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Performance of Cytoplasmic Male-Sterile Cotton Under Natural Crossing in New Mexico[1]

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ABSTRACT

Cytoplasmic male-sterile (*Gossypium harknessii* Brandg. × *Gossypium hirsutum*[6] L.) lines versus similar male-fertile lines of cotton were evaluated for yield and lint percentage under conditions of natural pollination for 2 years at Las Cruces, N.M. Two locations distinguished by their relative distance from a honeybee (*Apis mellifera* L.) apiary were used each year.

Bee populations appeared to be a major factor in these experiments. Fewer bees were apparently necessary for good pollination of the male-sterile lines than was previously reported. Eight of the twelve comparisons for yield between steriles and fertiles were statistically significant. The male-sterile lines produced less seed cotton and lint each year in the field more distant from the bee hives. Near the apiary, the steriles produced significantly more seed cotton and seed than the fertiles. No significant differences were detected for the interactions of sterility × pollinators or of sterility × nectaries for any of the agronomic traits studied, although there were some indications that there was a partial honey bee preference for nectaried plants.

Breeding programs which include improved cytoplasmic male-sterile strains and adequate populations of honey bees may provide the means for commercial production of hybrids.

Additional index words: *Gossypium hirsutum* L., *Gossypium harknessii* Brandg., *Gossypium barbadense* L., Honeybees, (*Apis mellifera* L.), Nectariless, Yield, Lint percentage.

CYTOPLASMIC male-sterile cottons, synthesized by crossing the genome of *Gossypium hirsutum* L. onto the cytoplasm of *G. harknessii* Brandg., have shown complete sterility at this location. Normal cultivars of cotton serve as nonrestoring B-lines when crossed onto the *G. harknessii*-derived male-sterile. A single dominant gene from the *G. harknessii* genome restored complete fertility in several *hirsutum* genotypes studied in Mississippi (10). With a complete cytoplasmic male-sterile and restorer system, the pathway for the production of commercial hybrid cotton has been opened.

Based on the discovery of excellent combining ability (1), the N. M. Agric. Exp. Stn. began to commit increasingly more resources into hybrid cotton research in 1973. In view of this commitment, it was imperative to determine whether or not there are gross differences in production between male-sterile and normally fertile cottons.

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Despite the development of a male-sterile restorer system, many investigators have had serious doubts as to whether or not sufficient quantities of hybrid seed can be produced on a profitable commercial scale. Those questions have arisen primarily because cross-pollination of cotton is largely due to bees; yet cotton is not the preferred pollen and nectar source of the major bee species. Certain investigators have suggested that bumble bees (*Bombus* sp.) are the most effective pollinators of cotton (17). Bumble bees are only occasional visitors to cotton fields in this area. Other reservations stem from observations of considerable season-to-season and within-season variation in the number of bees within fields (3, 11). Saturation of an area by introducing colonies of honeybees (*Apis mellifera* L.) has been attempted with some degree of success (3, 7, 11, 12). Removal of all nectar sources except those inside the flower has also been suggested (3). The latter can be accomplished by using sterile and pollinator lines that are homozygous for the genes ne_1 and ne_2 (8).

Precise answers to these and other questions concerning cross-pollination in cotton will require many years of study with carefully prepared materials under a wide range of environmental conditions. On the other hand, it is desirable to know whether or not there are gross differences in production between male-sterile and normally fertile cottons before committing resources too heavily to the production of male-sterile and restorer lines that would eventually serve as potential parents for commercial F_1 hybrids.

Previously reported data on production of hybrid seed on male-sterile lines has been conflicting. Weaver (18) has reported that excellent seed set could be obtained on a genetic male-sterile ($ms_{5\text{-}6}$) at a number of locations across the cotton belt. Stith (16) has also reported excellent seed set on various cytoplasmic male-steriles in Arizona. On the other hand, Kohel and Richmond (5) found that while a good seed set could be obtained on male-sterile plants of ms_2, at College Station, Tex. at a site having high natural crossing, that a corresponding site with low natural crossing had very poor seed set. Kohel and Richmond had levels of high and low natural crossing of 48 and 7%, respectively. Natural crossing in the Mesilla valley in New Mexico corresponds fairly closely to their low natural crossing site. Natural crossing at Las Cruces has ranged from 5 to 15% and averaged close to 10% over the years reported by Simpson (14) and in the N. M. Agric. Exp. Stn. Reports (unpublished data collected in 5 of the past 7 years). In view of the conflicting reports as to the probable success of producing hybrid seed at low natural crossing levels, it was decided to undertake some experiments in 1973 to determine seed yield at Las Cruces on the best male-sterile lines available at the time. It was vital to know if male-steriles would perform at approximately equal or significantly lower yield levels than our current strains and varieties. We also wanted to determine if hybrid seed production would be grossly affected by the presence or absence of nectaries in the A and B lines and if the major cultivated species (*G. hirsutum* and *G. barbadense*) would serve equally well as pollen sources.

