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Surfactants in Water Drown Honey Bees[1,2,3]

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ABSTRACT

Apis mellifera L. drowned in water containing as little as 25 ppmv of Multi-Film X-77®, and higher concentrations (100 ppmv and above) caused very heavy losses. Yet X-77 and 6 other surfactants (Brij® 30, Brij® 92, dimethyl sulfoxide, Span® 20, Tween® 20, and XF-1-3655 (nonionic water soluble silicone-glycol copolymer)) were relatively nontoxic to the bees as stomach poisons. Drowning occurred when the X-77 water was supplied in plastic buckets, dirt ponds, and cement ditches. The bees preferred water without X-77 to water containing X-77 by more than a 10 to 1 margin when given a free choice on drip boards or in cement ponds.

X-77 retained sufficient activity to drown bees in cement ponds for more than 60 days after it was added at 500 ppmv to the water.

During hot weather in June, July, and August, 1971, caged colonies with X-77 added to their water supply quickly ceased rearing brood, and all unsealed larvae died and some of the colonies died. Yet colonies receiving the same treatment during cooler weather did not suffer any observable damage although there was a continuous and heavy loss of water carriers.

Surfactants in small ponds, puddles, or irrigation ditches could cause bee losses. Given free choice, bees preferred water without X-77, but collected large quantities of water containing surfactant when confined to cages or when in apiaries with no other water source available.

Large numbers of bees drowned when they visited water containing commercial phenoxy herbicides which normally are nontoxic when sprayed or fed to adult honey bees, *Apis mellifera* L., (Moffett et al. 1972, Morton et al. 1972). Because these herbicides contained surfactant we decided to study the toxicity of the surfactants to honey bees, both as stomach poison and as surface-active agents in water.

Soap has been used for more than a century to kill sucking insects. Many authors have shown that most surfactants applied as a spray in high concentrations will kill soft-bodied insects such as aphids, mosquitoes, ants, and whiteflies (Ginsburg 1935, Dozier 1937, Wilcoxon and Hartzell 1931, Wolfenbarger 1957, and many others). Hard-shelled insects were generally not affected by these sprays.

McGovran (1929) found the addition of 1% sodium oleate soap allowed water to penetrate the larger tracheae of some honey bees, while water alone did not enter the tracheae. Water solutions containing either 0.5% penetrol or 0.5% sodium oleate penetrated ⅓ to ⅔ the length of the larger tracheae of the tomato hornworm, *Manduca quinquemaculata* (Haworth), while water alone did not enter the spiracles (Wilcoxon and Hartzell 1931).

Procedures and Results

Small-Cage Feeding Studies

In the 1st series of tests, approximately 100 bees which had emerged within the previous 24 hr were shaken into 2×2×6-in. screened cages. Frames of emerging brood from field colonies were placed in an incubator to obtain these young bees. Each cage was supplied with water, 60% sucrose solution, and 5 g of the pollen supplement consisting of 2 parts soy flour, 1 part pollen, 6 parts sucrose, and sufficient water to give this mixture the proper consistency. Both the water and sucrose solutions were fed in 5-dr plastic vials inverted over the screen wire top of the cages. Two holes were drilled in the cap of each vial so the bees could obtain the syrup and the water ad lib.

Dead bees were counted and removed daily. The surfactants were fed for 60 days or until all bees died in the cage. The number of days required for ½ of the bees in each cage to die (half-life) was calculated and used as an index of the toxicity of the surfactants to honey bees. Each surfactant was fed at concentrations of 0, 10, 100, and 1000 ppmw (parts per million by weight) in 60% sucrose solution. Five replications of each test were run. The surfactants fed were: Multi-Film X-77® (hereafter referred to as X-77), a blended nonionic surfactant containing alkylarylpolyoxyethylene glycols, free fatty acids, and isopropanol; Brij® 30, a polyoxyethylene (4)lauryl ether; Tween® 20, polyoxyethylene(20)sorbitan monolaurate; Span® 20, sorbitan monolaurate; Brij® 92, polyoxyethylene(2) oleyl ether; XF-1-3655, a nonionic water-soluble silicone-glycol copolymer; and a commercial solvent, DMSO (dimethyl sulfoxide).

