delle Colonie Italiane. Rivista di Parassito No. 1. supplemento.

RIOUX, JEAN A. 1958. Les Culicides du "Midi" Mediterraneen. Editions Paul Lechevalier, Paris, France.

SENEVET, G. and ANDARELLI, L. 1956. Les Anopheles de L'afrique du Nord et du bassin Mediterraneen. Editions Paul Lechevalier, Paris, France.

VERMEIL, C. 1953. Contribution á L'ètude des Cuclicides du Fezzan (Libie) Presence D'*Anopheles broussesi* E. a el Barka (Territoire de Rhat.) Bulletin de la Societé de Pathologie Exotique.

OVIPOSITION RESPONSES OF *CULEX PIPIENS QUINQUEFASCIATUS* SAY TO WATERS TREATED WITH VARIOUS CHEMICALS

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The female mosquito's choice of oviposition site is influenced by many factors. Among the species that lay their eggs singly or in rafts on the surface of the water, this choice may be affected by type of vegetation, degree of shade, and amount of protection from wind and wave action. The choice of oviposition site will also be affected by the physical and chemical properties of the water available.

The chemicals present in different types of water that may influence the female's choice of oviposition site have not been extensively investigated. Lund (1942) in studies of *Anopheles quadrimaculatus* Say found no evidence of any preference in water samples containing a series of different chemicals or differing in hardness, pH, and calcium ion concentration. Wallis (1954) showed that *Aedes aegypti* (Linnaeus) was repelled by a 3 percent salt solution but preferred a 0.25 percent salt solution over distilled water. A 1 percent sodium chloride solution was shown by Zuleta (1950) to be repellent to *Culex fatigans* Wied. (*Culex pipiens quinquefasciatus* Say) but a 1 percent magnesium solution was not. He also found that this species preferred well-matured grass infusion to rainwater. Gerhardt (1959) found that *Culex peus* Speiser laid eggs in field-test plots which contained fermenting dog food, casein, fat, or sugar and did not lay eggs in an untreated control plot. *Culex tarsalis* Coq. and *Anopheles freeborni* Aitken also laid eggs in these plots as fermentation subsided, but not in the control plots.

Materials and Methods.—One hundred and fifty-one chemicals were tested in water samples against gravid females of *Culex pipiens quinquefasciatus* Say to determine if they were attractive or repellent.[2] Some comparative tests of the attractiveness of natural breeding and distilled waters were also made.

The tests were made in screen cages measuring 12x12x12 inches that were fitted with a cloth sleeve on one side. Twenty to 25 blood-fed females, not over 5 days old, were used in each test. Acetone or water solutions of the chemicals were added to 350 cc. of distilled water in 400-cc. beakers. The chemicals were tested at 5, 25, and 50 p.p.m. In the multiple-choice tests three beakers containing different strengths of the chemical and one containing distilled water were used. The beakers were placed in the four corners of the cage. In the single-choice tests one beaker containing the chemical in 350 cc.

[1] A cooperative project between the Entomology Research Division of the U. S. Department of Agriculture and the Bureau of Vector Control of the California Department of Health. San Standring and Claude Hirst of the Bureau of Vector Control assisted with the project.

[2] Most of the chemicals tested were synthesized by chemists of the Entomology Research Division.

of water was used. A 10 percent sugar solution was available as food in all cages. The beakers containing the test solutions were placed in the cages on the second or third day after the females had taken their blood meal and removed 4 or 5 days later.

The cages were held in a room measuring 5x5x6 feet and lighted by one 100- and one 7½-watt bulb. The large bulb was turned on from 6 a.m. to 6 p.m. and the small one from 4 a.m. to 8 p.m. An air conditioner provided continuous moderate exchange of fresh air.

Results and Discussion.—In the multiple-choice tests in which the mosquitoes could deposit eggs in distilled water or in different concentrations of the chemical, 26 compounds appeared to be effective in preventing oviposition at 50 p.p.m. or less. The results are presented in Table 1.

The effectiveness of some of the above chemicals was explored in additional tests in which a single chemical solution was the only one present in the cage. Several of these were effective in preventing egg deposition at concentrations of 10 p.p.m. or less, but none at 1 p.p.m. The results of these tests are shown in Table 2.

