Seed yield per cone	Germi-nation	Reducing sugars	Total sugars	Lipids	Insoluble nitrogen
		Percent	Number	- - -	- - - -	- Percent - - - -		- - - -
September 15	0 98	156.2	...	...	0.64	4.7	29.2	4.0
1			0	64	.22	2.6	29.0	4.4
3			4	31	.17	1.3	26.2	3.1
5			33	20	.18	1.8	24.4	3.4
Average			13	38	.30	>2.6	27.2	3.7
September 22	.93	154.0	...	...	.56	3.8	27.4	3.8
1			9	71	.21	2.2	27.3	3.7
3			20	43	.04	2.0	26.4	3.8
5			37	43	.18	3.5	28.7	4.2
' Average			22	52	.25	2.9	27.4	3.9
September 29	.89	136.3	...	...	.46	4.4	27.8	3.8
1			0	73	.23	2.6	28.5	3.8
3			39	74	.12	2.9	28.5	3.7
5			59	60	.07	3.4	26.3	3.6
Average			33	69	.22	3.3	27.8	3.7
October 6	.85	128.5	...	...	.42	3.2	29.5	3.6
1			5	81	.11	2.3	30.4	4.0
3			54	74	.14	2.9	26.9	3.8
5			47	60	.20	3.3	25.9	3.8
Average			35	72	.22	2.9	28.2	3.8
October 13	.80	126.7	...	...	.19	2.8	29.4	3.7
1			23	91	.18	2.6	30.1	4.0
3			46	90	.12	3.7	27.4	4.0
5			56	91	.10	3.4	25.6	4.0
Average			41	91	.15	3.1	28.1	3.9

the first week of storage, after which the decreases were smaller.

Values for total sugar also varied with collection date and cone storage period but followed the trends observed for loblolly pine seeds. Although sugars in seeds from unstored cones decreased from 4.7 percent for the earliest collection to 2.8 percent for the latest, total sugar contents of seeds from stored cones tended to increase with length of storage.

Although lipid contents were not affected by collection date, levels decreased with increasing lengths of cone storage (table 2). Insoluble nitrogen was not affected by either collection date or storage period.

Slash

Morphological characteristics and viability.-Slash pine cone collections typically begin in early September; the present tests confirm that maturity occurs before September 15. Cone moisture contents were about 135 to 140 percent when specific gravity was 0.89.

Cone collections began about 8 weeks prior to maturity. Like loblolly and longleaf pine slash pine seed yields increased with each subsequent collection date and with longer cone storage periods. Acceptable yields were obtained when cones were collected in late August, provided the cones were held 3 or 5 weeks prior to processing (table 3). When cones were held for 5 weeks, seed yields from the August 23 collection (specific gravity 0.99) were 70 per cone compared to 85 fox cones that were picked on September 22 (specific gravity 0.81).

Germination levels were never high. Best germination (83 percent) was for seeds from cones collected on September 22 and held 5 weeks. No seeds from cones picked in July germinated, even after 5 weeks of storage, and viability was low for seeds from the August collections regardless of the cone storage period.

Slash pine cone collections normally begin in early September. Although yields are usually adequate by late August for cones with a specific gravity of 0.95, reduced viability occurs. To obtain maximum germination, cones must be fully mature when collected and stored.

Biochemical analyses.-Like loblolly and longleaf, reducing-sugar content in slash pine seeds from unstored cones appeared closely related to maturation. Levels dropped rapidly for the last three collection dates (table 3).

Total sugars followed similar trends; the highest level (2.8 percent) occurred for cones collected earliest. Total sugars of seeds from unstored cones dropped to 0.6 percent at maturity. Cone storage markedly lowered total sugar levels of immature seeds but not of mature ones.

Lipids constituted only about 2.6 percent of seed weight in early collected seeds but increased to about 20 percent with maturation.

