Published November, 1980

Effect of 2,4-D on Cotton Yield, Floral Nectar, Seed Germination, and Honeybee Visits

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ABSTRACT

In Arizona honey bees, *Apis mellifera* L., frequently visit cotton flowers, *Gossypium hirsutum* L., in sufficient numbers to adequately pollinate the male sterile flowers and produce hybrid cotton seed. Yet in some other states and at times in Arizona, honey bee visits to cotton flowers are low or erratic. Since hybrid cotton seed may be in demand in the near future, this study was made to determine how spraying 2,4-D [(2,4-dichlorophenoxy) acetic acid] on cotton plants affected honey bee visits, seed yields, and nectar secretion.

Small concentrations of 2,4-D were sprayed on five cotton cultivars at three locations in Arizona from 1975 to 1977. Each year, 1 ppm applied in 187 liters of water/ha at the beginning of bloom increased the volume of floral nectar more than 30%. An application of 10 ppm was harmful to the plants. Application of 2,4-D when plants started to flower increased the amount of nectar more than application 3 weeks earlier or application at both times. One ppm of 2,4-D had no significant effect on cotton yields or sugar concentration of the floral nectar. This dosage also had no significant effect on honey bee visits during the only year, 1977, that visits were counted. However, this year the visits were relatively high in both the check and unsprayed plots. Results might be different when the bees are not visiting the flowers well. None of the levels of 2,4-D applied (0.01, 0.1, 1.0, and 10.0 ppm) affected germination or weight of the seeds produced or caused damage to seedlings grown from the sprayed plants.

Additional index words: Apis mellifera L., *Gossypium hirsutum* L., Hybrid cotton, Pollination, Nectar secretion.

THE finding and development of a usable cytoplasmic male sterile cotton, *Gossypium hirsutum* L., by Meyer (1973) has greatly increased the probability of producing hybrid cotton commercially in the United States, practically, as plant breeders solve the fertility restorer problem. However, one of the problems remaining is having a consistently reliable insect pollinator. The most promising insect vector, honey bees, *Apis mellifera* L., has given variable results. For example, in 1977, in large-scale field studies in central Arizona (Moffett et al., 1978), honey bees visited and pollinated male sterile cotton flowers consistently and adequately until insecticides were applied. Yet in other years and in other fields, honey bee visits to such flowers have ranged from almost none to numerous (Moffett et al., 1975).

Moffett et al. (1976), in an investigation of the problem, found that honey bees preferred to visit cotton genotypes with a greater volume of floral nectar and higher sugar concentrations in the nectar. In fact, nectar sources with the highest concentration of sugar are usually preferred by bees (Vansell 1942, Vansell and Todd, 1946, and Jamieson and Austin, 1958). Unfortunately, the average sugar concentration (26%) of the floral nectar of most commercial cultivars of cotton is lower than that normally preferred by honey bees (30 to 50%) (Waller, 1972). Thus, increasing the sugar concentration of the nectar could make cot-

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ton flowers more consistently attractive to honey bees. Since previous studies (Feltner and Sackett, 1964) have shown that applications of phenoxy herbicides sometimes increase the sugar concentration and volume of nectar of certain plants, it was possible that they might do this for cotton plants, thereby helping solve the problem. However, King (1961) found that large doses of 2,4-D [(2,4-dichlorophenoxy) acetic acid] drastically reduced nectar secretion in poinsettia, *Euphorbia pulcherrima* Willd., although low concentrations seemed to stimulate it. King also found that 3 ppm of 2,4-D stimulated growth of some plants, but 100 ppm killed many other species. Likewise, Massengale et al. (1968) did not obtain significant differences in either sugar concentration or volume of nectar in alfalfa, *Medicago sativa* L., flowers after spraying the plants with low doses of 2,4,5-T [(2,4,5-trichlorophenoxy) acetic acid] in Arizona though Feltner and Sackett (1964) did when they sprayed alfalfa in Wyoming. Also, Staten (1946) reported extreme sensitivity of cotton to 2,4-D; and McIlrath and Ergle (1953a) found that seedling cotton is more sensitive to minute quantities of 2,4-D than older plants, though effect was dependent on the amount of herbicide applied. Therefore in 1975, 1976, and 1977 we examined the effect of 2,4-D on both the amount and sugar concentration of the nectar produced by cotton flowers. Seed germination tests were made in 1975, yields studies in both 1976 and 1977, and floral visits by honey bees in 1977.

METHODS AND MATERIALS

In all 3 years, the amine form of 2,4-D was applied to the test plants in 187 liter/ha of water by walking through the plots with a three-nozzle hand boom that was a meter wide. The two outside nozzles of the boom were open, and the middle nozzle was closed.

