EFFECTS OF SURFACE TENSION ON PUPAE OF *CULEX PIPIENS QUINQUEFASCIATUS* SAY AND *AEDES AEGYPTI* (L.)

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Surface tension phenomena are known to play an important part in the development and survival of aquatic insects. Some works on the ecology of animals stress the importance of surface tension of water to aquatic organisms (Shelford 1929, Chapman 1931, Welch 1935). Mosquitoes spend some of their life stages in water and these stages are known to be affected by changes in the surface tension of their aquatic environment. However, only a few workers have studied the relationship of this phenomenon to mosquito larvae (Miall 1903, Renn 1941). Miall gave an account of the processes involved and mentioned their importance in the life of mosquito larvae. Russel and Rao (1941) reported that *Anopheles* larvae fail to cling to the surface when the surface tension was reduced to 27–36 dynes/cm.

Singh and Micks (1957) studied the effects of surface tension on the development of several species of mosquitoes. They reported that four species of *Culex* develop normally in water with lower surface tension but *Aedes aegypti* prefer higher levels of surface tension.

During a study of the effects of several aliphatic amines, mostly cationic surfactants on pupae of *Culex pipiens quinque-*

fasciatus, we observed marked mortality of pupae and adults even at low concentrations. This high mortality was seemingly not due to the toxicity of the compounds. It was surmised that change in surface tension played a role in producing these effects. The present study, therefore, was undertaken to investigate the effects of the reduced surface tension on the pupae of *C. p. quinquefasciatus* and *A. aegypti*. A nonionic surfactant was used in these studies. Effects of changing surface tension on pupae of different ages were also studied.

MATERIALS AND METHODS. Four-ounce wax-treated cups were set with 100 ml of tap water in each. Fifty pupae of known age were added. For measuring the surface tension, additional cups containing 100 ml of tap water were arranged side by side. All cups except the controls were then treated with diluted solution of the nonionic surfactant polytergent B-200 (nonylphenoxy polyethoxy ethanol) in water. Various concentrations were employed for the reduction of surface tension of the water in the cups. Each treatment was run in duplicate with two cups of untreated water kept as checks. All cups were kept under observation at 25.5° C (water temperature in cups ranging from 23° to 24.5° C) for 3 days. The extent of pupal and adult mortality was recorded every 24 hours. The initial as well as subsequent surface tension of each of the solutions at 24, 48 and 72 hours was recorded.

The surface tension of the solution was determined with a Cenco Surface Tension Capillary Tube (CENCO No. 70506). The capillary tube was placed in 100 ml of the liquid to be tested in a narrow-mouthed bottle and the temperature of the liquid was recorded. The height to which the liquid rose in the tube was determined. The surface tension (δ) of the liquid was calculated by the following expression:

$$\delta = hrdg/_2$$

where δ is the surface tension expressed in dynes/cm,
h is the height of the liquid in cm in the surface tension tube,
r is the radius of the bore of the tube in cm,
d is the density at the test temperature expressed in gm/cm^3,
and g is the acceleration due to gravity expressed in cm/sec^2.

In order to determine the bore radius (r) some standard liquids of known surface tension (acetone, benzene and distilled water) were used. Temperatures of acetone and benzene were maintained at 21° and that of the water at 23° C, as the surface tension of these liquids at these temperatures was obtained from standard tables. The acceleration due to the gravity (g) was rounded to 980 cm/sec^2.

RESULTS AND DISCUSSION. Reduction in surface tension resulting from the addition of Polytergent B-200 and its effects on pupae of *C. p. quinquefasciatus* 10 hours old are shown in Table 1. The highest concentration of the surfactant caused high overall pupal and adult mortality. Adults began to emerge within 24 hours after treatment when the surface tension of the solution was lower than that of the water by about 3–6 dynes/cm. It is evident from Table 1 that only a few of the adults which emerged from the treated pupae survived at the highest concentrations. Some of the pupae yielded partially emerged imagoes. Adults which emerged completely could not fly. Due to the low surface tension of the water, these insects were unable to rest on the surface of the water. The surface film was broken and the adults readily drowned.

Three days after the treatment the surface tension of all the treated water was almost normal. Most of the adults emerging after this period were able to survive. More than 50 percent of the pupae exposed to the 15 ppm concentration died during the exposure period of 72 hours. This concentration also affected the adult survival to a great extent. Surface tension was depressed (not shown in table) at this concentration for 3 days. Adult mortality was lower in cups where the surface tension of the solution approached 70 dynes/cm, almost close to normal. Mor-

TABLE 1.—Effect of a surfactant (Polytergent B-200) on pupae and adults of *Culex p. quinquefasciatus*.[a]

Conc. (ppm)	Surface tension (Dynes/cm) hrs. aft. treat.[b]				No. of dead (d) pupae and flying (f) adults hours after treatment						Cumulative total mortality (no.) aft. (hrs.)		
					24		48		72				
	0	24	48	72	Pupa (d)	Adult (f)	Pupa (d)	Adult (f)	Pupa (d)	Adult (f)	24	48	72
1	66	70	71	71	1	33	0	41	0	21	5	5	5
3	..	..	..	..	3	30	0	25	1	15	20	29	30
5	63	68	69	71	4	9	1	23	2	16	39	49	52
7	..	..	..	..	5	1	5	8	0	22	41	67	69
10	62	67	68	70	22	0	6	8	0	15	63	74	76
15	..	..	..	..	32	0	20	0	1	5	56	89	95
ck	71	71	71	71	0	38	0	41	0	21	0	0	0

[a] Average pupal age—10 hours; 100 pupae used per treatment.
[b] Surface tension determinations of the solutions were made at 23–24.5° C. and that of untreated water (check) at 23° C.

