STUDIES ON THE FLIGHT RANGE OF CERTAIN *CULEX* MOSQUITOES, USING A FLUORESCENT-DYE MARKER, WITH NOTES ON *CULISETA* AND *ANOPHELES*[1]

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The problem of the flight range of *Anopheles* mosquitoes has been investigated rather extensively because of their known relationship to malaria. These studies have been well summarized recently by Eyles.[1] However, few reports are to be found which concern the flight range of the culicine mosquitoes, and we are acquainted with but one having to do with *Culex* (Clarke [2]). Certain species of *Culex* now are known to serve as vectors of some of the important virus encephalitides of man and horse, in addition to their well-known role as carriers of human filariasis and the avian malarias. Furthermore, their importance as pests cannot be minimized.

With the increased appreciation of the public health significance of *Culex* mosquitoes and with the beginning in California of extensive control programs directed particularly against *Culex tarsalis* Coquillett, it became necessary to acquire knowledge of the distances traveled by some of the commoner species of this genus in California. The studies herein reported were carried out during the summers of 1946 and 1947 in Kern County, California, an area in which the Western equine and St. Louis types of encephalitis are endemic. This same area has been a center for other epidemiological studies of these and other related infections by the Hooper Foundation, Neurotropic Virus Research Unit.

METHODS

A review of the literature convinced us that none of the methods in common use for marking, and later identifying the same marked specimens among collections, was quite suited to our over-all field research problem. If the large numbers of mosquitoes collected in the flight-range studies could also be subjected to tests for the presence of viruses or to dissection to determine sporozoite and oocyst rates in avian malaria surveys, we could accomplish more from the use of a limited group of trained field collectors. The use of aniline dyes, commonly employed by other investigators, required the moistening of specimens with a solvent such as alcohol or acetone, thus rendering the specimens useless for other studies. It was felt that the use of the alternative, metallic dusts, would necessitate such elaborate precautions in preventing contamination of clothing and laboratory equipment as to render field operations highly unwieldy. It was also recognized that the type of meticulous examination required for identifying specimens so marked was very time consuming. Therefore, a marker with the following characteristics was sought: (1) small amounts would be visible without the use of a solvent; (2) it could be handled easily with a minimum chance of contamination of equipment, contact with which might contaminate unmarked mosquitoes; and (3) close microscopic examination of individual specimens would not be required for its detection. The fluorescent dyes seemed best to fit the above characteristics.

[1] This investigation was carried out in collaboration with the Communicable Disease Center, U.S.P.H.S.; and the Commission on Virus and Rickettsial Diseases, Army Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D. C.; and under a contract with the California State Department of Public Health.

[2] Assigned to the George Williams Hooper Foundation through the California State Department of Public Health by the Communicable Disease Center, U.S.P.H.S.

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Beginning in San Francisco in May 1945 the following dyes were tested: * rhodamine, anthracene, fluorescein, uranium salts, uranin, riboflavine and fluorescent purple. Before the findings of these laboratory studies could be applied to field tests, Zukel [3] reported tne findings of laboratory studies of a somewhat similar nature. Following this Chang [4] published a report on laboratory studies of fluorescent materials as markers for mosquitoes. However, the above authors did not point out that fluorescent particles frequently are to be found on field-caught mosquitoes, and occasionally even on laboratory-reared specimens. In examinations under ultraviolet radiation of over 78,000 freshly killed field-collected mosquitoes in Kern County, numerous specimens have been observed to have minute fluorescent blue, purple, green, white, yellow or orange particles on their bodies. However, we did not observe on these mosquitoes any naturally occurring red-fluorescent materials. Therefore, rhodamine-B (National Aniline Division, Allied Chemical and Dye Corporation), a water-soluble dye producing red fluorescence under ultra-violet light was finally selected for use in all of the field studies herein reported.