Also, in all 3 years, all nectar samples were taken from cotton flowers that had been bagged the previous day in the late bud stage. These flowers had opened inside the bag when the nectar samples were taken. The nectar was always drawn from the nectaries (by the capillary action of 1.0 μl micropipettes) after 1300 hours Mountain Standard Time because the amount produced by cotton flowers increases almost linearly from a low point starting at 0900 hours and peaking about 1600 hours (Moffett et al. 1976). Sugar concentration of the nectar was measured with a hand refractometer and reported as percent sugar.

1975 Test. The 1975 test was made with plants grown under irrigation on the Univ. of Arizona Agricultural Experimental Farm at Marana. The two cultivars studied were 'Deltapine 16' and an A-line 'Stoneville 213.' Stith developed the latter genotype from male-sterile M-8 stock using *Gossypium harknessii* Brandegee cytoplasm released by Meyer (1973).

Plots of each cultivar were replicated four times. They consisted of two double rows 200 m long divided into five 30-m subplots in the northern 150 m of the rows. On 23 June, or on 14 July, or on both dates, each subplot except the checks in each plot was sprayed with one of four concentrations (0.01, 0.1, 1.0, or 10 ppm) of 2,4-D. Then, usually, two nectar samples per subplot were taken on each of eight dates (15, 22, 29 July, 5, 22 Aug., 9, 23 Sept., and 21 Oct). Only 768 samples could be taken since flowers were not always open in every plot on each sampling day.

In a special sampling, from 30 July through 1 Aug., an additional 264 samples of nectar were taken from the four subplots that had been sprayed with 1 ppm 2,4-D and from the four corresponding unsprayed (check) subplots.

On 17 November, five open bolls of cotton selected at random from each subplot were harvested. Seeds were separated from the lint by hand and weighed. These seeds were planted in vermiculite, and germination was recorded. The seedlings were observed for symptoms of injury resulting from the application of 2,4-D.

Table 1. Average influence of applying four concentrations of 2,4-D spray to two cotton cultivars at prebloom, bloom, or at both times on the amount of floral nectar produced. Marana, AZ. 1975.

Date of application	Floral nectar/flower after indicated dose (ppm) of 2,4-D†					Mean nectar/ flower all treatments	Avg sugar in nectar‡
	Check	0.01	0.1	1.0	10.0		
	μl						%
23 June	8.0	9.1	8.4	11.6	9.8	9.7*	26.9
14 July	7.2	10.4	9.0	15.2	9.0	10.9*	27.5
Both	9.4	9.6	9.7	11.8	9.6	10.2	27.8
Average§	8.2[a]	9.7[a]	9.0[a]	12.9[b]	9.5[a]	10.3*	27.4

* Significantly different from check at the 0.05 level of significance.
† Excluding control. ‡ All treatments. § Means followed by same letter do not differ significantly at the 5% level of significance (Duncan's New Multiple Range Test).

1976 Test. The 1976 test was made with 'Deltapine 61' grown in an irrigated commercial cotton field near Sacaton, AZ. Test plots were 30 m long and two 1.015 m rows wide. They were located in the middle of a large cotton yield planted with alternating 48-row blocks of Deltapine 61 and 'Pima S-5.' There were 10 test plots of Deltapine 61 and 10 matching unsprayed check plots. The 2,4-D spray was applied at a rate of 1 ppm on 24 June when the cotton was just starting to bloom. At maturity, the cotton from each of the 20 plots was hand picked and weighed.

Twenty nectar samples (two/plot) were taken from both the sprayed and the check plots on each of 11 dates (weekly from 1 July to 25 Aug. and then again on 16 and 30 Sept.).

1977 Test. The 1977 test was made at Aguila, AZ with irrigated A-line Stoneville 213 and B-line Stoneville 213 in a 30 ha field that a large farming corporation used to produce hybrid cotton seed. Flowering was late due to cool weather and a late planting. In early July 400 colonies of honey bees were moved within 100 m of the field. At the same time another 100 colonies were put close to a nearby 7 ha field planted alternately with A- and R-line cotton. Thus, about 300 ha of cotton were blooming within 2 km of these 500 colonies.

The plots of A-line cotton in the test field consisted of two rows 400 m long with skip rows on both sides of the two rows. The B-line blocks were similar except that they were each four rows wide. (This two-skip-four-skip planting pattern was normally used on this farm.) On 26 July, at the beginning of flowering, 1 ppm 2,4-D was sprayed on six A-line plots and on six B-line plots. Six similar plots of each cultivar were unsprayed.

