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# Pollination by Honey Bees of Male-sterile Cotton in Cages[1]

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## ABSTRACT

**In 1969 and 1970, honey bees (*Apis mellifera* L.) successfully pollinated the caged A-line flowers of a three-species cross between *Gossypium arboreum* L., *G. thurberi* Todaro, and *G. hirsutum* L., which exhibits cytoplasmic male sterility at moderately high temperatures. The pollen source was an isogenic B-line. The supply of seeds was increased from a few to more than 80 kg. The A-line plants produced 84% as much seed cotton as the B-line plants. Some 2% of the seeds produced on A-line plants were from self-fertile flowers; 98% were the result of crossing. A reduction in sunlight of 47% lowered yields of seed cotton 50%, compared with plants subjected to a 27% reduction.**

***Additional key words:* *Apis mellifera* L., *Gossypium arboreum* L., *G. thurberi* Todaro, *G. hirsutum* L., Shading.**


INCREASES of 25% or more in cotton yields due to heterosis in certain hybrids are reported in the literature. The development of cytoplasmic male-sterile cotton (*Gossypium* sp.) flowers makes the production of hybrid cottonseed practical if an insect pollinator can be found to transfer pollen to the male-sterile flowers. Honey bees were used in this study to see if they would pollinate male-sterile flowers growing in screened cages.

Meyer (7) describes the feasibility of using male-sterile lines to produce hybrid cotton. Kearney (3), Peebles (9), and Kime and Tilley (4), among others, have reported large increases in yield from some hybrid cottons. The strain used in this study is a potential parent of hybrid cotton.

## MATERIALS AND METHODS

The strain used in the tests was obtained from Vesta Meyer, Associate Geneticist, Delta Branch, Mississippi Agricultural Experiment Station, Stoneville.

This strain was produced by first crossing *Gossypium arboreum* L., a source of cytoplasmic genetic male sterility, with *G. thurberi* Todaro. The chromosome number of the offspring was then doubled by using colchicine to obtain a chromosome complement of 26 pairs. Repeated backcrosses were made with *G. hirsutum* L. ('Deltapine M-8') until a strain was obtained that resembled the recurrent Deltapine parent but possessed the *G. arboreum* cytoplasmic male-sterility factor. The pollen from the A-line plants produced 17 to 19 days after the maximum daily temperature reaches 32 C (6) is sterile, while the pollen from the B-line plants is not sterile until 17 to 19 days after the maximum daily temperature exceeds 40 C. In 1969 the seeds were planted in a small (3.66 by 7.32 by 1.83 m; 26.79 m²) saran mesh cage. The seed harvested in 1969 was planted April 7, 1970 in a greenhouse and was transplanted May 14 to ½ of a large (43.87 by 74.34 by 2.74 m; 3,261.30 m²) cage covered with copper wire. The number of bee visits was determined by an observer walking slowly down the row and glancing at each open flower. If eight bees were seen when looking briefly at 100 flowers, the visitation rate was said to be 8%. Yield was determined at harvest by picking the seed cotton from each plant, weighing it separately, ginning the cotton, and counting and weighing the seed from the A- and B-lines separately to obtain an average seed weight.

All colonies had continuous access to water, pollen supplement, and sugar sirup while in the cages.

## RESULTS

*1969 Test.* Twenty-six A-line plants and 46 B-line plants were enclosed in the cage just before the plants started blooming. The planting was arranged so that each A-line plant had a B-line plant on each side to insure maximum reproduction of the limited A-line seed available. The additional B-line plants were also planted to insure maximum pollination. On July 7 one colony of honey bees was placed in the cage. Every 2 weeks it was rotated with a second colony from outside the cage. The dates of observations and the results of counts of bee visitations (made between 9 and 10 AM) are shown in Table 1. By August 1 there was a heavy boll set, and blooms after this date were scarce.

*1970 Test.* The planting pattern and arrangement of the large cage in 1970 are shown in Fig. 1. The 19 rows ran north and south and were 0.91 m apart. Even-numbered rows began with two B-line plants followed by 10 A-line plants, and the remainder of these rows was planted with two B-line plants alternating with 20 A-line plants. Odd-numbered rows began with two B-line plants followed by 20 A-line plants, and the remainder of these rows was planted with 2 B-line plants alternating with 20 A-line plants. The rows were thus offset, and no male-sterile plant (A-line) was more than five plants from a pollen (B-line)

**Table 1. Honey bee visits to cotton flowers in a small cage, Tucson, Ariz., 1969.**

| Dates observed | No. flowers observed | % flowers with honey bees |
|---|---|---|
| 7/8-11 | 233 | 10 |
| 14-18 | 256 | 16 |
| 21-25 | 151 | 18 |
| 7/29-8/1 | 75* | 9 |
| 8/4-7 | 32 | 22 |
| 8/15, 29 | 12 | 17 |
| 9/2-5 | 18 | 28 |
| 9/12 | 14 | 14 |
| Total | 791 | Avg 14.7 |

* After Aug. 1, the blooms were scarce because of the heavy early set.