In the laboratory, attempts to obtain adequately marked adults from larvae reared in 1:1,000 to 1:10,000 solutions of rhodamine-B in water were unsuccessful, either because of high mortality among larvae or of failure of the dye to carry over into the adult stage. Similar unsatisfactory results were reported by Chang.[4]

It was found, however, that 25 to 50% of adults feed readily on 1:1,000 to 1:10,000 solutions of rhodamine-B in sugar water. No toxic effects were noted on comparison with controls fed on sugar water alone. Adults which had fed on such a solution retained a characteristic carmine-red coloration of the abdomen readily visible in ordinary light. When irradiated with the dark light, a brilliant red fluorescence was observed. The female mosquito retained this fluorescence for at least 7 days in the laboratory, even following a blood meal and the subsequent formation of eggs. It also has been noted that the dye is still visible after death of the mosquito for at least 19 days. Although in field tests, several recoveries were made of mosquitoes which had fed thus on the dye solution, this method is not too well suited to flight range studies, principally because of the difficulty in getting a large proportion of specimens to feed on the solution. However, it would appear that this method does offer certain advantages for use in tagging mosquitoes for other biological studies, since there is a minimal chance of damaging specimens or of contaminating unmarked ones.

The application of fluorescent dyes to the bodies of mosquitoes as dusts has been found to be the method of choice. Zukel's [3] method of preparing and applying the fluorescent materials was used. Briefly, this consists of mixing one part of the dye with 6 parts of gum arabic, adding water until the mixture is of a pasty consistency, allowing this to dry, and then grinding it to a fine powder in a mortar. The powder is applied to adult mosquitoes in the holding cage, by means of a hand-operated powder blower (DeVilbiss, #118), after which the humidity in the cage is raised nearly to saturation. The dye-gum arabic particles, absorbing moisture, adhere readily to the mosquitoes' bodies, and apparently are not lost after drying. The minimum amount of dust necessary to clearly mark the mosquitoes should be used. An excess will result in increased mortality and decrease in flight power. To determine the proper amount requires some preliminary experience.

All mosquitoes used in the flight-range experiments were reared in the laboratory from field-collected larvae and pupae. During the first year's study, adults, as they emerged, were transferred by means of a sucking tube from the emergence cages to a wooden, screen-topped, holding and

* We are greatly indebted to Dr. Louis A. Strait, of the University of California Medical Center, for valuable advice and assistance in selecting these dyes and a proper source of ultra violet light of suitable wave length.

release cage. At this time they were counted. Since pure cultures of any one species of mosquitoes ordinarily were not available, a sample of adults from the emergence cages was identified. By this means the approximate number of each species was estimated. During the entire holding period the adults were offered solutions of rhodamine-B in sugar water on cotton pads. Within 48 hours after emergence, the adults were transported to the point of release in the holding cage, which was wrapped in wet burlap sacks in order to maintain a humid atmosphere. In spite of all precautions, however, it was found that the mortality of mosquitoes in the holding cage was about 25%. Ten minutes before release the mosquitoes were dusted with the gum arabic-dye particles.

Several modifications were made in the procedure for the second year's studies. Larval and pupal stages of mosquitoes, principally of the species *Culex tarsalis* but including small numbers of *Culiseta incidens* Thomson and *Anopheles pseudopunctipennis franciscanus* MacCracken were collected simultaneously in a prolific breeding area. The proportion of each species was determined by the identification of samples of larvae and adults from the cultures. These were held in the laboratory in white enamel pans. As the pupae appeared they were transferred in accurately counted lots of approximately 500 into 50-pound shortening cans which had been modified by replacing the centers of the covers with bobbinet and by brazing short copper tubes into the bases of the cans as water outlets (Fig. 1). Adults were permitted to emerge in these cans and were offered a solution of rhodamine-B and sugar as food. Specimens were held in the cans for from 3 to 7 days before release. It was found that the mortality in pupae and adults amounted to less than 5%, probably because of the relatively small numbers of insects for the available space and also because a high humidity was maintained. In preparing for the release of the mosquitoes, the water in the bottom of the rearing cans was drained. The dead or live pupae remaining in the water at this time were counted to determine the actual number of adults emerged. Following this, the rhodamine-B gum arabic dust was introduced in the same manner as previously described and wet towels were placed over the top of the can to maintain a high humidity. Then, the cans were transported from the laboratory to the release point, a distance of about 20 miles. After release all adults remaining in the cans were identified, counted and checked for presence of the fluorescent marker.