The 7 surfactants were relatively nontoxic to honey bees when fed in sucrose syrup at a rate of 1000 ppmw or less (Table 1). XF-1-3655 caused the most marked reduction in half-life, 15 days when fed at 1000 ppmw. The next lowest half-life of any of the other 6 surfactants was 32 days. Standifer (1972)

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Table 1.—Half-life of caged honey bees fed 4 concentrations of 7 different surfactants, Tucson, Ariz. 1971.

Test	Surfactant	Concentration[a] (ppm) 0 (check) days	10 days	100 days	1000 days
1	X-77	57.4 a	53.0 a	59.6 a	32.4 b
2	Brij 30	45.3 ab	44.1 ab	48.2 a	39.0 b
3	Tween 20	52.2 a	52.8 a	54.4 a	55.2 a
4	Span 20	42.4 a	45.0 a	46.3 a	46.1 a
5	Brij 92	41.2 a	41.1 a	42.1 a	34.0 a
6	XF-1-3655	31.2 ab	33.1 ab	25.2 b	15.0 c
7	DMSO	39.2 a	38.1 a	42.4 a	38.4 a

[a] Means in the same row followed by same letter do not differ significantly at 5% level of probability as calculated by Duncan's multiple range test. These tests were not run concurrently. Therefore, direct comparisons cannot be made between tests.

Table 2.—Daily comparison of drowning of bees when 1000 ppmw X-77 or 2,4,5-T were added to the water supply of colonies confined to cages, June 1971, Tucson, Ariz.

Days after test started	No. dead bees[a] in water containing: No additive	1000 ppm 2,4,5-T	1000 ppm X-77
1	17	249	531
2	6	64	402
3 & 4	8	106	477
5	6	181	535
6	6	171	318
7	8	73	68[b]
Weekly total	51	844*	2,331*

[a] Total of 3 colonies. Counts were made between 7:30 and 8:30 AM.
[b] Almost no bees were left to go to the water.

reported that the surfactant Triton® X-100 (Isooctyl phenyl polyethoxy ethanol) was toxic to bees when fed at 1250 ppm while dosages of 10,000 ppm or higher were extremely toxic.

The addition of small amounts of surfactant increases longevity. Caged bees fed 100 ppm surfactant in their sirup lived longer in 6 of the 7 tests (Table 1). Similarly, feeding nontoxic herbicides at 100 ppm increased bee longevity in 20 out of 22 tests when the herbicides showing significant toxicity were eliminated (Morton et al. 1972).

X-77 Added to Water in Plastic Buckets

In the 6 tests in this experiment a single colony was placed in each 12×12×9-ft Saran® mesh cage. Water was supplied to each colony in 5-qt plastic buckets. Four small pieces of wooden lath were placed in each bucket to provide a landing for the bees while they were collecting water.

The water was changed every day, and all colonies were fed pollen supplement continuously. Sugar sirup was also fed to all the colonies in Tests 1 through 4. Daily counts were made of the dead bees in each bucket at 7:30 each morning, and these deaths were credited to the preceding day. Three colonies (replications) received each treatment.

In the 1st test, 1000 ppmv (parts per million by volume) X-77 was added to the water in one treatment. The 2nd treatment had 1000 ppmv 2,4,5-T added to the bee's water.

In Tests 2–4 lower dosages of X-77 were studied to determine the dosage which did not cause bees to drown. Tests 5 and 6 were a repeat of the high doses of Tests 1 and 2 except these were done during cooler weather.

The addition of X-77 to the water buckets caused extensive drowning of the bees (Tables 2 and 3).