MATERIALS AND METHODS

This research was conducted in two separate experimental cotton fields, D and N, for 2 consecutive years (1973 and 1974) at Las Cruces, N.M. The fields were characterized by their relative distances from a small commercial honeybee apiary which served as a major source of pollination for crops in the immediate area. Field N (near) was located within 350 m of the apiary, while field D (distant) was about 1,500 m away. The experimental areas were portions of larger fields of cotton, varying from 2.02 to 14.18 ha.

In the 1973 experiments, the checks used were the highest producing cotton types available, but only slightly related to the male sterile lines. In 1974, check strains were used which were more closely related to the male-sterile lines. All the male-steriles used in these experiments were derived by crossing 'Acala 1517' types onto a source of *G. harknessii* × *G. hirsutum*[4] obtained from Vesta Meyer of the Delta Branch Exp. Stn., Stoneville, Miss. in 1970. Plants used in 1973 were a BC_1 generation of Acala nectariless onto the male-sterile source. The nectaried progeny of this backcross was designed as B522-2$^+$ and the nectariless line as B522-2$^-$. The symbol + and − refer respectively to the presence and absence of leaf, bract, and involucral nectaries. The sterile lines used in 1974 were derived by an additional backcross onto B522-2$^-$. One of these, designated c45, was derived by using a nectariless pollinator, the other designated c1-1 was derived by using a nectaried pollinator line. The companion fertile checks were the pollinator lines 45 and 1-1, respectively.

In the D experiments, the responses of nectaried and nectariless lines were measured in both years; and in 1973, both *G. hirsutum* and *G. barbadense* ('Pima S-4') pollinators were used. The experimental design for Field 73-D was a modified split- split-plot consisting of two main plots, nectaried and nectariless; two subplots per main plot, *G. hirsutum* and *G. barbadense*; and four lines, steriles B522$^+$ and B522$^-$ and fertile B8784$^+$ and B8784$^-$, which were randomized within each subplot. Data were collected from only the male-sterile lines and the fertile checks. Experiment 73-D was planted on 9 May 1973. Each plot was a single row 6-1 m long and 0.96 m apart with a 1.5 m alley. Sterile and fertile lines were thinned to an equal stand averaging 46 cm between plants. Plots were harvested in mid-October.

Experiment 73-N measured only the performance of male-sterile vs. male-fertile lines. A paired-plot design was utilized in this experiment with eight replications and two treatments (sterile B522 vs. fertile Acala 1517-70$^+$ and B7862$^-$). The nectaried segregates (B522$^+$) were paired against Acala 1517-70 while nectariless (B522$^-$) was paired against B7862$^-$. Acala 1517-70$^+$ is a high yielding commercial variety and B7862$^-$ a high yielding experimental nectariless Acala line. Each plot was a single row 16.8 m in length and spaced 0.96 m apart. This experiment was planted on 8 May 1973. Within each replication, plot stands were equalized by thinning, maintaining about 15 cm between plants. Plots were harvested in mid-October which was expected to be the termination date for harvest of good planting seed. The second harvest was therefore not deemed worthwhile.

Both experimental areas in 1974 were 17 rows wide. The sterile vs. fertile treatments were allotted to rows 3 and 11 in odd numbered ranges and 7 and 15 in even numbered ranges so that they did not fall back to back. The treatments in the 1974 experiments consisted of one yield row flanked by two pollinator rows on each side. The remaining rows were planted to open-pollinated male-steriles. Stands were thinned to approximately 10 plants/m.

Experiment 74-D was planted on 2 May 1974, in a split-plot design with five replications. Two main plot treatments (nectaried and nectariless) and two subplot treatments (sterile c45 and fertile 45) were used. Plots were single rows 7.3 m long and 0.96 m apart with a 1.8 m alley. All stocks used in this experiment were derived from *G. hirsutum*.

Experiment 74-N was planted on 3 May 1974. The experimental design used was a split-plot with four replications. The two main plot treatments were *G. hirsutum* and *G. barbadense*, which served as pollinator rows for each of the two subplots (sterile c1-1 and fertile 1-1). The presence of ample pollen at all times was assured by using four different strains of both *hirsutum* and *barbadense*. The wide difference in maturity of the pollinators resulted in continual bloom. The rows were 7.9 m in length separated from each other by a distance of 0.96 m. Alleys were 1.2 m wide.

