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An Evaluation of Honey Bee Foraging Activity and Pollination Efficacy for Male-Sterile Cotton[1]

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ABSTRACT

The effectiveness of honey bees, *Apis mellifera* L., as pollinators of male-sterile cotton, *Gossypium hirsutum* L., was evaluated using 649 colonies located adjacent to 53 ha of cotton grown on Pullman clay loam (fine, mixed, thermic Torrertic Paleustoll) near Plainview, TX. The planting pattern used was 2A (male-sterile seed parent): 2B (male-fertile maintainer) rows throughout. Colonies were evaluated for population, brood, and weight changes. Observers recorded bee visits to flowers of both A-line and B-line plants. Flowers were tagged over a 5-week period to determine the percentage of bolls setting and the yield of seed and lint. Most colonies had less than 10 frames of bees and less than 3 frames of brood. Honey bee visits to the A-line flowers varied from 0.4 to 1.7% over a 5- week period of observation. The bee activity on the B-line flowers was much less, with a range of 0.1 to 1.2% visits. The authors concluded that 5 colonies/ha with 28 000 bees per colony would adequately pollinate male-sterile cotton under the conditions of this test.

***Additional index words:* *Apis mellifera, Gossypium hirsutum,* Pollination, Nectar, Pollen.**

SCIENTISTS have discussed the potential advantages of hybrid cotton (*Gossypium* sp.) during the past 90 years (Mell, 1894; Cook, 1909; Kearney, 1924). Few practical advances toward commercial production of hybrid cotton were made before the development of male-sterile (A line) cotton, although India has been growing hybrid cotton on 800 000 ha annually by producing hybrid seed using hand labor for pollination (Srinivasan et al., 1972).

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The genetic-cytoplasmic male-sterility system described by Meyer (1975) is currently being used in hybrid cotton breeding programs. Cotton pollen must be transferred by insects (usually a bee) from the pollen parent to the male-sterile seed parent to produce hybrid seed. Meyer (1969) earlier concluded that one of the main obstacles to practical production of hybrid cotton seed was the use of bees for effective and economic pollination of the A-line parent.

Moffett and coworkers studied both honey bee and wild bee visits to A-, B- (maintainer), and R-line (restorer) genotypes and commercial cultivars of *Gossypium hirsutum* L., in Arizona and Texas (Moffett, 1983). Satisfactory seed yields were obtained in Arizona on A-line plants when the area was saturated with honey bees (Moffett et al., 1978). Wild bee populations fluctuated too much from year-to-year and between fields during a given year to be dependable pollinators (Moffet et al., 1976a, 1980).

Farmers on the Texas High Plains annually plant cotton on a million ha and several seed companies are developing cotton hybrids for this area. Because of the need for more information on the use of bees for pollination, we studied honey bee management for production of cotton seed on male-sterile A- line cotton near Plainview, TX in 1980. The results of this study are presented in this paper.

MATERIALS AND METHODS

A total of 649 honey bee colonies were moved into a 3km^2 area. One 38 ha field in 2 blocks (North Field) had 534 colonies placed on all sides of one block and on three sides of the other. A second nearby field of 15 ha (South Field) had 115 colonies located across a roadway 20 m directly north of the field. The cotton was planted in a 2 A line (genetic-cytoplasmic male sterile):2 B line (fertile maintainer) alternating row pattern on Pullman clay loam (fine, mixed, thermic Torrertic Paleustoll) near Plainview. The colonies were moved into the area in mid-July when both the A and B lines were in early bloom. The Delta-type cotton used in this study has a tendency for the A-line plants to bloom approximately a week earlier than the B-line plants.

Colonies were evaluated at the beginning (mid-July) and near the end of the pollination period (late August) by recording the number of frames of bees, frames of brood and colony weights. One-third of the 649 colonies were examined using a non-random selection method of check two and skip four until data for 216 colonies had been recorded.