Several surfactants were found to prevent oviposition at 10 p.p.m. when single beakers were offered for egg deposition. The most effective of the series tested was Triton X-155. This material was effective at 2 p.p.m. in distilled water. The results of these tests are shown in Table 3.

This species of female mosquito rests on the surface of the water while laying her eggs. It seemed possible that the repellent action of some of the surfactants might be due to the reduction of surface tension

TABLE 1.—Results with compounds that appeared to reduce *Culex pipiens quinquefasciatus* egg deposition in multiple-choice tests

Compound	Number of egg rafts at indicated dosage in p.p.m.[a] during 4-day exposure period		
	0	5	25
Acetonitrile, 2-allyl-4,5-methylenedioxyphenyl-	6	3	0
Allethrin	1	0	0
Benzoic acid, *o*-chloro-, 4-methylcyclohexyl ester	2	1	0
Benzoic acid, *o*-chloro, *o*-tolyl ester	1	0	0
Butyric acid, 3-(*m*-methoxyphenoxy)-, propyl ester	6	0	0
Chrysanthemumic acid, benzyl ester	4	2	0
Chrysanthemumic acid, 2,4-dimethylbenzyl ester	5	3	0
Chrysanthemumic acid, *m*-ethoxybenzyl ester	4	0	0
Chrysanthemumic acid, *p*-methoxybenzyl ester	5	0	0
Chrysanthemumic acid, *o*-methylbenzyl ester	1	0	0
Chrysanthemumic acid, 3,4-methylenedioxyphenethyl ester	6	0	0
Chrysanthemumic anhydride	4	2	0
4-Cyclohexene-1,2-dicarboximide,*N*-octyl-	6	2	0
Ether, gamma-bromopropyl *m*-methoxyphenyl	4	6	0
m-Toluamide, *N,N*-dibutyl-	7	1	0
m-Toluamide, *N,N*-diisopropyl-	11	1	0
m-Toluamide, *N,N*-dipropyl-	2	3	1
Oleic acid	9	3	0
Oleic acid, butyl ester	3	3	1
o-Veratrylamine, *N*-ethyl-*N*-heptyl-	6	1	0
Phosphonic acid, ethyl-, ethyl ester, anhydride with chrysanthemumic acid	2	2	3
Piperonal, 6-allyl-	2	2	0
1-Propanol, 2-amino-3-isobornyloxy-2-methyl-	3	3	0
Propionic acid	5	3	4
Succinamic acid, *N,N*-dipropyl-, *sec*-butyl ester	1	1	0
Succinic acid, 2-piperonyl-, 4-ethyl 1-propyl ester	3	4	0

[a] No egg deposition at 50 p.p.m.

TABLE 2.—Egg deposition by *Culex pipiens quinquefasciatus* in cages containing a single compound in distilled water (no-choice tests)
(Average of one to three tests per concentration)