The center of the experimental area was selected as the release point.

For recoveries mosquities were collected from preselected stations by means of sucking tubes. Those from each collecting station were placed in separate small carrying cages. As far as possible, collections were made in all directions from the release point at distances varying from 0.2 miles up to 3 miles. Upon return to the laboratory the mosquitoes were lightly chloroformed, and examined immediately in a dark-room under a low power dissecting microscope for the presence of rhodamine particles. For a source of ultra violet light an H_4 Mercury lamp (Keese Engineering Company, Shannon No. 92-LS) equipped with two Corning heat resisting glass filters, a blue and a red-purple, was used. This lamp produced light with an average wave length of 3400 Å and a range of 3100 to 4000 Å. Detailed examination and handling of individual specimens was not necessary. The rhodamine particles adhering to marked mosquitoes were readily visible as brilliant red pin points of light. Marked specimens were separated and identified. Using this technique it was possible to examine over 1,000 specimens per hour. The short period of exposure to this intensity and wave length of ultra violet apparently does not inactivate virus in the mosquitoes for numerous virus isolations were made in both 1946 and 1947 from mosquitoes which had been examined for fluorescence in flight range studies.

The 28 square-mile experimental area is level and consists of farmyards and

cultivated fields. No communities are included in the area. The principal crops are cotton, potatoes, tomatoes, alfalfa, cereals and deciduous fruits, chiefly peaches. All crops are constantly irrigated during the summer months, and normally there are present large numbers of the mosquito species included in this study. The area is characterized by high summer temperatures and low relative humidities. During the time of these studies daily maximum temperature ranged from 81° to 105° F. and relative humidity ranged from 8–73% with the mean below 40%.

The entire area had been carefully mapped during preceding years for other studies, and complete records of the distribution of buildings were available. Also, the preferred adult mosquito resting places were well known. The collection stations were determined by the occurrence of preferred resting places for adult *Culex*. All stations were man-made shelters, such as: porches, chicken houses, pump houses, out-door privies and garages.

1946 STUDY AND RESULTS

From May 20 to June 28, 1946, five releases were made totaling approximately 47,700 mosquitoes belonging to the species *Culex tarsalis, Culex stigmatosoma* Dyar and *Culex quinquefasciatus* Say, with approximately equal ratios of males to females. These releases were all made between the hours of 5 and 7 p.m. Between May 21 and July 2, collections were made on 13 days in an attempt to recover marked specimens. The distances at which all recaptures were made are summarized in Table 1.

A total of 11,800 marked *Culex tarsalis* were released. Of 14,873 specimens of this species collected after the releases only 5 marked females were recaptured. Two of these were taken at a distance of 0.5 mile and 3 at 0.2 mile from the point of release (Fig. 2).

About 7,500 marked *Culex stigmatosoma* were released, and 3,048 specimens of this species collected subsequently. Ten marked specimens were recaptured: 6 females and 3 males at 0.2 mile, and 1 female at 1 mile from the release point.

Of approximately 26,700 *Culex quinquefasciatus* released and 18,244 specimens collected, 58 marked specimens were recovered. The distances at which recaptures were made are summarized in Table 2. Twenty females and 27 males were recovered at 0.2 mile, 2 females and 1 male at 0.5 mile, and the maximum distance at which a recovery was made was 1 female at 2.5 miles.