Observers determined the foraging activity of honey bees and wild bees during a 5-week period between 20 July and 23 August. This was done by counting flowers with or without a visiting bee while walking through the field as described by McGregor (1959). Bee visitation counts were made only between 1000 and 1700 (CDT) because cotton flowers secreted little or no nectar before 1000 (Waller and Moffett, 1981; Waller et al., 1981) and because the closing of many flowers in late afternoon made counting floral visitors difficult. In the South Field, bee visitation counts were taken on rows running east and west at three distances from the northern boundary of the field where all the colonies for this field were located. Counts were made in a similar manner in the North Field on rows running north and south near the west edge, center, and east edge of the larger portion of this field.

Each day that flower and bee counts were taken, A- and B-line flowers on the rows where the bees were counted were tagged to determine the percentage boll set. In early October the resulting bolls were collected from more than 3000 flowers tagged throughout the summer, the lint and seed weighed, and the seeds counted.

Nectar production was also evaluated on the days that flower and bee counts were taken in each field. Five exposed flowers and five bagged flowers were collected hourly between 1000 and 1700 CDT from each of the two lines. Nectar samples were removed from these flowers and measured using capillary pipets and sugar percentages (total dissolved solids, TDS) were determined by refractometer.

Pollen was collected weekly using modified O.A.C. pollen traps (Waller, 1980) placed on eight colonies widely separated around both fields. The pollen was weighed and 200 pollen pellets were randomly taken from each sample for identification using a light microscope at 400×. A reference pollen collection was made by collecting honey bees on flowers from plants near the cotton fields.

Honey bee visits were examined by analysis of variance using both bees counted per row and bees counted per 100 flowers. However, the bee visitation data presented are primarily descriptive showing hourly, weekly, or spatial trends and not treatment and effect relationships. Seed and lint yields for A-line and B-line plants were compared using Students *t-test.*

RESULTS AND DISCUSSION

Colony Evaluation

At the beginning of the pollination period we recorded means of 6.61 frames of bees per colony and 2.56 frames of brood. Colony populations were probably low because some had been weakened from insecticides applied to crops near their previous locations. The use of 649 honey bee colonies on 53 hectares might have been considered a "saturation" pollination level if these colonies had contained bee populations considered normal for July and August. If we assume 1400 bees per frame (Simpson, 1969) and 28 000 bees per "strong" colony, then the honey bees present in the 649 colonies at the beginning of these studies were equivalent to 214 "strong" colonies. This reduced the effective colony populations to 4.9 and 1.8 "strong" colonies/ha on North and South Fields, respectively.

During the 5-week pollination season, the frames of brood per colony increased slightly (0.71), while the adult bee populations decreased slightly (0.25 frames) and a mean of only 4 kg weight gain occurred. Insecticide treatments on nearby fields of cotton, corn (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], and sunflower (*Helianthus annuus* L.) were the probable cause of these small colonies not performing better during this period.

Table 1. Honey bee visits to A- and B-line cotton flowers in two fields at different times of day, Plainview, TX, 1980.

Starting time (C.D.T.)	A-line		B-line		A line:B line	
	North Field	South Field	North Field	South Field	North Field	South Field
	Bees/100 flowers				ratio	
1000	1.26	0.40	0.46	0.15	3.1	2.7
1100		0.57		0.17		3.4
1300	1.43	0.70	0.34	0.14	4.2	5.0
1400		0.65		0.15		4.3
1500	0.80	0.55	0.24	0.14	3.3	3.9
1600		0.53		0.10		5.3

Bee Foraging Activity

We observed 323 135 flowers in the North Field with 2497 honey bees and 10 wild bees, an average of 0.77% for the season which is well above the 0.50% minimum bee visitation level suggested by Moffett et al. (1976c). In the South Field 195 304 flowers were observed with 854 honey bees and 119 wild bees for an average of 0.50% visitation (0.044% by honey bees, and 0.06% by wild bees). Foraging activity on A-line flowers peaked in the early afternoon between 1300 and 1500, but the visitation to B-line flowers was slightly higher early in the day in the North Field but was relatively uniform throughout the day in the South Field (Table 1). Hourly differences were not statistically significant.

