STAGES OF FLOWER DEVELOPMENT AND CONTROLLED-POLLINATED SEED YIELDS FOR AMERICAN SYCAMORE

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Abstract.--Six stages of bud and flower development of American sycamore are described and used to determine optimum controlled-pollination procedures. Flowers on trees from southern origins developed about three days ahead of flowers on trees from more northerly origins in Mississippi when all were grown at the same site, but flowers from all origins were susceptible to frost kill during stages 4 and 5. Yields of seed balls and full-seed percentages indicated that: (1) female flowers should be bagged by mid stage 4 and control-pollinated at mid to late stage 5, (2) bagging was effective in preventing outside pollination and should not be removed until flowers reach mid stage 6, and (3) up to 24 days of bagging had no detrimental effect on flower survival or seed yield. Most mortality of bagged flowers occurred during the three weeks after bag removal, and this was also when most growth of the seed ball occurred. Male flowers should be collected during early to mid stage 5, and they will yield approximately 0.01 ml of pollen per flower ball.

Keywords: Platanus occidentalis, flowering, controlled-pollination.

INTRODUCTION

Controlled pollinations among selected trees are often required for basic genetics studies and for multiple-generation improvement programs. Effective pollinations are dependent on: (1) recognition of the developmental stages of flowers and (2) knowledge of when to isolate female (pistillate) flowers, when to collect male (staminate) flowers, when to apply pollen to the female flowers, and when to remove isolation bags. Such information has been illustrated by Bramlett and O'Gwynn (1980) for southern pine flowers. Publications on flowering and fruiting of American sycamore (Platanus occidentalis L.) (Bonner 1974, Wells and Schmidtling 1990) do not provide sufficient detail for controlled pollination purposes. The objectives of this paper are to: (1) describe morphological stages of sycamore flower development and (2) identify optimum stages for bagging, pollen collection, pollination, and release.

METHODS

Bud appearance and degree of flower emergence were categorized into developmental stages and recorded at approximately two-day intervals from March

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20 to April 10, 1987, and from March 23 to April 28, 1988, on 18 clones in a grafted sycamore orchard in east-central Mississippi (33°17'N, 88°54'W). Six clones had origins in southwest Mississippi and central Louisiana, six had origins in central Mississippi and southern Arkansas, and six had origins in northern Mississippi and central Arkansas.

In 1988, single-pair matings with reciprocal crosses were made within each of the three origin groups. Self-pollinations were also made when there were sufficient flowers. Female flowers were bagged in sausage casing (14-cm diameter; from TEE-PAK, Inc., Oakbrook, IL) during April 2-8, and stage of female flower development was recorded at time of bagging. Male flower balls were collected during April 2-13, and stage of male flower development was recorded at time of collection. Male flowers were dried in sausage-casing packages under warm lights and then shaken through a fine wire screen into vials. The cotton-stoppered vials were stored in a dessicator containing "Drierite" (anhydrous $CaSO_4$) in a refrigerator to maintain moisture content at one percent and temperature at 2-3°C until used. Controlled pollinations were done with a cyclone pollinator (ERI Machine Shop, Iowa State University, Ames, Iowa), and female flower stages at time of pollination were recorded. When sufficient flowers were available, some bags were not pollinated to provide a check of the effectiveness of bagging. Bags were removed at six to 24 days after pollination, depending on stage of female flowers in bags, and flower stages were recorded.

Seed ball survival, peduncle length, and ball diameter were recorded at monthly intervals from three weeks after bag removal until November 7-9, 1988, when the seed balls were harvested. Open-pollinated seed balls from the clones were collected at the same time. The percent full seeds was determined for each "cross" of each clone (outcrossed, selfed, no pollen, and open-pollinated) from x-ray radiographs. Four 50-seed samples and a 20-seed sample from each of 52 "crosses" were placed on double-sided cellophane tape attached to 20cmx25cm paper cards. These were x-rayed on Kodak Industrex Type M film with an exposure of 25kVP, 3mA, 65-cm film-focus distance for 60 seconds. The 20-seed sample was subsequently used for cutting tests to confirm empty- or full-seed status of radiograph images.

The yield of seed balls harvested (expressed as a percentage of the female flowers pollinated) and the yield of full seeds (expressed as a percentage of the total number of seeds in a seed ball) were averaged across clones for (1) the female flower stages when pollinated, (2) the female flower stages when bagged (when no pollen was put in the bags), and (3) the number of days that the cross-pollinated female flowers were bagged. Means across all clones were also used to illustrate seed ball survival and growth at monthly intervals following removal of bags. SAS procedure REG (SAS Institute, Inc., 1988) was used to determine the overall relationship between number of male flower balls collected and pollen yields for flowers collected at two stages of development. A balanced subset of seven clones having seeds from all four types of "crosses" (open, cross, self, and no pollen) was used in the SAS ANOVA procedure for a Duncan's test of ranked "cross" means.