In the June 10–17 test, the 3 colonies with 1000 ppmv X-77 in their water supply suffered a heavy loss of bees, and 2 colonies died. All colonies receiving either X-77 or 2,4,5-T in this test lost all their unsealed larvae, but the colonies receiving 2,4,5-T recovered quickly once the test ended and they were moved outside. The check colonies reared brood normally. In the 2nd test, concentrations of 250 and 500 ppmv of X-77 were studied to see if they caused drowning. The bees again drowned in large numbers, and all unsealed larvae died in the X-77 treatments during the 2 days of the test.

In the 3rd study, the concentrations of X-77 were lowered to 50 and 100 ppmv, and the bees collected this water for 9 days (Table 3). Although still high, the drowning rate decreased, and 2 of 3 colonies in each treated group reared brood normally. The concentration was again reduced in the July 28 to Aug.

Table 3.—Summary of 6 tests on the drowning of honey bees when X-77 was added to their water supply, Tucson, Ariz., 1971–1972.

Amount of surfactant added (ppm)[a]	Avg no. bees drowned daily/colony[b]	Avg daily ambient temperature, °C Min	Max
Test 1: June 10–17			
1000	130*		
1000 (2,4,5-T)	40*	16	37
None	2		
Test 2: June 22–24			
500	311*		
250	556**	23	41
None	3		
Test 3: June 30–July 9			
100	126*		
50	52	22	39
None	4		
Test 4: July 28–Aug. 6			
25	16*		
10	1	21	37
None	1		
Test 5: Nov. 2–Dec. 23			
1000	158**		
None	2	2	19
Test 6: May 17–June 6			
500	151**		
None	2	13	33

[a] X-77 except as marked in Test 1.
[b] Three colonies in each treatment; counts made between 7:30-8:30 AM.

9 test to 10 and 25 ppmv. The drowning rate was sharply reduced for the colonies receiving 25 ppmv in their water supply and not significantly different from the checks (40 bees to 22) in the water buckets containing 10 ppmv X-77. Brood rearing was poor in both the checks and these 2 treatments.

A repeat of the higher concentration studies (500 and 1000 ppmv X-77) was made during cooler weather (Tests 5 and 6, Table 3), except no sugar sirup was fed. Although large numbers of bees drowned in the buckets containing X-77, this did not appear to adversely affect the colonies. The temperature of the brood nest was recorded 3 times each day in these last 2 tests. Brood rearing proceeded similar to that of the check colonies. In Test 6, an additional 3 colonies were studied. These colonies were treated similarly to the check colonies except they received no water for the 19 days of the test. All their unsealed larvae died almost immediately, and no new brood was reared. Yet these colonies survived in a much weakened condition. When placed outside after the test they recovered and raised brood.

Death probably occurred by the extreme wetting of the bee and by the penetration of the larger tracheae by the water. When bees collected water by standing on the wood lath, the water containing X-77 seemed to engulf or creep over the bees.

Soil Pond Studies

Ten small soil ponds ca. 3 ft diam and 18 in. deep were dug. They were paired so that there were 8 ft between the centers of the 2 ponds in each pair. The pairs were various distances apart but averaged ca. 30 ft. To prevent excessive water loss, we placed a plastic liner on the bottom of each pond. Two inches of soil were shoveled on top of this liner to simulate a small earthen pond. Five of the ponds were filled daily with water containing 500 ppmv X-77 while the other pond in each pair was filled with untreated water. Approximately 30 colonies were situated within 100 yards of the ponds.

Twenty-two days after the test started, the ponds were emptied, the plastic liners replaced, and fresh soil was placed in the ponds. Then the treatments were reversed. Ponds previously receiving water containing X-77 were filled with untreated water, while the ponds that had received the untreated water previously, now were filled with X-77-treated water. These reversed treatments were terminated after an additional 11 days.

The number of bees collecting water were counted at 8:30 AM, 11:30 AM, and 2:30 PM Mountain Standard Time on week-days. Dead bees removed from the water daily at 7:00 AM were considered to have drowned the previous day.