Competition from abundant and highly attractive nontarget pollen and nectar sources within flight range were probably responsible for a seasonal decrease in visits to cotton flowers. Honey bee visitation declined as the season progressed; 50% on the A line and 90% on the B line between the 1st and 5th week of the observation period (Table 2). Honey bees showed a slight preference for the A line at the beginning of the flowering season and this selectivity increased steadily over the 5-week period of observation. The increasing preference for A-line cotton flowers over B line cotton flowers may be because honey bees learn to avoid cotton flowers having pollen. Moffett et al. (1975) showed that the most attractive cotton genotypes were deficient in pollen.

A statistically significant reduction in the level of honey bee activity occurred with increased distance from the colonies in the South Field (Table 3) with

Table 2. Honey bee visits to A- and B-line cotton flowers in two fields over a 5-week period, Plainview, TX, 1980.

	North Field			South Field		
Dates	A line	B line	A line: B line	A line	B line	A line: B line
	Bees/100 flowers		ratio	Bees/100 flowers		ratio
20-26 July	1.70	1.23	1.4	0.93	0.86	1.1
27 July-2 Aug.	1.09	0.47	2.3	0.40†	0.20†	2.1
3-9 Aug.	1.00	0.23	4.3	0.72	0.12	6.0
10-16 Aug.	0.73	0.14	5.2	0.54‡	0.09‡	5.9
17-23 Aug.	0.85	0.10	8.5	0.42	0.10	4.3

† Beekeeper placed supers on his colonies the previous day.
‡ Irrigation prevented bee counts in rows nearest the bee colonies on this date.

Table 3. Honey bee visits to cotton flowers at three distances from honey bee colonies (South Field), Plainview, TX, 1980.

	Rows 18 to 21 (Near Side)		Rows 234 to 237 (Middle)		Rows 406 to 409 (Far Side)	
Dates	A line	B line	A line	B line	A line	B line
	bees/100 flowers					
20 July	1.40	1.07	0.99	1.04	0.63	0.55
31 July†	0.64	0.26	0.40	0.24	0.21	0.11
8 Aug.	0.97	0.17	0.66	0.09	0.49	0.08
14 Aug.‡	–	–	0.59	0.07	0.48	0.13
22 Aug.	0.70	0.17	0.42	0.00	0.19	0.09

† Beekeeper placed honey supers on his colonies the previous day.
‡ Irrigation prevented counting on the rows nearest the colonies on this date.

flowers near the south edge of this field receiving only one-half as many honey bee visits as the flowers near the north edge, and flowers in the middle of the field being visited at intermediate levels. With an effective honey bee population of only 1.8 colonies per hectare on the target field one might expect such a reduction in forager activity over this distance. The drop in production with increased distance from the bees was less than the drop in bee activity (see Vaissiere et al. 1984). This supports the idea that 0.5 bees per 100 A-line flowers is adequate for effective pollen transfer, as only 2 counts were below 0.4 bees per 100 flowers.

Nectar Production

Floral nectar was collected in measurable amounts only after 1100 because of the small volume available previous to this time. Nectar became increasingly abundant as the day progressed and reached a maximum of 5 to 15 μL per flower by 1700 (see Waller and Moffett, 1981). This dearth of floral nectar until mid-morning with increasing amounts available as the day progressed agrees with a similar study done in Arizona by Moffett et al. (1976b).

Mean total dissolved solids (TDS) in the cotton floral nectar samples ranged between 29 and 60% for the various sources (Table 4). The bagged B-line flowers and both the unbagged and bagged A-line flowers tended to have similar TDS levels on any given date. However, the unbagged B-line flowers were 5 to 18% higher in TDS than the average of the other 3 sources. Relatively higher TDS levels in nectar from unbagged B-line flowers were likely due to higher evaporation caused by relatively fewer bees collecting nectar from these unbagged B-line flowers.

Table 4. Mean percent sugar concentration (TDS) of nectar removed on four dates from flowers of A-line and B-line cotton plants, either uncovered and exposed to foraging bees or covered with paper bags to exclude bees, Plainview, TX, 1980.