Table 3.--Characteristics of slash pine cones and seeds after various collection dates and storage periods

Collection date (1971) and storage period (weeks)	Cone specific gravity	Cone moisture content	Seed yield per cone	Germination	Reducing sugars	Total sugars	Lipids	Insoluble nitrogen
		Percent	Number		- - - - - - - - Percent - - - - - - - -			
July 12	1.05	206.6	...	...	0.79	2.8	2.6	1.3
1			0	0	.19	.5	2.4	1.4
3			12	0	.04	.2	.9	1.0
5			13	0	.02	.1	.6	.8
Average			8	0	.26	.9	1.6	1.1
July 26	1.01	186.3	...	...	.75	2.8	7.7	2.0
1			0	0	.22	1.0	7.1	2.0
3			1	0	.04	.5	6.5	1.9
5			53	0	.03	.5	6.3	2.3
Average			18	0	.26	1.2	6.9	2.0
August 9	1.04	175.9	...	...	.77	2.7	13.8	2.9
1			0	6	.20	1.5	13.9	2.8
3			35	11	.01	.7	13.2	2.4
5			51	24	.00	.9	12.5	2.8
Average			29	14	.24	1.4	13.3	2.7
August 23	.99	160.7	...	...	.56	2.3	18.4	3.4
1			1	14	.25	1.4	19.7	3.7
3			36	58	.01	1.1	19.1	3.7
5			70	68	.08	1.1	20.3	4.3
Average			36	47	.22	1.5	19.4	3.8
September 7	.92	138.5	...	...	.49	1.5	20.6	4.1
1			6	49	.08	1.1	19.0	3.9
3			64	71	.00	1.3	21.7	4.4
5			70	67	.00	1.4	21.5	4.7
Average			47	62	.13	1.3	20.7	4.3
September 22	.81	126.8	...	...	.16	.6	19.6	4.3
1			70	54	.00	1.1	21.2	4.6
3			75	73	.03	1.3	21.5	4.4
5			85	83	.10	1.1	21.6	4.6
Average			77	70	.07	1.0	21.0	4.5

Cone storage did not affect lipid content. A 20 percent lipid content was necessary to ensure viability of 60 percent or greater. Sugars were apparently converted into lipids at maturation.

Protein-nitrogen contents also significantly increased with successive collection dates (table 3). For early collections, levels decreased with longer cone storage periods but thereafter increased only slightly or not at all. At maturity protein-nitrogen levels were slightly above 4 percent.

X-rays.-X-rays of slash pine seeds showed that the embryo is well-developed and the coat cavity filled when the seeds are mature and viable. There were large differences in the amount of the seed cavity filled by the embryo and megagametophyte between early and late collections (fig. 1). Seeds from the July collections showed little differentiation into embryonic and megagametophytic tissue; at this early stage, seed content appeared milky, indicating high sugar and protein-nitrogen contents. These observations were consistent with the germination data and the biochemical analyses.

CONCLUSIONS

Collections of slash and loblolly cones can begin 2 or 3 weeks before maturity if specific gravity is 1.0 or less, but yields will probably decrease even after cone storage. Acceptable yields and germination can be obtained when collections of loblolly cones begin about mid-September (specific gravity 1.0) and when collections of slash cones begin in late August (specific gravity 0.95). However, optimum yields and germination are possible only if the cones are mature when collected. Early collections are advisable only if large quantities of seed are needed immediately or if labor or the collectable crop is limited. Seeds from immature loblolly cones are apparently mature when extractable, and slash pine seeds continue ripening during cone storage. However, viability of longleaf seeds from immature cones decreases during storage. Therefore, only mature longleaf cones should be collected. Once ripe, longleaf cones can be stored from 3 to 5 weeks to increase seed yields without reducing viability, but the storage period should not exceed 8 weeks.

Moisture contents varied slightly among species but were usually near 120 to 140 percent at maturity.

For the species evaluated, reducing sugars offered the best potential biochemical index to maturity. For all three species, levels decreased to about 0.20 percent at maturity-when specific gravities were 0.88.