TABLE 3.—Effect of a surfactant (Polytergent B-200) on pupae and adults of *Aedes aegypti*.[a]

Conc. (ppm)	Surface tension (Dynes/cm) hrs. aft. treat.[b]				No. of dead (d) pupae and flying (f) adults hours after treatment						Cumulative total mortality (no.) aft. (hrs.)		
					24		48		72				
	0	24	48	72	Pupa (d)	Adult (f)	Pupa (d)	Adult (f)	Pupa (d)	Adult (f)	24	48	72
1	66	70	71	71	3	30	3	36	1	15	7	16	19
3	..	..	..	..	3	28	5	25	2	18	15	25	29
5	63	68	69	71	12	25	9	17	2	3	35	52	54
10	62	67	68	70	33	0	12	1	1	2	75	96	97
15	..	..	..	..	41	0	10	0	0	0	79	98	100
ck	71	71	71	71	2	0	0	40	0	21	2	2	2

[a] Average pupal age—10 hours; 100 pupae used per treatment.

TABLE 2.—Effect of a surfactant (Polytergent B-200) on the pupae and adults of *Culex p. quinquefasciatus*.[a]

Conc. (ppm)	Surface tension (Dynes/cm) hrs. aft. treat.			No. of dead (d) pupae and flying (f) adults hours after treatment				Cumulative Total mortality (no) aft. (hrs.)	
				24		48			
	0	24	48	Pupa (d)	Adult (f)	Pupa (d)	Adult (f)	24	48
1	66	70	71	5	92	0	1	7	7
3	..	..	..	15	70	2	3	25	27
5	63	68	71	13	53	0	4	43	43
7	..	..	..	18	29	1	0	68	71
10	62	67	68	21	5	..	..	95	95
ck	71	71	71	2	95	0	3	2	2

[a] Average pupal age—one day; 100 pupae used per treatment.

tality of adults was highest in cups where the surface tension was lower than 70, indicating relationship between mortality and surface tension.

Results of the effect of surfactant on older pupae are shown in Table 2. These pupae were about a day old and most of the adults emerged within 24 hours after treatment. Heavy adult mortality occurred in the solution having the lowest surface tension 24 hours after treatment. Almost all the adults emerging from the pupae treated with the lower concentrations survived and flew off the water surface even though surface tension was slightly reduced in these treatments from the normal.

Table 3 presents the effects of the surfactant on 10-hour old pupae of *A. aegypti*. Mortality of pupae was observed to be higher than that of the pupae of *C. p. quinquefasciatus* of same age. The adult mortality was also comparatively higher in solutions of high concentrations of surfactant.

Singh and Micks (1957) observed similar effects of surface tension change on pupae of *Aedes aegypti, Anopheles quadrimaculatus* and four species of *Culex*. However, they observed that none of the *Culex* species were affected significantly by the reduction of the surface tension down to 53 dynes/cm. Pupal survival and adult emergence were affected considerably when the surface tension was lowered to 45 dynes/cm. From their studies it became apparent that *Culex* pupae were more capable of surviving in water having lower surface tension (45–53) as compared to *Aedes* or *Anopheles* species.

From the present studies it is evident that the pupae of *Culex p. quinquefasciatus* died either due to toxic effects of the compound or due to a slight lowering of surface tension of the water. Pupae of *A. aegypti* were found more susceptible to toxic effects or reduction of surface tension or both, than those of *C. p. quinquefasciatus*. Singh and Micks (1957) noticed a similar trend in their study.

The dead and dying adults showed various degrees of disintegration of their bodies while floating on the surface of the water. Adults drowned showed sloughing off of scales from both wings and the body. Most of the dead adults had crumpled legs and wings, some of them with characteristic "balloon shaped" wings. The drowning of emerging adults was inversely related to surface tension. More adults drowned during the first 24 hours, when surface tension was low, than at subsequent intervals when the surface tension was high.

Some studies were also conducted on 4-hour-old pupae of *C. p. quinquefasciatus*. Results of these studies are not presented here, but it was found that 4-hour and 10-hour old pupae were equally affected by the effective concentrations of 5-15 ppm. A marked difference, however, was observed between the mortality of 10-hour old pupae and that of the 1-day old pupae, suggesting that the mature pupae

are more susceptible to the change of surface tension of the water.

The results of the present experiments indicate that developmental stages of mosquitoes among other things are affected by a reduction of the surface tension. Manipulation of surface tension for mosquito control merits further investigation. This technique if found practical will provide additional tools to combat immature mosquitoes in certain types of breeding sources.

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