The bee visits to the soil ponds started slowly. The counts increased gradually from 38 the 2nd day to 1186 the 23rd day (Table 4) in the check ponds. Meanwhile, the visits to the ponds containing X-77 increased slowly until the 13th day (26 to 115 bees counted) and then gradually declined to 20. On the 23rd day, 59 times as many bees were counted visiting the untreated water as were visiting the water containing 500 ppm X-77. The overall drowning rate per bee visit was 42 times higher in the ponds containing X-77 as in the untreated ponds. This high drowning rate occurred even though most bees collected water on the dirt slopes above the water level. Bees falling into untreated water usually struggled to the edge and crawled out within a few minutes. One bee took 10 minutes to get out. In contrast, bees falling into the X-77 ponds struggled only a few seconds and then ceased moving and died. However, bees spent about the same time collecting water at either the treated (48.8 sec[5]) or the untreated ponds (50.7 sec).

On the 1st day of the reversal, the bees' visits to the newly treated ponds were still very high (997 observed) while visits to the newly untreated ponds were low (19). Bee visits to the newly treated ponds gradually declined to 16, eleven days after the reversal. Meanwhile, visits to the untreated water had gradually increased to 509 (Table 5). The death rate was 34 times higher per bee visit in the ponds with X-77 added, compared with the untreated ponds in this reversal study.

Cement Pond Study

Ten cement ponds were poured. The location of these ponds was similar to the soil ponds. They were 3 ft square at the top, 1 ft deep, and 1 ft square at the bottom. All 4 sides had a 45° slope.

Table 4.—Daily visitation and mortality of honey bees on or near soil ponds of water with and without X-77, Tucson, Arizona, April–May, 1972.

	Water[a]		Water + 500 ppm X-77[a]		
Date	Dead bees[b]	Observed visitations[c]	Dead bees[b]	Observed visitations[c]	Mortality/ bee visit[d]
April 24	1		7		
25	12	38	35	26	4.3
26	7	66	36	42	8.1
27	7	101	37	54	9.9
28	10	138	48	60	11.0
May 1	8	274	48	69	23.9
2	8	399	90	95	47.2
3	25	537	123	105	27.0
4	13	645	143	112	63.4
5	9	654	94	115	59.4
8	20	810	77	72	43.3
9	24	994	93	75	51.4
10	19	993	76	52	76.4
11	16	993	49	40	76.0
12	22	1093	53	40	65.8
15	24	1186	40	20	98.8
Total	224	8921	1042	977	42.48

[a] Total of 5 ponds.
[b] Dead bees were counted and removed at 7:00 AM the following morning.
[c] Bees visiting the ponds were counted at 8:30 AM, 11:30 AM, and 2:30 PM except on Saturdays and Sundays.
[d] Ponds containing X-77 ÷ ponds containing water only.

[5] Average of 55 water collectors.

Table 5.—Visitation and mortality of honey bees on or near soil ponds of water with and without X-77 when the treatments were reversed 22 days after the start of the test, Tucson, Ariz. 1972.

	500 ppm X-77 + water[a] (formerly water only)		Water[a] (formerly 500 ppm X-77 in water)		
Date	Dead bees[b]	Observed visitations[c]	Dead bees[b]	Observed visitations[c]	Mortality/ bee visit
May 16	1263	997	0	19	
17	464	649	1	130	92.94
18	275	393	9	176	13.68
19	141	234	6	194	19.48
22	44	53	6	164	25.39
23	56	30	8	200	44.17
24	52	23	7	305	98.51
25	45	17	9	334	98.24
26	58	16	14	509	131.79
Total	2398	2392	60	2031	33.93

[a] Total of 5 ponds.
[b] Dead bees were counted and removed at 7:00 AM the following morning.
[c] Bees visiting the ponds were counted at 8:30 and 11:30 AM, and 2:30 PM except on Saturdays and Sundays.
[d] Ponds containing X-77 ÷ ponds containing water only.

These ponds were kept filled with untreated water starting June 2 so the bees would start visiting them. Each pond held 30 gal of water. At 7:30 AM on June 20, 57 ml of X-77 was added to ½ the ponds to give a concentration of 500 ppmv of X-77.