Twenty nectar samples were taken from the sprayed B-line plots on both 9 and 23 Aug., but lack of bloom prevented later sampling of this cotton. Similar samples were taken from the A-line flowers on 2, 16, and 30 Aug. and on 7 Sept. An equal number of samples were taken from the check flowers. The cotton from the plots was machine-harvested and weighed.

In 1977, the attractiveness of the cotton flowers to honey bees was determined once each week by walking slowly through the field and counting the honey bees visiting open cotton flowers (McGregor, 1959). Only bees visiting inside the flowers were counted. Few honey bees were observed visiting leaf or extra-floral nectaries of cotton. This is in direct contrast to some areas like the High Plains of Texas. These honey bees frequently visit extra-floral and leaf nectaries in large numbers and sometimes almost completely ignore the floral nectaries.

RESULTS

1975 Test. Spraying with 1 ppm at the beginning of bloom increased the average volume of floral nectar (Table 1) significantly over that of the check flowers. Spraying as flowering began also produced more nectar per flower (10.9 μl) than spraying 3 weeks earlier (9.7 μl) or spraying on both dates (10.2 μl).

The increase in floral nectar continued throughout the flowering season in the cotton plants sprayed with

Table 2. Effect of applying four concentrations of 2,4-D spray on the amount of floral nectar produced by cotton plants. Marana, AZ. 1975.†

Treatments: ppm 2,4-D	Floral nectar/flower							Avg sugar in nectar	Avg wt/ seed‡	Germination‡
	7/15	7/22	7/29	8/5	8/22	9/9	Mean*			
	μl							%	mg	%
0.0	6.7	6.3	10.8	8.1	10.7	6.6	8.2[a]	27.7	111	73
0.01	6.4	7.9	11.1	10.9	10.6	8.9	9.3[a]	26.6	117	77
0.1	6.6	6.7	12.7	7.1	11.8	7.9	8.8[a]	28.5	109	69
1.0	6.8	8.8	14.8	14.4	18.3	10.2	12.2[b]	26.8	114	75
10.0	8.5	8.4	11.7	8.8	11.0	8.0	9.4[a]	27.3	114	71
Average	7.0	7.6	12.2	9.9	12.5	8.3	9.6	27.4	113	73

* Means followed by the same letter do not differ significantly at the 0.01 level of significance (Duncan's New Multiple Range Test).
† The data are polled for the 3 applications. ‡ No significant differences.

Table 3. Effects of spraying Deltapine 61 at beginning of bloom with 1 ppm 2,4-D. Sacaton, AZ. 1976.

Treatment	Total yield of seed cotton		Volume of nectar		Sugar in nectar	
	10 fields	Difference	Flower	Difference	Concentration	Difference
	kg/ha	%	μl		%	
Unsprayed	2,314		8.3		27.2	
Sprayed	2,344	+1.3	10.8**	+30.8	26.5	−2.5

** Significantly different from unsprayed flowers at the 0.01 level of significance.

Table 4. Effect of spraying A- and B-line 'Stoneville 213' cotton plants at beginning of bloom with 1 ppm 2,4-D. Aquila, AZ. 1977.

Cotton genotype and treatment	Honey bee visits/flower observed†	Nectar volume/ flower	Sugar concentration	Yield seed cotton
	%	μl	%	kg/ha
A-Line				
Unsprayed	4.58	12.6	30.4	2,172
Sprayed	4.34	16.3**	29.3	2,216
B-Line				
Unsprayed	3.48	10.8	27.6	3,627
Sprayed	3.58	14.8**	27.7	3,510

** Significantly different from unsprayed flowers at the 1% level of significance. † Data for first 5 weeks of bloom. Insecticide applications after that date reduced bee visits to a low level.

1 ppm 2,4-D. However, only the nectar samples taken between 15 July and 9 Sept. were used in evaluating the effects of treatments in 1975 (Table 2) because the volume secreted by the cotton plants in late Sept. and Oct. was small and erratic (1.9 and 0.4 μl). In addition, the data on nectar secretion obtained for the two genotypes are pooled in Tables 1 and 2 because the two genotypes reacted similarly. For example, the 240 A-line Stoneville 213 flowers from the unsprayed plots averaged 7.79 μl of nectar compared with 8.56 μl for the 240 unsprayed Deltapine 16 flowers. The 480 A-line Stoneville 213 flowers from the sprayed plots averaged 9.43 μl/flower compared to 10.42 μl/flower for the Deltapine 16 A-line. In contrast, during the special sampling 30 July to 1 Aug. (data not shown), the 66 A-line Stoneville 213 flowers produced more nectar per flower than the Deltapine flowers in both the check plots (8.8 vs. 7.7 μl) and in the plots sprayed with 1 ppm 2,4-D (11.6 vs. 11.4 μl).

