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Influence of Phenotype, Season, and Time-of-day on Nectar Production in Cotton[1]

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ABSTRACT

Twenty-five cultivars and breeding stocks of *Gossypium hirsutum* L. and two cultivars of *G. barbadense* L. were studied for rates of flowering and diurnal and seasonal patterns of floral nectar production. Nectar secretion began ca. 0800 hours and production increased linearly until the flowers closed at ca. 1700 hours. In mid-season, *G. hirsutum* produced 25 μl nectar/flower for at least 5 weeks. Neither nectar volume nor sugar concentration differed significantly among the 25 entries of *G. hirsutum*. When samples were collected between 1300 and 1700 hours, sugar concentrations were mostly ca. 20%. Flowers of *G. barbadense* produced about three times this amount of nectar with only slightly lower sugar concentrations. Honeybee (*Apis mellifera* L.) visits to both species of cotton were negligible, even though we provided nearly 10 colonies/ha during the flowering season.

Additional index words: *Gossypium hirsutum* L., *Gossypium barbadense* L., *Apis mellifera* L., Floral nectar, Nectar volume, Nectar sugar, Pollination, Pollen.

When cultivars that are normally self-fertile are replaced by male-sterile and restorer lines to produce hybrids, there is often an increased emphasis on and concern about pollination, especially if production of these hybrids requires insects for pollen transfer. Flowers of plants that have lost their dependency on insect pollinators may also have lost their ability to attract them. Cotton (*Gossypium* spp.) may fall into this category.

Vansell (1944a) reported that honeybees (*Apis mellifera* L.) did not collect cotton (*G. hirsutum* L.) pollen and that cotton nectar was low in sucrose. He also reported that when both cotton and alfalfa (*Medicago sativa* L.) were available to honeybees, the bees usually collected alfalfa nectar. Vansell reasoned that bees preferred alfalfa over cotton because cotton nectar contained only 25 to 30% sugar. However, Vansell (1944b) also reported that cotton honey crystallized rapidly because of its high glucose content. Butler et al. (1972) confirmed the high glucose content of both floral and extra-floral nectars from both *G. hirsutum* and *G. barbadense* L. Waller (1972) showed that of the three principal nectar sugars, honeybees were least likely to collect sugar mixtures high in glucose.

Butler et al. (1972) reported that *G. hirsutum* produced floral nectar containing 36.5% total sugars but only 2.2 μl/flower as compared with *G. barbadense* with 13 μl/flower at 21.5% sugar. Unfortunately, Butler et al. (1972) collected cotton nectar mostly between 0700 and 1100 hours. Neither Vansell (1944a, 1944b) nor Butler et al. (1972) appear to have excluded insects from the flower prior to collection of their samples.

By contrast, Moffett et al. (1976) bagged unopened flowers the day before collecting cotton floral nectar samples and reported means of 8.5 and 52 μl floral nectar between 1300 and 1400 hours for *G. hirsutum* and *G. barbadense*, respectively. They also reported that nectar volume increased steadily throughout the day to reach a peak at about 1700 hours. In their study, sugar concentration of nectar was more important than nectar volume in determining floral attractiveness to honeybees; the 12 most attractive genotypes contained a mean of 37.4% sugar compared with a mean of 26.9% sugar in the 20 least attractive genotypes.

Cotton flowers are only marginally attractive to honeybees. An average of less than one bee/100 flowers is commonly reported during August in Arizona, when flowering is maximal (Moffett et al., 1975a). Moffett et al. (1975b) also found that many of the types of cotton that were most attractive to honeybees carried cytoplasm from species other than *G. hirsutum* or *G. barbadense*.

The prospects for producing hybrid seed on male-sterile cotton plants would be improved if visits to the cotton flowers by honeybees could be assured. The major objectives of the study reported in this paper were to measure rate of flower production and diurnal and seasonal fluctuations in floral nectar volume and sugar concentration in 27 very early- to late-maturing cotton cultivars and breeding stocks. An additional objective was to assess honeybee preference among the 27 cultivars and breeding stocks. However, floral visits by honeybees were so few that it was not possible to reach this objective.