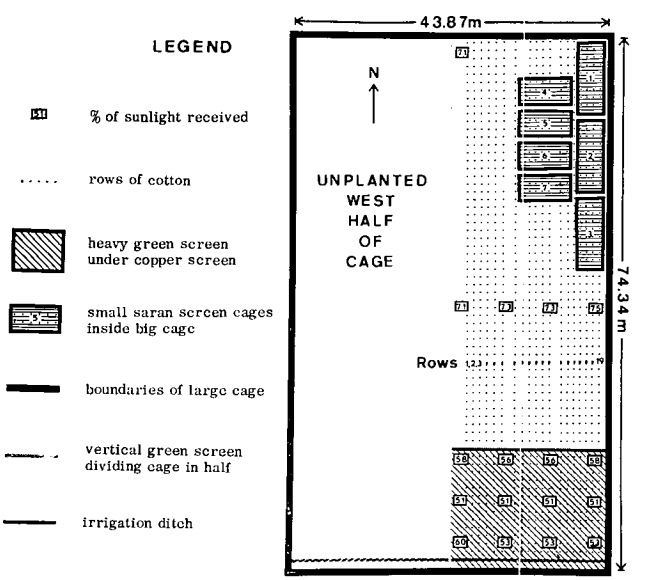


**Fig. 1. Map of cotton planting pattern, shading, and double caging used in 0.327-ha cage, Tucson, Ariz., 1970.**

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plant. The first blooms appeared June 23, and strong colonies of bees were moved into the cage starting June 25, until eventually 12 colonies were placed in the large cage.

Once the blooms appeared, scoring of flowers for sterility began with a system developed by Justus and Leinweber (2) in which 0 = no pollen and 4 = 100% fertility. Between June 23 and July 6, all A-line plants dehiscing pollen and all off-type plants were rogued. In 18 such evaluation 12,343 flowers were scored, and 4.16% were found to be shedding pollen grains. The mean weekly scores for the 7 weeks were 1.59, 1.02, 0.30, 0.44, 0.45, 0.33, and 0.03, respectively. The values for the first 2 weeks include the pollen shedders that were rogued when they were first observed.

Also, we verified whether enough pollen grains were produced to set bolls on the A-lines by selfing done during the 8-week flowering period. A total of 3,488 flowers were selfed (collodian was used to seal the flowers), and 118 (3.38%) of these flowers set bolls. The average number of seeds in the selfed bolls was four. From 1,500 A-line flowers tagged at random throughout the season 489 bolls were set (32.6%) with an average of 17 seeds per boll. Therefore, approximately 2.4% of the seeds produced on the A-line plants resulted from the viable pollen produced due to selfing.

The colonies of bees placed in the cage lost most of their bees within 2 days after they were moved into the cage; these bees flew to the top of the cage and apparently did not return to the hive. On July 14-16 heavy green plastic screen was placed as a second roof below the copper screen over the south 14.6 m (Fig. 1); however, the green screen did not help the problem appreciably. Seven small saran mesh cages were erected inside the large cage from July 19 to 23, and single colonies of bees were placed in each cage. The bees outside the small cages consumed their food sparingly, while those in the small cages consumed their food avidly. Colonies in the small cages remained strong. Any of the 12 colonies in the large cage that weakened were moved out and others were brought in.

The colonies of bees placed in areas covered only by the copper screen visited individual cotton flowers three times more frequently during the last half of the blooming period than during the first half (Fig. 2). Bees under the green screen visited individual flowers 14% more frequently than bees under the copper screen; however, the screen affected the plants adversely. They grew rank and produced only about half as many flowers as the plants in the rest of the cage. Bees confined to the small cages visited the cotton flowers avidly at an average visitation rate of 33.4/100 flowers, or 33.4%.

The average visitation rate for bees under only the copper screen was 8.05% for the 9,271 flowers observed during the blooming period. The peak flowering period was July 16 through August 24.