TABLE 2. Summary of Recoveries of Marked *Culex quinquefasciatus*, 1946

Distance in miles from release point	Females recovered	Males recovered
0.2	20	27
0.5	2	1
0.75	2	0
1.0	3	2
2.5	1	0
Totals	28	30

Although detailed records of direction and intensity of air movement are not available for the period during which the studies were made, it was noted at the release point that in the early evening there was usually a light to heavy northwest breeze which died down by 10:00 p.m. United States Weather Bureau reports from Bakersfield, about 20 miles south of the study area, recorded a prevailing air movement from the northwest with daily maximum velocities ranging from 13 to 31 miles per hour during the study period. The two releases of *Culex quinquefasciatus* were made on relatively quiet evenings. Two releases of *Culex tarsalis* and *Culex stigmatosoma* were made on evenings when a strong northwest breeze was blowing; the third was made on a quiet evening. Only 13 of the 73 recoveries were made to the north or west of the release point. The greatest distance at which recoveries were made in the direction against the prevailing wind were as follows: *C. quinquefasciatus*, 2 females at 1 mile and 2 males at ¼ mile; *C. tarsalis*, 2 females at ½ mile.

TABLE 1. Summary of Marked Mosquitoes Released and Specimens Recovered in 1946

Date of Release	Species Released	Approx. No. Released	Date of Collection	Total No. of Mosquitoes Collected	Marked Specimens Collected		
					Species	Number	Distance Travelled
			5–21	432	C. tarsalis	1	0.2 mi.
					C. stigmatosoma	1	0.2 mi.
			5–22	584	C. stigmatosoma	2	0.2 mi.
5–20	C. tarsalis	2,800	5–23	274	C. stigmatosoma	1	0.2 mi.
	C. stigmatosoma	5,800			C. stigmatosoma	1	1.0 mi.
			5–24	943	C. stigmatosoma	1	0.2 mi.
					C. tarsalis	1	0.5 mi.
			5–28	815	C. tarsalis	1	0.2 mi.
					C. stigmatosoma	4	0.2 mi.
					C. tarsalis	1	0.5 mi.
6–11	C. tarsalis						
	C. stigmatosoma	3,400	6–12	1,976	C. quinquefasciatus	1	0.2 mi.
	C. quinquefasciatus	1,200			C. quinquefasciatus	1	1.0 mi.
				2,153	C. quinquefasciatus	6	0.2 mi.
6–13	C. quinquefasciatus	6,500	6–14		C. quinquefasciatus	1	0.2 mi.
					C. quinquefasciatus	1	0.5 mi.
			6–18	3,964	C. quinquefasciatus	11	0.2 mi.
					C. quinquefasciatus	6	0.2 mi.
					C. quinquefasciatus	3	1.0 mi.
					C. quinquefasciatus	1	1.0 mi.
					C. quinquefasciatus	1	0.5 mi.
					C. quinquefasciatus	1	0.75 mi.
6–18	C. quinquefasciatus	19,000			C. quinquefasciatus	1	0.5 mi.
			6–19	4,165	C. quinquefasciatus	11	0.2 mi.
					C. quinquefasciatus	1	1.0 mi.
					C. quinquefasciatus	1	2.5 mi.
			6–20	5,968	C. quinquefasciatus	9	0.2 mi.
					C. quinquefasciatus	2	0.2 mi.
			6–29	2,490	C. tarsalis	1	0.2 mi.
6–28	C. tarsalis	9,000	7–1	5,616	no recoveries		
			7–2	3,719	no recoveries		

1947 STUDY AND RESULTS

This study was conducted between June 15 and July 23, 1947. During this period a total of 23,021 mosquitoes consisting of 93.6% *Culex tarsalis*, 4.5% *Anopheles pseudopunctipennis franciscanus* and 1.9% *Culiseta incidens* were released on 8 different occasions (Table 3). Approximately equal ratios of males and females of each species were included in the releases. Collections were made on each of

TABLE 3. Number of Mosquitoes Released in Each Week of the 1947 Study

Week	Total Specimens Released
June 15–21 *	3,611
June 22–28	4,522
June 29–July 5	4,675
July 6–12 *	6,251
July 13–19	3,962
Total	23,021

* Two releases made this week.