MATERIALS AND METHODS

Twenty-seven cotton cultivars and breeding stocks were planted on 19 Apr. 1979 at the Arizona State Univ. Farm, Tempe, in a randomized complete block design with four replications. Each plot had four rows 9 m long spaced 1 m apart. Plants were hand thinned to ca. 30-cm spacing within each row. Twenty-one of the cultivars and breeding stocks used were selected because of their early maturity, including three that lacked extrafloral nectaries: 'Stoneville 701,' 'Stoneville 825,' and 'Stoneville 887' (*G. hirsutum*). Six commercial cultivars of normal maturity were also included: 'Pima S-4' and 'Pima S-5' (*G. barbadense*), and 'Stoneville 213,' 'Stoneville 256,' 'Deltapine 16,' and 'Deltapine 61' (*G. hirsutum*).

Sixty colonies of honeybees were moved near the cotton field

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Table 1. Daily rate of flowering for five categories of cotton cultivars and breeding stocks at Tempe, Ariz. 1979.

Entry	Flowers/day/100 plants Week 1	2	3	4	5	6	7	9	10	Mean
Pima S-4 (*G. barbadense*)	0.6	3.6	22.2	32.2	72.4	37.9	49.0	31.4	102.0	35.1
Pima S-5 (*G. barbadense*)	7.3	21.1	42.6	36.1	91.7	51.5	34.9	33.1	86.9	40.5
Regular maturity (4 *G. hirsutum*)	12.0	22.7	46.6	146.0	151.0	110.0	93.2	32.2	6.8	62.1
Early maturity (16 *G. hirsutum*)	19.5	29.5	58.0	145.0	144.0	108.0	83.5	15.9	5.0	60.8
Very early maturity (5 *G. hirsutum*)	26.9	41.0	73.9	168.0	134.0	79.0	64.6	2.1	2.6	59.2

(6.1 ha) and placed under Saran® [4] screen shades (94% light removed) to reduce heat stress. Colonies were delivered to the field on three dates as follows: 26 June, 10 colonies; 28 June, 20 colonies; and 10 July, 30 colonies. Supers of drawn comb were placed on each colony to provide storage space for honey, and combs of honey were removed, extracted, and returned before crowding of the brood nest could occur. Two colonies were fitted with modified O.A.C. pollen traps (Waller, 1980) to provide samples of bee-collected pollen for analysis. Pollen traps were emptied each week, and samples of 200 pollen pellets were identified with a light microscope at a magnification of 400×.

Flowers on all 108 experimental units were counted from late June until the end of August. Early season counts included all flowers seen on every plant, but during August only the flowers on the two center rows of each plot were counted. Every flower counted was simultaneously checked for the presence of a honeybee.

Nectar was collected by removal of a flower from a plant and insertion of a disposable glass micropipet (5 or 20 μl capacity) between the calyx and corolla. Dragging the pipet around the flower several times, alternately stopping when the pipet was filling and moving again when the filling action ceased, removed most of the nectar. The pipet was moved gently to avoid injury to the flower, which might have caused cell sap to be released, and the petals were left intact to prevent the release of cell sap at the point of petal attachment. A 5-μl aliquot of nectar from each flower was sealed in the pipet with Critoseal® [4] and held on ice until the following day when the pipet was broken and the nectar sugar (actually total dissolved solids) was determined with a hand refractometer.

We first analyzed volume and sugar concentration of nectar collected throughout 1 day from flowers exposed to normal bee visitation and from flowers covered with paper bags since early morning (0600 to 0800 hours). These comparisons were made on a commercial cultivar, Deltapine 61.

Weekly nectar collections from each of the 27 cottons (ca. four bagged flowers from each) provided information about changes in nectar volume and sugar concentration over the period of bloom. A total of eight dates spanned a time period from late June to mid-September.

We then calculated total daily nectar production as follows: n = pfv where n = nectar (μl/ha), p = plants/ha (23,156), f = flowers/plant, and v = nectar volume/flower. Similarly, we calculated sugar production: s = ncd where s = sugar (g/ha), n = nectar (ml/ha), c = sugar concentration and d = density of this nectar (g/ml).

RESULTS

The mean density of the 9,728 plants on the 108 plots was 23,156 plants/ha. *Gossypium hirsutum* bloomed earlier and more profusely than *G. barbadense* (Table 1). Peak bloom occurred during the latter part of July for all 25 *G. hirsutum* cottons. Those entries of *G. hirsutum* previously classified as regular, early, or very early in maturity tended to bloom as expected. In *G. barbadense* two flowering peaks occurred, one in late July and one in late August.