The readings of temperature and humidity obtained during the test were considered typical of the Arizona environment. The lowest daily maximum temperature was 32 C in June, 29 C in July (2 days only), and 33 C in August. These were almost all higher than the 32-C threshold temperature essential for male sterility in the A-line strain of this cotton (7). Actually, the 32 C is not an exact temperature requirement that must be

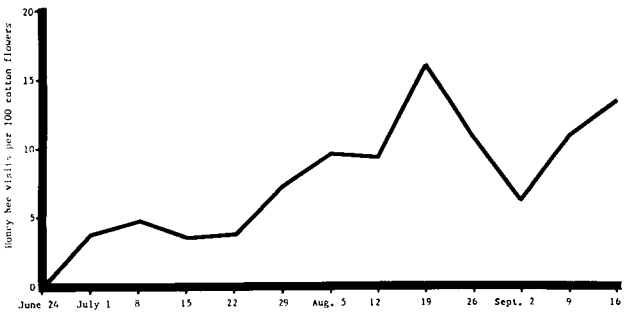


**Fig. 2. Weekly average of honey bee visits to cotton flowers in a large cage, Tucson, Ariz., 1970.**

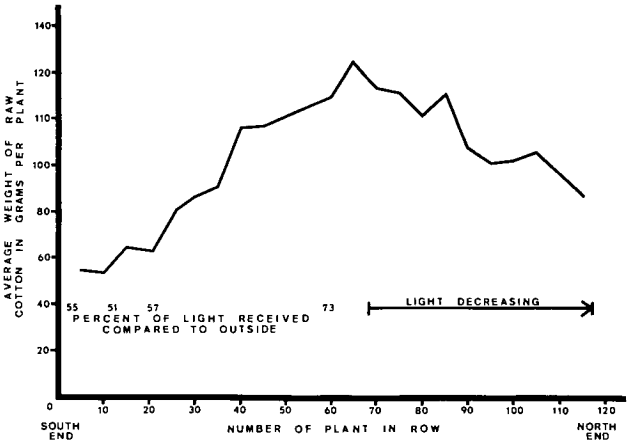


**Fig. 3. Effect of shading on yields of seed cotton in a large copper screen cage, Tucson, Ariz, 1970.**

**Table 2. Yield of A-line cotton plants confined under single copper screen in a large cage in relation to their distances from B-line plants, Tucson, Ariz., 1970.**

| Distances of A-line plants* from B-line plants | Yield/plant,† grams | |
|---|---|---|
| | Seed cotton | Seed |
| Adjacent | 128 | 78 |
| 2nd plant | 129 | 77 |
| 3rd plant | 115 | 69 |
| 4th plant | 114 | 68 |
| 5th plant | 117 | 70 |

* 27 plants for each distance. † Differences in yield were not significant.

attained in all environments. In the Arizona environment of humidity and temperature sterility was evident at 24 C.

No other pollinating insects were observed on the cotton flowers. Although no insecticides were applied, insect damage to the cotton was negligible. Pink bollworms [*Pectinophora gossypiella* (Saunders)], did infect the very late bolls that were too small and too green to harvest, but they appeared too late to damage the other bolls.

When yields were calculated for A-line plants, starting with those adjacent to the B-line plants through those five plants from a B-line plant (Table 2), the immediately adjacent plants were found to average 9% more cotton than plants that were five plants away; however, the variability between plants was high, and the differences were not statistically significant at the 5% level of confidence. The bees flew readily from A- to B-line plants and vice versa.

Under both the heavy green screen and the copper screen, light was reduced 47%, and yields were reduced 50% compared to yields in areas under only the copper screen where the light was reduced 27%. The A-

line plants in cages 4 through 7 produced an average of 67 g of raw cotton/plant, compared with 136 g/plant produced by these plants in cages 1 through 3. Apparently, the extra shading that occurred in cages 4 through 7 was detrimental to the plants. This result is in agreement with that of Knight (5) and others (1 and 8), who reported that partial shading greatly reduced cotton yield.

The 217 B-line plants produced an average of 120 g of seed cotton/plant compared with 100 g produced by 1,584 A-line plants. The seed weighed 71 g/B-line plant and 60 g/A-line plant. The B-line plants averaged 18 seeds/boll; the A-line plants averaged 17 seeds/boll. The percentage seed by weight in the seed cotton was 59% for both the A- and B-lines. If similar yields can be obtained under field conditions, hybrid seed production will be economically feasible. Honey bees can be effective pollinators when present in sufficient numbers.

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