The results of the tests (Table 2) indicated that 1 ppm 2,4-D (0.2 g/ha) increased nectar secretion significantly compared with the control; the other doses did not. (The differences were apparent through the flowering season, even in late Sept. and Oct.)

During the special sampling 30 July to 1 Aug., the flowers from plants sprayed with 1 ppm 2,4-D on 14 July yielded more nectar per flower than flowers from plots sprayed 23 June (13.0 vs. 10.4 μl) or flowers from plots sprayed twice with 1 ppm 2,4-D on both dates (13.0 vs. 11.0 μl). Thus, the 198 flowers from plants from all plots sprayed with 1 ppm 2,4-D yielded 37% more nectar per flower than flowers from unsprayed plants.

There was a slight nonsignificant decrease in sugar concentration in the floral nectar (Table 2) of sprayed plants compared with unsprayed plants (27.3 vs. 27.7%). Plants sprayed with 10 ppm 2,4-D suffered severe damage to the leaves and growth that persisted throughout the season. However, none of the four concentrations affected the germination or weight of the seeds produced by the sprayed plants. In addition, seedlings grown from seeds of plants treated with these four concentrations showed no observable damage. Fibers from plants treated with 10 ppm 2,4-D were more easily separated from the seeds than fibers from plants treated with the lower concentrations or untreated.

1976 and 1977 Tests. Again in both 1976 and 1977, the volume of nectar increased in plants treated with 1 ppm 2,4-D, and this increase occurred as long as samples were taken (30 Sept. in 1976 and 7 Sept. in 1977). It averaged 31% higher than the controls (Tables 3 and 4). However, the data in Table 4 on the B-line reflect only the first 5 weeks of bloom since applications of insecticide in the test field so reduced the visits of honey bees that interactions were invalid thereafter. The persistent effect of 2,4-D on the amount of nectar secreted confirms reports by McIlrath and Ergle (1953b) concerning the long lasting effect of 2,4-D on cotton.

There was a slight decrease in sugar concentration of the nectar in flowers from sprayed plants in both 1976 and 1977 (Tables 3 and 4). This may be because a higher percentage of water evaporated from the smaller volume of nectar taken from the check flowers than evaporated from the larger volume of nectar in the 2,4-D treated flowers when the nectar is placed on the Abbe refractometer.

There were no significant differences in yield of seed cotton between the plants sprayed with 1 ppm 2,4-D and unsprayed plants (Tables 3 and 4); sprayed plants averaged 1.3% more yield in 1976 and 0.8% less yield in 1977.

The increase in nectar volume did not significantly change honey bee visits to the flowers of the sprayed plants (Table 4). Bee visits to sprayed A-line flowers decreased slightly (5%) while bee visits to the sprayed B-line flowers increased slightly (3%) compared to visits to flowers from similar unsprayed plants.

DISCUSSION

Small amounts of 2,4-D sprayed on cotton plants in Arizona field tests conducted between 1975 and 1977 increased the volume of floral nectar secreted by the plants. One ppm applied in 187 liters of water/ha (0.2 g actual 2,4-D/ha) gave the largest increase (30% or more) of the four doses studied. This increase in volume of nectar when 1 ppm 2,4-D was applied occurred consistently in the five cotton cultivars tested and in three widely separated locations; it also persisted throughout the flowering season. No cultivar, yearly, or location interactions were detected.

The slight decrease in sugar concentration noted for treated flowers (1.1% in 1975, 2.5% in 1976, and 1.8% in 1977) may reflect measurement error. The readings were taken outdoors where temperatures usually exceeded 33 C. When only nectar samples with volumes of 8 μl or more were considered, the sugar concentrations of floral nectar from sprayed plants (1 ppm 2,4-D) were slightly higher than those from unsprayed plants.

A single spray applied at the beginning bloom was more effective in increasing nectar secretion than an application made 3 weeks earlier or applications made at both times.

None of the applications of 2,4-D reduced germination of the cotton seed harvested from the sprayed plants.

Spraying cotton plants with 1 ppm 2,4-D at the beginning of bloom did not cause significant differecnes in either yield of seed cotton or the sugar concentration of the floral nectar. This dosage also did not significantly alter honey bee visits to the cotton flowers during the only year, 1977, that visits were counted. Bee visits were high that year, and results might be different when the honey bees are not visiting unsprayed cotton flowers well.

The large and persistent increase in the amount of floral nectar secreted by cotton flowers when plants were sprayed with 2,4-D demonstrated that it is possible to influence nectar secretion by the use of 2,4-D. Other herbicides probably influence nectar secretion in various ways.

Although 1 ppm of 2,4-D did not increase honey bee visits in this study, some other herbicides and/or 2,4-D applied under other conditions might attract more honey bees to cotton flowers.

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