	B-line		A-line	
Dates†	Unbagged	Bagged	Unbagged	Bagged
27 July-2 Aug.	56.5 ± 3.3	42.9 ± 13.3	40.0 ± 11.5	39.5 ± 8.3
3-9 Aug.	59.8 ± 0.4	37.3 ± 9.3	45.8 ± 2.5	41.5 ± 7.6
10-16 Aug.	36.1 ± 4.6	33.5 ± 3.6	29.0 ± 3.3	31.2 ± 4.1
17-23 Aug.	43.8 ± 7.7	29.1 ± 7.6	35.6 ± 7.5	32.1 ± 5.5

† No nectar was collected during the 1st week of this study.

Table 5. Weekly breakdown of percentage bolls setting, seeds per boll and lint per boll on A-line and B-line cotton, Plainview, TX, 1980.

	North Field Week					South Field Week				
	1	2	3	4	5	1	2	3	4	5
	Boll set (%)									
A line	83	68	–†	33	40	86	55	49	58	43
B line	91	84	–	37	25	–	76	22	44	20
	Seeds/boll (no.)‡									
A line	23	18	–	19	21	20	19	20	11	17
B line	29	23	–	26	23	–	29	26	13	20
	Lint/boll (g)‡									
A line	1.4	1.1	–	0.9	0.7	1.7	1.1	0.9	0.7	0.6
B line	1.9	2.1	–	1.5	0.9	–	1.9	1.6	1.0	1.0

† – indicates no data are available.
‡ For these traits paired *t-test* significant at $P \leq 0.01$ for A vs. B line.

The sugar concentration of the cotton floral nectar was generally above the 30% level that is preferred by honey bees (Waller, 1972).

Pollen Collection

Amounts of pollen collected and weighed weekly varied from 403 to 1316 g per colony per week. Smartweed (*Polygonum* sp.) provided the single largest source of pollen (31.5%). Large amounts of pollen were also collected from 3 cultivated crops: sorghum, sunflower, and pearl millet [*Pennisetum americanum* (L.) Leeke]. During late August, ragweed(*Ambrosia* sp.) became the main source of incoming pollen. Only four cotton pollen pellets were found among more than 10 000 examined. Thus, honey bees obviously preferred pollen from floral sources other than cotton.

Boll Set and Yield

More than 80% of the A-line flowers that were tagged during the first week of our study set bolls, but this percentage dropped to near 40% by the 5th week (Table 5). The B line set a higher percentage of bolls than the A line during early bloom, but only about half as many as the A line in the 5th week. The greater reduction in set by the B line was probably because the B line set relatively more bolls than the A line did earlier. McGregor and Todd (1956), reported that cotton can compensate for a poor early set by setting more bolls later in the season.

The B line produced significantly more seeds per boll ($t = 5.3$, $P \leq 0.01$) and more lint per boll ($t = 6.0$, $P \leq 0.01$) than did the A line, in each of the 8 pairs of data (Table 5). The number of seeds per boll averaged 23.6 vs. 18.5 and the grams of lint per boll averaged 1.5 vs 0.9 for the B line and A lines, respectively. Despite more production per boll by the B line, the seed yields of the A and B lines in the North Field were almost identical, as measured by both plot yields (see Vaissiere et al., 1984) and also from harvest weights reported by the seed company.

CONCLUSIONS

The objective of this study was to demonstrate the effect of a saturation level of honey bee colonies for pollination of male sterile cotton. However, examination of these colonies showed that we actually had the equivalent of about 5 colonies/ha and 2 colonies/ha on the North Field and South Field, respectively. Consequently, the results indicated that the former level of colony rental was fully adequate and that the latter resulted in a somewhat reduced level of honey bee foraging activity. The authors concluded that honey bee visitation level of 0.5 to 1.0% to the male-sterile flowers provided adequate pollen transfer.

The numbers of bees provided for pollination of these commercial plantings, the levels of floral visits recorded, and the yield of seed resulting from this pollinator activity provide encouragement to those actively engaged in developing hybrid cotton. Further work is needed to relate levels of bees present to actual pollen transfer and to establish recommendations for economic use of bees in producing hybrid cotton seed commercially.

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