Compound	Number of egg rafts at indicated dosage in p.p.m. during 4-day exposure period					
	50	25	10	5	2	1
Acetonitrile, 2-allyl-4,5-methylenedioxyphenyl-	1	..	..	..	..	..
Allethrin	..	..	..	0	..	..
Barthrin	..	..	0	..	..	..
Benzoic acid, *o*-chloro, 4-methylcyclohexyl ester	0	0	..	1	..	..
Benzoic acid, *o*-chloro-, *o*-tolyl ester	5	..	..	..	..	..
Butyric acid, 3-(*m*-methoxyphenoxy)-, propyl ester	3	..	..	..	..	..
Chrysanthemumic acid, benzyl ester	2	..	..	..	..	..
Chrysanthemumic acid, 2,4-dimethylbenzyl ester	0	0	0	3	..	..
Chrysanthemumic acid, 3,4-dimethylbenzyl ester	..	..	..	2	..	..
Chrysanthemumic acid, *m*-ethoxybenzyl ester	0	..	..	0	..	1
Chrysanthemumic acid, *m*-isopropoxybenzyl ester	0	..	..	1	..	..
Chrysanthemumic acid, *p*-methoxybenzyl ester	..	..	..	0	1	..
Chrysanthemumic acid, *o*-methylbenzyl ester	0	0	..	0	2	..
Chrysanthemumic acid, 3,4-methylenedioxyphenethyl ester	5	..	..	..	..	..
Chrysanthemumic anhydride	6	..	..	..	..	..
4-Cyclohexene-1,2-dicarboximide,*N*-octyl-	3	..	..	..	..	..
Ether, gamma-bromopropyl *m*-methoxyphenyl	3	..	..	..	..	..
Fencholic acid, piperonyl ester	0	..	..	2	..	..
Malathion	..	..	2	1	..	..
Oleic acid	11	..	..	..	..	..
Oleic acid, butyl ester	3	..	..	..	..	..
Phosphonic acid, ethyl-, ethyl ester, anhydride with chrysanthemumic acid	2	..	..	..	..	..
Piperonal, 6-allyl-	2	..	..	..	..	..
1-Propanol, 2-amino-3-isobornyloxy-2-methyl-	3	..	..	..	..	..
Propionic acid	2	..	..	..	..	..
Succinamic acid, *N,N*-dipropyl-, *sec*-butyl ester	2	..	..	..	..	..
Succinic acid, 2-piperonyl-, 2-ethyl 1-propyl ester	3	..	..	..	..	..
m-Toluamide, *N,N*-dibutyl-	0	..	..	..	..	..
m-Toluamide, *N,N*-diisopropyl-	0	..	..	4	..	..
m-Toluamide, *N,N*-dipropyl-	1	..	..	..	..	..
o-Veratrylamine, *N*-ethyl-*N*-heptyl-	0	..	..	3	..	..

so that the water surfaces would no longer support the weight of the female. Reduction of surface tension might also conceivably affect the repellency of these materials in other ways. Surface tension determinations were therefore made of a number of the repellent and nonrepellent surfactants in this series (Table 3). The surface tensions of the nonrepellent surfactants were found to extend below those of the repellent materials. This would indicate that reduction of surface tension does not prevent the female from resting on the surface of the water.

Partial mortality of adults occurred in a number of cages in this series when toxic materials were used. Some of the more effective repellents caused partial mortality at low dosages and others did not.

Beechwood creosote and *N*-butyl-*N*-ethly-*o*-veratrylamine were the only compounds of the 151 tested that appeared to increase the attractiveness of distilled water. A review of the literature relating to the use of creosote for insects by Hocking (1960) shows that it has been widely used as a repellent for several insects but has not previously been found to be attractive. Both creosote and *N*-butyl-*N*-ethyl-*o*-veratrylamine were more than three times as attractive as distilled water. Comparative tests of water from a pond where *C. p. quinquefasciatus* was breeding showed that the pond water was also more attrac-

TABLE 3.—The repellent effect of surfactants on oviposition of *Culex pipiens quinquefasciatus* when single beakers were available per cage. Average of one to three tests per concentration

Surfactants [a]	Number of egg rafts at indicated dosages in p.p.m. during 4-day exposure period			Surface tension (Dynes per cm. at 10 p.p.m.)
	10	2	1	
Alkaterge C	1	..	..	27.8
Antarox A-200	0	..	1	..
Atlas 32344	12	..	..	34.9
Atlox 1045A	6	..	..	..
B-1956	3	..	..	..
D-40-FG	3	..	..	..
Igepon AP-76	1	..	..	..
OPE 1	12	..	..	..
Renex 20	3	..	..	44.1
Santomerse DT	5	..	..	..
Span 20	10	..	..	42.3
Tergitol NPX	0	2	..	..
Triton CF21	0	4	11	40.7
Triton F10	0	4	3	40.7
Triton Gr-5	3	..	..	53.6
Triton QS-15	3	..	..	59.3
Triton X-45	4	..	..	40.1
Triton X-100	0	12 [b]	3	53.6
Triton X-151	0	..	12	44.5
Triton X-155	0	0	1	31.9
Triton X-161	1	..	..	50.0 [c]
Triton X-171	8	..	..	50.0
Triton X-300	9	..	..	55.0
Triton X-301	7	..	..	..
Triton 114	1	..	..	..
Tween 20	4	..	..	..
Tween 80	5	..	..	..
Tween 618	8	..	..	45.2
Vatsal OT	2	..	..	..
Triton X-100 + *o*-methylbenzyl ester of chrysanthemumic acid, 1:1 ratio	11	3	13	..