In 1974, the bee population was estimated in both experimental fields by counting bees/100 flowers between 1000 and 1100 hours biweekly during the peak bloom period. Plots were harvested on 20 Oct. and 14 Nov.

RESULTS AND DISCUSSION

In plantings near bee hives sterile cotton lines outproduced fertiles in seed cotton, but the fertiles were superior when the plantings were distant from the hives (Table 1). Differences in seed cotton yields between two of these four treatment comparisons were significant at the 0.05 or higher level of probability. The same trend was evident for seed and lint yield and five of eight comparisons were significant at the same level of probability. Bee population levels may well be the deciding factor in determining whether commercial hybrid seed production is feasible.

Differences in bee populations were noted between the N and D fields when measured in 1974. Number of bees/100 flowers averaged 0.42 for both sterile and fertile lines in field D. Counts actually decreased to zero for a 2-week period in that field. The mean number of bees in Field N was 2.14 for the male-fertiles and 3.43 for the male-sterile plants.

The pattern of fluctuation in bee numbers resembled that reported by Moffett et al. (13) who also found a sharp decrease in mid-season (Aug.). This drop in bee numbers was associated with an observable gap in boll setting on many sterile plants and probably explains why the 1973 data showed the steriles to be

Table 1. Yields and lint percentage between similar male-sterile and male-fertile lines within each experiment.

Exp.	Entries	Seedcotton	Seed	Lint	Lint
		kg/ha			%
73-D	Sterile	968	683	285	29.4
	Fertile	1,041	621	420	40.3*
73-N	Sterile	1,310	913	397	30.3
	Fertile	947	589**	358	37.8**
74-D	Sterile	2,420	1,667	753	31.1
	Fertile	3,182**	2,035**	1,147**	36.1
74-N	Sterile	3,188	2,238	950	29.8
	Fertile	2,690**	1,711**	979	36.4**

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.
† Significance statements refer to paired comparisons of sterile vs. fertile lines within each experiment.

Table 2. Yields, lint percentage produced by nectaried vs. nectariless lines, and the effect of *G. hirsutum* versus *G. barbadense* pollinators.

Exp.	Seedcotton	Seed	Lint	Lint
	kg/ha			%
73-D				
G. hirsutum Poll.	1,044	707	337	32.3
G. barbadense Poll.	965	622	343	35.5
Nectaried	1,154	747	407	35.3
Nectariless	855*†	560*	295	34.5
74-D				
Nectaried	2,935	1,940	995	33.9
Nectariless	2,667	1,779	888	33.3
74-N				
G. hirsutum Poll.	3,221	2,129	1,092	33.9
G. barbadense Poll.	2,657	1,799	858	32.3

* Significant at the 0.05 level of probability. † Significance statements refer to paired comparison within each experiment. ‡ Nectaried vs. nectariless comparisons were made ignoring sterility and pollinator source; *G. hirsutum* vs. *G. barbadense* pollinator comparisons were made ignoring sterility and nectar characters.

10 to 13% higher in shedding, which was a significant difference.

The higher incidence of bees on the male-steriles is not surprising since Moffett (11) has indicated that *G. harknessii*-derived male-sterile lines were preferred by bees over most other genotypes he tested. It is possible that bee preference for the *harknessii* male-sterile could account for the high yield of hybrid seed in spite of the relatively low and fluctuating numbers of pollinators. However it appears that fewer bees than the 10/100 flowers estimated by McGregor (6) will be needed to produce good seed set in cotton when the ratios of male-fertiles to steriles is approximately equal. Natural crossing between male-fertile lines recorded at Las Cruces (unpublished data) was 13 and 12% for 1973 and 1974, respectively.

Lines with *G. harknessii* cytoplasm not carrying fertility restoration genes are inherently lower in lint percentage than similar lines carrying fertility restoration genes (9). In these experiments the male-sterile ranged from 5.0 to 10.9% lower in lint percentage than did the corresponding fertile lines. When the major production objective is high-value planting seed the low lint percentage factor is not nearly as serious as it is in conventional breeding where lint is the major objective. The low lint percentage does not carry over to F_1 progeny when the male-sterile is pollinated by a restorer line.

Differences due to pollinator lines were significant in one experiment. In this experimental design, pollinators were summed over both steriles and fertiles (Table 2). In experiment 73-D, plots pollinated by nectaried lines were significantly higher in yield. However the interaction, sterility × pollinators, was not significant at the 0.05 probability level for any of the characters measured.