RESULTS AND DISCUSSION

Six morphological stages of bud and flower development of sycamore were devised for this study and are described in Table 1. The early part of a stage

Table 1. Six stages of bud and flower development in sycamore.

Stage	Description	Time Period in central MS
1	Tight buds; no swelling; vegetative buds indistinguishable from floral buds.	Winter to mid March
2	Swollen buds; bud scales splitting; sap extrusion may occur from buds; vegetative buds indistinguishable from floral buds.	3-4 days in late March
3	Leaf emergence from under bud scales, but "flower ball" (globose head of flowers of one sex) (which may be in same bud) not visible; on some trees the flower ball emerges immediately when scales split, so that stage 3 is skipped.	0-2 days in late March
4	Starts (stage 4-) when flower balls can first be identified as they emerge from bud (female is red, from stigmas, and male is green); females are more frequent on branch tips in top of crown, while males occur throughout crown and are more frequent on branchlets 20-100 cm back from branch tips; by late stage 4 (=stage 4+) the female is a 5-8 mm diameter bright purplish red ball on a 2-3 cm peduncle, and the male is a 1-cm diameter dark green or purplish-green ball on a 2-cm peduncle.	5 days in late March to early April
5	Starts (stage 5-) when the female flower ball enlarges to 1-cm diameter on a 3-4 cm peduncle, color may change to greenish white, and the ball has a soft "waxy" feeling when rubbed between fingers; male flower ball at stage 5- shows some yellow or gold color, and less than 10% of balls on tree have started to open (i.e. pollen released when ball thumped); during stages 5 and 5+ the female flowers retain the soft waxy feeling and the peduncle elongates to 5-6 cm, while 10-50% of the male balls are open during stage 5 and 50-90% are open during stage 5+; stages for males and females may not be the same on the same tree.	Females = 7-10 days in early April; Males = 2-7 days in early April
6	Starts for female flowers (stage 6-) when the ball starts turning brown and is not as waxy feeling; by stage 6 the female is brownish red and dry to touch, with diameter of 10-15 mm; by stage 6+ the stigmas have dried to dead brown and shriveled, so that the background green color of core becomes the dominant color of the young seed ball (diameter of 15-20 mm and peduncle of 5-12 cm); stage 6- for males occurs when 90% of balls have opened and shed pollen and stamens to leave only the flower ball core; by stage 6 for the male flowers, only cores remain on the tree.	Females = last 2 weeks in April; Males = 3rd week in April

is designated by "minus" following the stage number, the late part is indicated by "plus," and the middle part has only the stage number without plus or minus.

In 1987, clones from the southern-origin group were about three days ahead of the other two groups in stage of development (Table 2). There was variation

Table 2. History of bud and flower development for 18 sycamore clones from three latitudinal origins prior to and following a late frost on March 30, 1987, in east central Mississippi.

	Stage of Bud & Flower Development							
	March				April		No. Flowers/Tree	
Origin & Statistic	20	25	27	31	3	6	Males Collected	Females Counted
Southern (30°45'-31°50'N Latitude) (s.w. MS and e. LA):								
Maximum	3-	4+	5	5+	5+	dead	119	10+
Minimum	2-	4-	4+	5	5	dead	3	2
Average	2.0	4.0	4.6	5.0	5.0	dead		
Central (33°7'-33°40'N Latitude) (c. MS and s. AR):								
Maximum	2+	4	5-	5	5	dead	95	10+
Minimum	1	2	3	4-	4-	dead	none	1
Average	1.7	3.1	3.8	4.5	4.6	dead		
Northern (34°20'-34°35'N Latitude) (n. MS and c. AR):								
Maximum	2	4+	5	5+	5+	dead	209	10+
Minimum	1-	2+	3-	3+	3+	alive	none	none
Average	1.3	3.1	3.7	4.3	4.3	dead		
Overall Avg.	1.7	3.4	4.0	4.6	4.6	dead		

among clones within the same group for rate of development, and there were clonal differences in male and female precociousness of these five-year-old grafts (13-year-old ortets). However, temperatures dropped to 26°F (-3.3°C) on March 30, when all but one of the clones had floral buds in stages 4 or 5. All of these buds were killed, as indicated by arrested bud development and discoloration by April 6. The one clone with stage 3+ buds eventually produced floral buds and seed balls. Apparently, both male and female buds are extremely susceptible to frost kill during stages 4 and 5. Trees with flowers at stage 6 on the nearby Mississippi State University campus did not exhibit frost kill.