Bee visits to the cement ponds dropped 60% within an hour after X-77 was added to these ponds. On the 2nd day, visits to the X-77 ponds were only 1% as great as to the untreated ponds and then dropped to virtually zero (Table 6). Although no X-77 was added to any of these cement ponds after the initial addition, 84 days later less than 1% as many bees were collecting from the treated ponds as the untreated ponds.

Flies drowned in large numbers in the cement ponds containing X-77. Ants, wasps, moths, and other insects also drowned in these treated ponds.

Seventeen days after the X-77 was added to these ponds, 5 bees were dropped into the treated ponds. They struggled weakly, and movement ceased in an average of 21.4 sec. Ten seconds later these bees were taken from the ponds, but all failed to revive.

The 5 bees dropped into the check ponds swam vigorously and all reached shore. The average time to reach shore was 38 sec (range 7–61 sec).

There was sufficient surfactant in the cement ponds to drown bees 60 days after the X-77 was added to these ponds. During this period untreated water was added to both the check and treated ponds to replace the water lost by evaporation and 3 of the treated ponds had been diluted by minor flooding.

In studies by Davis (1967) pond water degraded completely 4 of 5 surfactants tested in 15 to 21 days and reduced the 5th surfactant by 85%.

X-77 Drip-Board Study

Ten drip-boards were placed in a circle around two 55-gal barrels. Water was piped from the barrels by copper tubing and was turned on at 7:00 AM and off at 7:00 PM by an automatic timer. One barrel supplied untreated water to 5 of the drip-boards, while the 2nd barrel supplied water containing X-77 at a concentration of 500 ppmv to the other 5. The drip boards were 2 ft wide, 4 ft long, and sloped to an angle of approximately 20° from horizontal.

Water was trickled slowly down the boards starting late in the afternoon of May 25. Counts of bees collecting water were made from May 30 until the test was terminated after the June 2 observations.

Thirteen times as many bees (447) were counted visiting boards wet with untreated water as were counted on boards wet with water containing X-77 (34 bees). These counts were made at 8:30 and 11:30 AM and 2:30 PM daily for 4 days.

The change was almost immediate. First-day counts of bee visits were 98 to 9 in favor of the untreated boards.

Discussion

None of the 7 surfactants studied were toxic to bees when fed orally in low dosages. In fact, they appeared to increase bee longevity slightly. Yet low concentrations of one of the surfactants, X-77, caused extensive drowning of bees collecting water. There are times when the water in small ponds and irrigation ditches could exceed the concentrations that cause drowning. This is particularly true in irrigation ditches which are often sprayed repeatedly with herbicides to kill weeds.

When given free choice, the bees collected un-

Table 6.—Honey bee visits and mortality following the addition of X-77 to water in small cement ponds.[a] Tucson, Ariz. 1972

	Bee visits[b]		Dead bees	
Date	Water	Water + X-77	Water	Water + X-77
June 16[c]	184	203	7	7
19	277	328	8	8
	X-77 added			
20	413	73	9	8
21	102	1	29	293
22	271	6	20	81
23	330	4	6	49
July 5	703	0	34	19
19	285	0	8	6
Aug. 2	346	0	29	3
16	424	2	9	17
30	469	3	11	4
Sept. 13	618	2	22	7
27	728	6	6	4
Oct. 11	731	6	12	0

[a] Untreated water was added to these ponds as it evaporated.
[b] Bees visiting these ponds were counted at 8:30 AM, 11:30 AM, and 2:30 PM.
[c] Daily readings are given through-June 23, subsequent readings are listed at 14-day intervals.

treated water in preference to water containing X-77. The bees changed much faster and more completely from cement ponds with water containing X-77 to untreated cement ponds than from soil ponds with water containing X-77 to untreated soil ponds. The change was almost complete in one day in the cement ponds, while it occurred less completely over a period of 3 weeks in the soil ponds, even though X-77 in water was added continuously to the soil ponds while no X-77 was added to the cement ponds after the 1st day.

The apparent preference of the bees for untreated water might help prevent bee loses in the field when several sources of water are available. Additional studies with other surfactants and under other conditions would be valuable.

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