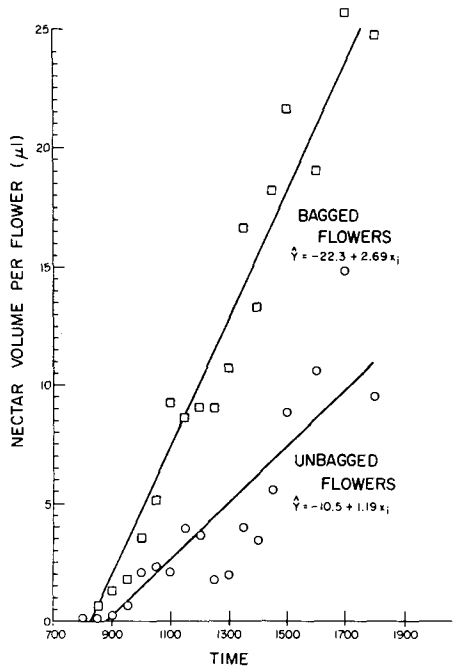

Fig. 1. Comparison of cotton (*G. hirsutum*) nectar volume for samples collected throughout 1 day from flowers covered by paper bags and flowers left uncovered.

No honeybees were seen visiting flowers on the date we took our first 10 colonies to the field (26 June). During the 1st week of data collection, with 30 bee colonies placed near the field, we noted a burst of bee activity (3.2 bees/100 flower) in the cotton flowers. Subsequent counts during July and August (with 60 colonies present) showed few bees visiting cotton flowers. Thus, these data are not presented, and no attempt has been made to compare honeybee visits to different cotton types. One surprising observation was that on 18 July, most honeybees observed visiting the cotton flowers were gathering cotton pollen. This observation was also supported by the analysis of pollen collected from traps on two colonies; one had 18% cotton pollen during Week 3 and another had 44% cotton pollen during Week 6.

Nectar first became collectible in the flowers of Deltapine 61 at 0800 hours, and the amounts collected increased significantly faster in bagged flowers than in unbagged flowers ($t = 8.9^{**}$, 185 df) (Fig. 1). Mean nectar volumes from both bagged and unbagged flowers showed a leveling off between 1100 and 1300 hours followed by a sharp increase. Nectar volume correlated

[4] This paper reports the results of research only. Mention of a commercial product in this paper does not constitute an endorsement of this product by the USDA.

Table 2. Mean nectar volumes and sugar concentrations from cotton flowers sampled over the duration of bloom at Tempe, Ariz. 1979.

		G. hirsutum						*G. barbadense*					
		Nectar volume			Sugar conc.			Nectar volume			Sugar conc.		
Week	Date	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE
			µl			%			µl			%	
1	29 June	48	5.3	0.9	45	42.6	1.8						
2	03 July	98	5.2	0.5	75	35.2	1.3	6	16.1	4.2	6	23.9	2.5
3	11 July	100	12.4	0.7	99	23.1	0.8	7	36.5	9.2	7	18.6	0.9
5	27 July	95	27.3	1.3	96	20.2	0.4	7	61.7	9.0	7	18.3	0.9
6	01 August	99	26.8	0.9	99	18.9	0.2	8	74.7	5.1	8	18.6	0.4
7	07 August	96	25.0	0.9	97	21.4	0.3	8	104.1	4.7	8	19.1	0.4
9	22 August	111	25.3	0.9	111	20.8	0.3	11	68.8	5.1	11	22.1	0.6
13	17 September	41	12.7	1.2	41	31.3	1.5						

Table 3. Volume and sugar concentration in cotton floral nectar lected from flowers of 25 cultivars and breeding stocks of *G. hirsutum*, Tempe, Ariz. 1979.

		Nectar volume			Sugar conc.		
Entry	Maturity†	Rank	Mean‡	Range	Rank	Mean	Range
			µl			%	
Acala SS-126	V	1	33.8	23-48	20	19.4	17-22
AET BR 2 8	E	2	33.6	18-52	16	19.6	17-25
DPL 70	E	3.5	30.9	18-41	6	20.9	19-26
DES 4-44	E	3.5	30.9	20-41	24	18.4	16-25
DES 56	E	5	30.7	12-46	18.5	19.4	17-22
AET BR 2 7	E	6	29.9	14-85	17	19.5	16-24
7203 14 104	E	7	29.4	18-47	15	19.7	18-24
DPL 61	R	8	28.9	15-40	2	22.6	16-31
GH8 10 75	V	9	28.7	17-39	21	19.2	16-21
ST 887	E	10	27.4	15-40	13.5	19.8	16-24
ST 256	R	11	27.1	14-43	11.5	20.2	17-24
7209 30 104	E	12	27.0	17-52	18.5	19.4	16-22
DPL 16	R	13	26.1	10-42	13.5	19.8	17-26
ST 506	E	14	25.3	12-46	23	18.8	14-23
Acala SS 95	V	15	24.8	8-51	25	17.9	14-23
DES 4-22	E	16.5	24.1	15-31	10	20.4	15-24
ST 213	R	16.5	24.1	13-43	3	22.1	17-31
AET BR 2 1	E	18	23.1	14-37	4	21.7	19-27
ST 701	E	19.5	23.0	11-38	11.5	21.2	18-24
DES 24	E	19.5	23.0	12-46	22	19.0	15-21
ST 825	E	21	22.9	12-40	7.5	20.8	15-27
AET 5	E	22	21.9	6-38	5	21.1	16-27
CAMD E	V	23	21.5	10-49	7.5	20.8	16-30
DES 4-30	E	24	21.2	11-33	9	20.7	19-26
D3 75	V	25	16.1	7-29	1	24.2	18-31
Mean			26.2			20.2	