In 1988, collection of male flowers before stage 5- yielded very little pollen, while pollen yield from males collected throughout stages 5-, 5, and 5+ was approximately 0.01 ml per ball (Figure 1). Female flowers cross pollinated before stage 4+ produced few seed balls, and those balls had a lower percentage of full seeds than flowers pollinated in stages 4+ to 5+ (Table 3). Pollination bags prevented open pollination of flowers when (1) the bags were placed over the

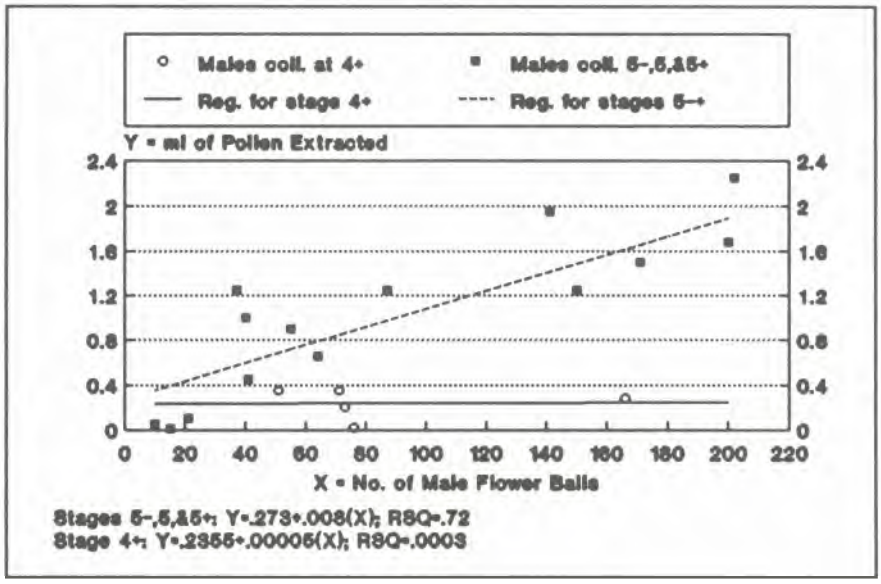

Figure 1. Pollen yields from male flower balls collected at two stages of development.

Table 3. Yields of seed balls and full seeds from female flowers cross-pollinated at different flower stages.

Female Flower Stage When Pollinated	No. of Flower Balls Pollinated	Seed Balls Harvested (% of flowers pollinated)	Full Seeds (% of total seed from ball)
4-	4	25	Not Meas'd.
4	7	14	24
4+	41	41	34
5-	61	51	31
5	15	73	31
5+	34	85	31
6-	2	50	14

female flowers before male flower development reached stage 5- on surrounding trees or (2) late-flowering trees were bagged before female flowers passed stage 4-. This was indicated by full seed yields of only 1-4 percent when flowers were bagged and not pollinated (Table 4), as compared with 23 percent for open-pollinated flowers and 27 percent for cross-pollinated flowers (Table 5). Self-pollinated flowers gave only four percent full seeds. The few full seeds in non-pollinated and selfed seed balls are similar to the results of Beland and Jones (1967), and reconfirm self incompatibility and lack of apomixis in the species. However, flower-ball survival was apparently not dependent on pollination in the present study, as the non-pollinated flowers produced as high a percentage of harvested seed balls as did the cross-pollinated and self-pollinated flower balls. There was no detrimental effect on seed ball survival or full seed yield from keeping cross-pollinated flowers bagged for as long as 24 days (Table 6).

Table 4. Yields of seed balls and full seeds from female flowers bagged at different flower stages and not pollinated.[a/]

Female Flower Stage When Bagged	No. of Flower Balls Bagged	Seed Balls Harvested (% of flowers bagged)	Full Seeds (% of total seed from ball)
4-	15	40	0.8
4	5	100	4.0
4+	5	60	1.8
5-	7	71	1.5

[A/] All female flower balls were bagged during April 2-4, except for five stage 4- flowers that were bagged on April 7-8 and one stage 4+ flower that was bagged on April 5. Male flower balls reached early shedding stage (5-) on two of 18 clones on April 2 and on six of the 18 clones by April 4.

Table 5. Effects of no pollination, controlled-cross pollination, self-pollination, and open-pollination on yields of seed balls and full seeds from seven sycamore clones.

	Seed Balls Harvested		
Type of Pollination	Number	% of Flowers Pollinated	Full Seeds (% of total seeds from ball) [a/]
No Pollen	14	88	2 b
Self	15	94	4 b
Cross	60	71	27 a
Open	147		23 a

[/] Means followed by different letters are significantly different at the 0.05 probability level according to Duncan's Test.