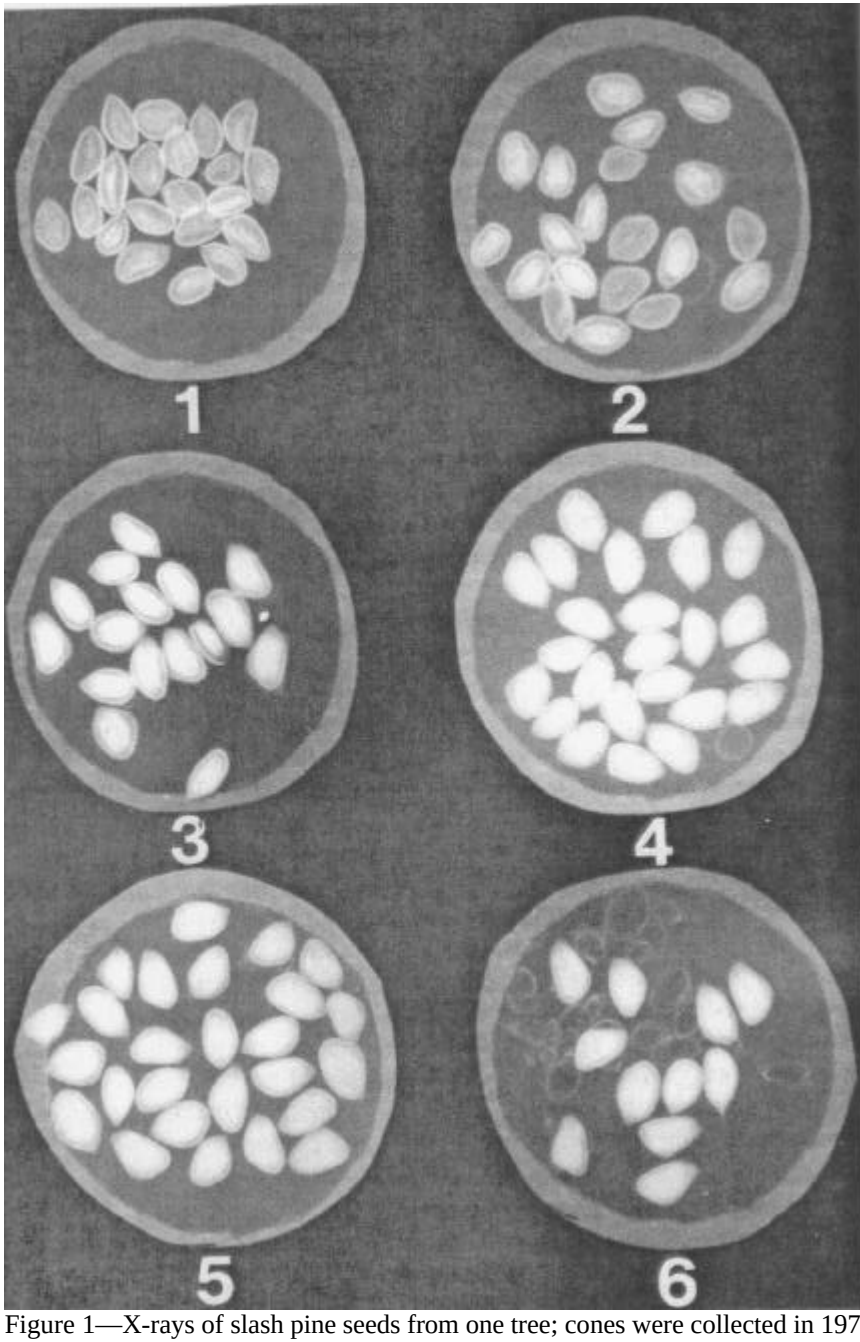

Figure 1—X-rays of slash pine seeds from one tree; cones were collected in 1971 on six dates as follows: (1) Jnly 12, (2) July 26, (3) August 9, (4) August 23, (5) September 7, (6) September 22.

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