[a] The inclusion of proprietary names of chemicals does not imply the endorsement of the U. S. Department of Agriculture.
[b] Effective (no egg rafts) at 5 p.p.m.
[c] Distilled water 68 dynes/cm. Temperature 74–78° F.

TABLE 4.—Relative attractiveness of distilled water and various solutions for *Culex pipiens quinquefasciatus* oviposition. Four replications per solution

Material	Total number of egg rafts laid at indicated dosage in p.p.m. during 4-day exposure period			
	50	25	5	0
N-butyl-*N*-ethyl-*o*-veratrylamine	11	11	11	3
Beechwood creosote	18	13	12	5
Comparison of pond and distilled water				
Pond [a]	..	..	..	37
Distilled	..	..	..	8

[a] From a grassy pond where *C. pipiens quinquefasciatus* were breeding.

tive than distilled water. The results of these tests are shown in Table 4.

The materials that were found to prevent or reduce oviposition in this study might not be practical under field tests because of their cost and short period of effectiveness. However, the results obtained suggest that effective chemicals might be found. The possibility of finding effective repellents for other mosquito species because of their more limited range of breeding conditions in nature seems even more favorable.

SUMMARY. One hundred and fifty-one chemicals were tested in water samples against gravid *Culex pipiens quinquefasciatus* Say to determine if they were attractive or repellent. Thirty-three of these materials prevented oviposition at 50 p.p.m. or less in distilled water in single-beaker cage tests. Thirteen were effective at 10 p.p.m. or less but only Triton X-155 (a surfactant) was effective at 2 p.p.m. Beechwood creosote and *N*-butyl-*N*-ethyl-*o*-veratrylamine were the only compounds that increased the attractiveness of distilled water.

References Cited

GERHARDT, RICHARD W. 1959. The influence of soil fermentation on oviposition site selection by mosquitoes. Mosquito News 19(3):151–5.

HOCKING, BRIAN. 1960. Smell in Insects: A Bibliography with Abstracts. Dept. of National Defence, Canada, FP Technical Report No. 8. 266 pp.

LUND, H. O. 1942. Studies on the choice of medium for oviposition by *Anopheles quadrimaculatus* Say. Jour. Nat. Malar. Soc. 1:100–11.

WALLIS, R. C. 1954. The effect of population density and of NaCl concentrations in test series in laboratory experiments with ovipositing *Aedes aegypti*. Mosquito News 14(4):200–4.

ZULETA, DE JULIAN. 1950. Comparative oviposition experiments with caged mosquitoes. Amer. Jour. Hyg. 52(2):133–42.

SOME OBSERVATIONS ON BITING FLIES ATTACKING SHEEP

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With the initiation of studies by the U. S. Department of Agriculture on the transmission of bluetongue disease of sheep with the fly *Culicoides variipennis* (Coquillett), one of the important steps in incriminating this species as the vector was establishing that it attacked sheep in the field. An examination of the literature showed no published records of *variipennis* biting sheep. In the fall of 1960 a number of field collections were made with a portable animal-bait trap, using a sheep. This paper presents some of the records obtained; these were made near Grand Junction, Colo.,[2] on the western slope of the Continental Divide.

The animal-bait trap consisted of an 8-foot-square piece of olive-drab canvas stretched on a pipe frame, and a pyramidal white cloth tent whose base was attached to an 8-foot-square pipe frame of lighter construction. These two frames were hinged together along one side, and in the open position they lay on the ground side by side. The frames were connected by heavy springs, and the tent side was held down with a weight. A sheep was staked out in the center of the canvas, and the trap was sprung from a distance to avoid contamination by human odor. As soon as the trap was closed the flies were collected, with special attention being given to those that remained engorging on the sheep.

This study was preliminary, since methodology was being developed at the same time for serological work, and for epidemiological studies in which many of

[1] The Simuliidae were determined by Alan Stone of this Division, who also corrected the author's determinations of the Culicidae. The author is indebted to his assistant, J. E. Wright, for his help in both the design and construction of the animal-bait trap.

[2] At the Leo James farm.