Table 6. Effects of number of days that female flowers were bagged on yields of seed balls and full seeds from controlled cross pollinations.

Number of Days that Flowers Bagged	No. of Female Flower Balls Bagged	Seed Balls Harvested (% of flowers bagged)	Full Seeds (% of total seeds from ball)
10-14	82	60	34
15-19	25	32	29
20-24	54	63	34

Most mortality of bagged female flowers occurred during the three weeks following removal of the bags (Figure 2). Six percent of the flowers died while

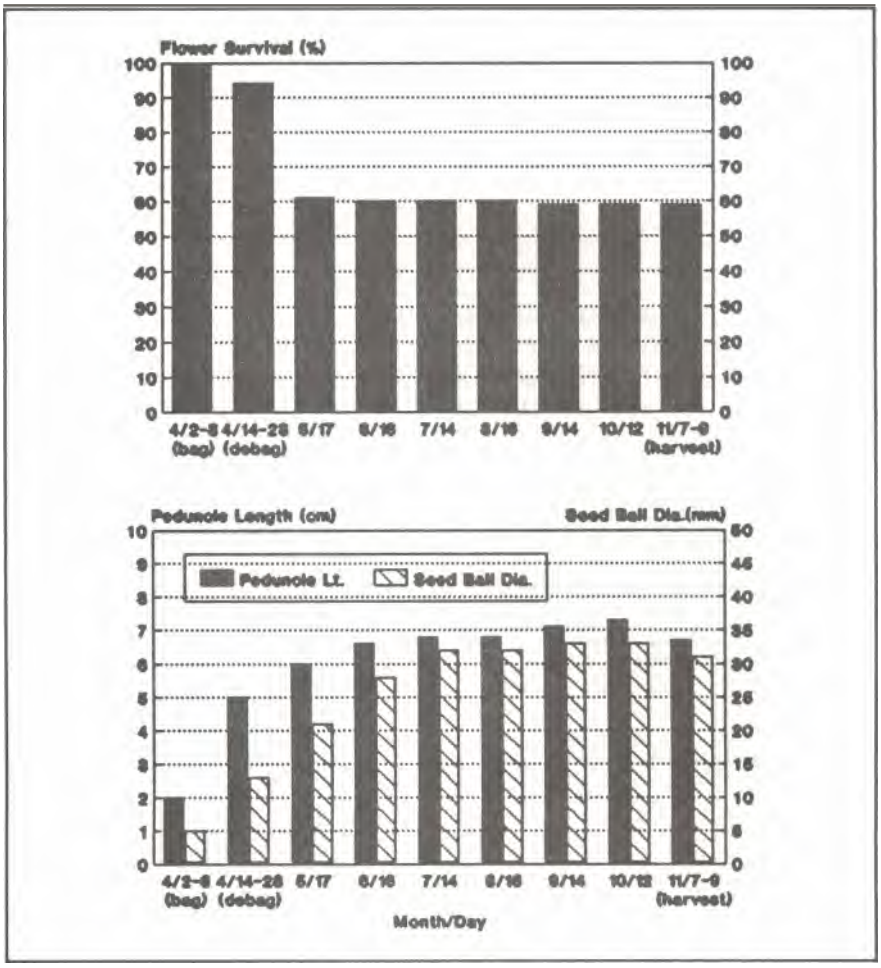

Figure 2. Female flower survival and seed ball development from bagging to seed collection.

in the bags, another 33 percent died during the three weeks after release, and only two percent died after that time. Either some flower balls were dying at time of release, or the flowers were very susceptible to the shock of release. The surviving seed balls had 90 percent of their mature peduncle length and about 70 percent of their mature diameter by three weeks after release from the bags. They continued to grow slowly through mid October, before shrinking slightly in late October as they dried at maturity.

SUMMARY AND CONCLUSIONS

(1) Six stages of sycamore bud and flower development can be used to determine the best times for bagging, male flower collection, pollination of female flowers, and bag removal.

(2) Trees from more southerly origins flower earlier, but floral buds in stages 4 and 5 from any origin are susceptible to frost kill.

(3) Female flowers should be bagged by stage 4 before pollen dispersal. Controlled pollination of these flowers should be done at stages 5 to 5+.

(4) Male flowers should be collected during stages 5- and 5. Pollen yield during these stages will be approximately 0.01 ml per flower ball.

(5) Bagging is effective in preventing outside pollination. Bags should not be removed until the female flowers reach stage 6. Up to 24 days of bagging has no detrimental effect on yields of seed balls or seeds.

(6) Most mortality of bagged flowers occurs during the three weeks after bag removal. The seed balls are about two-thirds of mature size at that time and are attached to peduncles that are nearly the mature length.

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