† Maturity rating based on percentage of seed cotton harvested at first of several picks (Wilson and George, 1980); R = regular, E = early, V = very early.

‡ Means based on ca. 12 flowers.

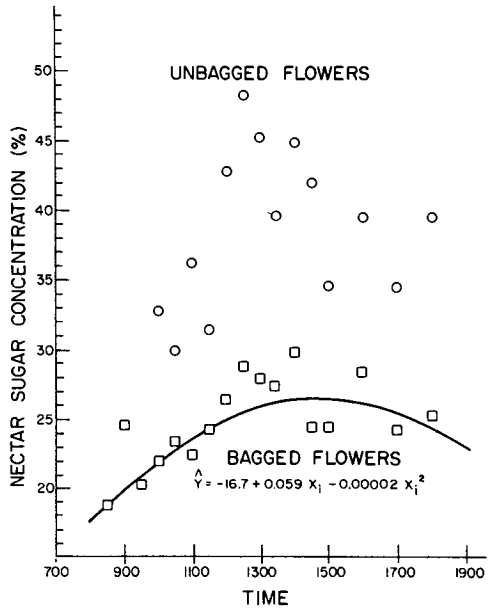

Fig. 2. Comparison of cotton (*G. hirsutum*) nectar sugar concentration for samples collected throughout 1 day from flowers covered by paper bags and flowers left uncovered.

better with time of day (93 df) for bagged flowers (r^2 = 0.83) than for unbagged flowers (r^2 = 0.56). The greater variability in nectar volume from unbagged flowers probably resulted from sampling of some flowers from which bees had removed nectar and others that had not been visited by bees. The significantly lower nectar accumulation in unbagged flowers was probably caused both by nectar removal by bees and evaporation of water. Sugar concentration was highest at midday in both bagged and unbagged flowers, probably because of evaporation and a reduced level of nectar secretion (Fig. 2). The data for sugar concentration in nectar from bagged flowers had a significant quadratic relationship with time of collection, but that from unbagged flowers was nonsignificant in tests of linearity and higher order tests.

Nectar volumes for *G. hirsutum* increased from about 5 to 25 µl/flower during the 1st month of bloom and then remained at this latter level for at least 5 weeks. *Gossypium barbadense* produced about three times more nectar than did *G. hirsutum* (Table 2).

Nectar sugar concentration for *G. hirsutum* started at more than 40% in late June but decreased to ca. 20% during peak production. Nectar sugar concentration of *G. barbadense* was generally somewhat lower and less variable than that of *G. hirsutum* (Table 2).

We compared the data for three dates (Weeks 5, 6, and 7) for 25 cultivars and breeding stocks of *G. hirsutum* (Table 3). These dates were selected because they represented a period of nearly uniform nectar production (Table 2), and all cottons had reached a peak in flowering (Table 1).

The mean nectar volume of the 25 cottons varied from 33.8 to 16.1 µl/flower; however, the range was large within many of these cottons (Table 3). Mean sugar concentrations varied from 24.2 to 17.9%. Spearman's coefficient of rank correlation for nectar volume vs. sugar concentration (Steel and Torrie, 1960) was $r_s = -0.364$ (probability 0.05 to 0.10). No significant differences among *G. hirsutum* entries for either nectar volume or sugar concentration were detected by the analysis of variance. For these dates, nectar volume increased con-

Table 4. Mean daily production of floral nectar and sugar by *G. hirsutum* and *G. barbadense*, Tempe, Ariz. 1979.