Since isogenic lines could not be used in these studies, the significant differences obtained cannot conclusively be attributed to the effects of male-sterile vs. male-fertile lines. However the large magnitude and consistent direction of the trend, plus the fact that several fertile and sterile lines were compared, lead us to accept the result that proximity to bee hives is a significant factor in hybrid seed production.

In interpreting the data it must be kept in mind that the plot units were too small to insure that all pollen carried to the male-sterile rows was of the designated type. However Johanssen (4), Green and Jones (2), and Stephens and Finkner (15) have shown that cross-pollination falls off very rapidly from the point source of pollen collection. The bulk of the pollen reaching the male-sterile plots should have been from the surrounding designated pollinator rows. In 1975 we were able to make a preliminary check on this assumption. Seed harvested from field 74-N (*hirsutum*-pollinated male sterile) and 74-D (nectariless-pollinated male-sterile) were grown out and checked for off-types. It was found that only 9% *barbadense* pollen was carried to the plots designated *hirsutum*-pollinated; while 88% nectaried pollen was carried to the plots designed to be pollinated by nectariless. The nectariless yield plots had been rogued once and were presumed to be reasonably pure types. The difference in within-plot pollination was very highly significant. Such a great difference may indicate that there is a preference for nectaried vs. nectariless plants by honeybees. Yield differences between nectaried and nectariless were significant in 1973; however, these differences were of relatively small magnitude and in these early generation materials, they might have been due to inherent differences in the yield potential of the individual lines. Isogenic lines must be prepared and larger plots must be used to precisely determine whether or not there are nectariless cottons that will be as effective as nectaried cottons in serving as pollen sources for male-sterile lines.

In general, under the conditions prevalent in southwestern New Mexico, a program to produce hybrid cotton seed using cytoplasmic male-sterile lines and natural pollination appears to be economically feasible if adequate numbers of honeybees can be maintained within the seed production fields.

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REFERENCES

1. Davis, D. D. 1974. Synthesis of commercial F_1 hybrids in cotton. I. Genetic control of vegetative and reproductive vigor in *Gossypium hirsutum* L. × *G. barbadense* L. crosses. Crop Sci. 14:745-49.
2. Green, J. M., and M. D. Jones. 1953. Isolation of cotton for seed increase. Agron. J. 45:366-368.
3. Grout, R. A. 1955. Honeybees make hybrid cotton possible. Am. Bee J. 95:10-11.
4. Johansson, T. S. K. 1959. Tracking honeybees in cotton fields with fluorescent pigments. J. Econ. Entomol. 52: 572-577.
5. Kohel, R. J., and T. R. Richmond. 1962. An evaluation of seed yield potential of completely male-sterile cotton in areas of high and low natural cross-pollination. Agron. J. 54:525-528.
6. McGregor, S. E. 1959. Cotton-flower visitation and pollen distribution of honeybees. Science 129:97-98.
7. ———, C. Rhyne, S. Worley, Jr., and F. E. Todd. 1955. The role of honeybees in cotton pollination. Agron. J. 47:23-24.
8. Meyer, J. R., and Vesta G. Meyer. 1961. Origin and inheritance of nectariless cotton. Crop Sci. 1:167-169.
9. Meyer, Vesta G. 1973. A study of reciprocal hybrids between upland cotton (*Gossypium hirsutum* L.) and experimental lines with cytoplasm from seven other species. Crop Sci. 13:439-44.
10. ———. 1975. Male sterility from *Gossypium harknessii*. J. Hered. 66:23-27.
11. Moffett, J. O. 1974. Honeybees and other insect visitors to normal and male sterile cotton. Ph.D. Thesis. Univ. Wyoming, Laramie, Wyo.
12. ———, and L. S. Stith. 1972. Pollination by honeybees of male sterile cotton in cages. Crop Sci. 12:476-478.
13. ———, ———, C. C. Burkhart, and C. W. Shipman. 1975. Honeybee visits to cotton flowers. Environ. Entomol. 4: 203-206.
14. Simpson, D. M. 1954. Natural cross-pollination in cotton. USDA Tech. Bull. 1094.
15. Stephens, S. G., and M. D. Finkner. 1953. Natural crossing in cotton. Econ. Bot. 7:257-269.
16. Stith, L. S. 1974. The potentials for hybrid cotton production. Beltwide Cotton Production Research Conferences. p. 145-146. Natl. Cotton Council. Memphis.
17. Theis, S. A. 1953. Agents concerned with natural crossing of cotton in Oklahoma. Agron. J. 45:481-484.
18. Weaver, J. B. 1971. Methods of utilizing male-sterility in cotton. Beltwide Cotton Production Research Conferences. p. 60-61. Natl. Cotton Council. Memphis.