Week	Date	*G. hirsutum*		*G. barbadense*	
		Nectar	Sugar	Nectar	Sugar
		ml/ha	g/ha	ml/ha	g/ha
1	29 June	24	12	-	-
2	3 July	37	15	46	12
3	11 July	169	43	274	55
5	27 July	907	198	1,172	230
6	1 August	642	131	773	154
7	8 August	475	111	1,011	209
9	22 August	96	22	514	124

siderably and sugar concentration decreased slightly for the late afternoon (1500 to 1700 hours) samples compared with the early afternoon (1300 to 1500 hours) samples.

Our calculations of nectar production indicated a maximum of about 1 liter/ha per day on 27 July (Table 4). This result agrees with Butler et al. (1972), who estimated 0.6 to 3.8 liter/ha per day, including extrafloral nectar, from a population of 50,000 plants/ha. Our data indicated production of sugar in amounts less than 200 g/ha per day from cotton floral nectar.

Gossypium hirsutum and *G. barbadense* differed in productivity of nectar, but not to the extent stated by Vansell (1944b), that "1 acre of Pima cotton is equivalent to at least 30 acres of Acala." If one uses total sugar production and assumes 17.5% moisture for honey, the yield of floral honey for *G. hirsutum* was 28.6 kg/ha (25.6 lbs/acre) and for *G. barbadense* was 43.6 kg/ha (38.9 lbs/acre). These figures are for a plant population of 23,156/ha over a 9-week period.

DISCUSSION

The extremely low honeybee activity on cotton flowers and the variability in nectar volumes were noteworthy. The short period of bee activity following the introduction of colonies to the area may have been because the bees were first attracted to the flowers and then discovered the extrafloral nectar sources later. Bees probably are not attracted to extrafloral nectaries in the same way that they are attracted to flowers since they lack the colors and aromas that are normally associated with flowers. However, once the extra floral nectaries were "discovered" by the bees they learned rather quickly to find these and began visiting them with considerable efficiency.

The observation that honeybees visited cotton flowers for pollen was quite unexpected, because cotton pollen has been generally considered to be unacceptable to honeybees. The relatively high levels of cotton pollen removed from incoming foragers by the pollen trap supports the field observations. We know of no published data on pollen trapped from colonies located in areas where cotton is the principal crop.

The rather uniform increase in nectar volume between 0800 and 1700 hours agrees with earlier work reported by Moffett et al. (1976). However, the collection of 26.2 and 80.1 μl/flower from *G. hirsutum* and *G. barbadense*, respectively, indicates much greater nectar production than had been reported previously for these species. Means of hourly collections from Deltapine 61 cotton were 10.7 μl/flower at 1300 hours but 25.7 μl/flower at 1700 hours (Fig. 1), so our later collection times at least partially explain the greater quantities of nectar that we found. Moffett et al. (1976) reported that Stoneville 213 reached a maximum production of ca. 16 μl at 1700 hours, whereas we collected a mean of 24.1 μl/flower from this cultivar, 17.0 μl between 1300 and 1500 hours and 34.1 μl between 1500 and 1700 hours.

The extreme fluctuation in nectar volume within most cottons studied appear to support the concept that flowers run a "lottery" for the bees (Heinrich, 1979). Any flower that consistently provides nectar loads of the maximum volume reported here would likely cause bees to visit few flowers during a given foraging bout. However, the gradually increasing availability of nectar throughout the day would tend to reduce this effect. The variability that we observed will make it difficult to discover the cultivars and breeding stocks that are consistently better than others. Factors that contributed to the experimental error included date of collection, time of collection, and operator efficiency. What is needed is a method for collecting many flowers at the same time on the same date and storing them so that one person will remove all the nectar. Under those conditions differences between phenotypes may become significant.

The higher sugar percentages in unbagged vs. bagged flowers during the early afternoon probably resulted from increased evaporation during the time of highest temperatures. The paper bag probably caused an artificially humid environment around the bagged flowers that reduced nectar sugar concentration. Thus, low sugar concentrations reported previously for cotton may be an artifact of bagging. Unbagged flowers in this study had sugar concentrations of 30 to 49%. This is the range most suited to the "tastes" of foraging honeybees (Waller, 1972).

Our inability to provide adequate numbers of pollinators by providing 60 colonies for 6.1 ha is a cause for concern. Attractiveness of cotton flowers to honeybees needs to be improved if pollination problems on hybrid cotton are to be avoided. Increasing nectar volume or sugar concentration may not be the answer, since both appear adequate. A study of the quality of cotton floral nectar and pollen might reveal some characteristics that cause negative responses by honeybee foragers.

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