26 days, and a total of 42,783 mosquitoes of all species were collected in an attempt to recover marked specimens. Thirty-two marked mosquitoes were recovered. These included 18 female and 10 male *C. tarsalis* (Table 4 and Fig. 1), 2 female and 1 male were partially engorged and gravid, and 2 were freshly engorged.

TABLE 4. Summary of Mosquito Collections Made in 1947 for Recovery of Marked Specimens: Results for *Culex tarsalis* Only

Study Ring	No. sq. mi.	No. collecting stations	No. collections made	No. mosquitoes all species collected	No. C. tarsalis collected			No. marked C. tarsalis recovered		
					F	M	Total	F	M	Total
0–½ mi.	.8	6	32	2623	667	255	922	7	6	13
½–1 mi.	2.4	16	126	13906	2316	900	3216	9	3	12
1–1½ mi.	3.9	22	205	17183	1868	540	2408	1	1	2
1½–2 mi.	5.5	9	40	2255	137	21	158	0	0	0
2–3 mi.	15.7	6	64	7042	923	133	1056	1	0	1
Total	28.3	59	467	43009	5911	1849	7660	18	10	28

DISCUSSION AND INTERPRETATION OF DATA

In these studies it soon became obvious that the number of marked specimens released represented but a very small part of the relatively enormous mosquito population naturally present in the 28 square miles of the study area, and that with the personnel available and territory to be covered, the number of recoveries would be relatively small. In 1946, of approximately 47,000 marked mosquitoes released, only 73 were recovered, or less than one out of 650 released; and in 1947 only one mosquito was recovered out of 720 released.

In Table 5 the collection data for 1947 have been presented on the basis of samples

A. pseudopunctipennis, and 1 male *Culiseta incidens.* The maximum range at which these species were recovered was: *C. tarsalis* female 2.5 miles, male 1.1 miles; *A. pseudopunctipennis* female 0.9 mile, male 0.9 mile; and *C. incidens* male 0.6 mile.

As in 1946 detailed records of direction and intensity of air movements are not available for the study area. United States Weather Bureau reports from Bakersfield recorded a prevailing northwest wind during the study period with daily maximum velocities ranging from 12 to 29 miles per hour. Of the 8 releases two were made on quiet evenings, 3 during gusty intermittent breezes, 2 during strong breezes, and for one, observations regarding the wind were not recorded.

The majority of the recoveries of marked *Culex tarsalis* (22 of 28) were made to the north or west of the release point (Fig. 1). The opposite was true in 1946 when only a small proportion of all recoveries (13 of 73) were made upwind.

It is interesting to note that 7 of 18 *Culex tarsalis* females recovered had succeeded in obtaining a blood meal subsequent to their release; 3 were gravid, 2

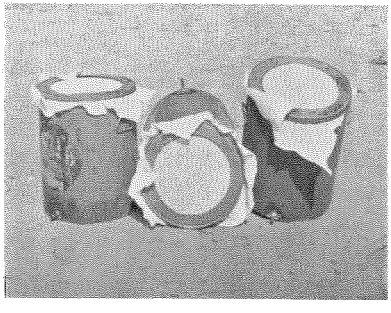


FIG. 1. Mosquito rearing cans used in 1947 study.

TABLE 5. Average Collection Figures per Square Mile of Study Area 1947

Study Ring	No. sq. mi.	Average No. stations per sq. mi	Average No. collections per sq. mi.	Average No. mosquitoes collected per sq. mi.	Average No. *C. tarsalis* collected			Recoveries marked *C. tarsalis* sq. mi
					F	M	Total	
0–½ mi.	0.8	7.5	40.0	3279	834	331	1165	16.25
½–1 mi.	2.4	7.5	52.5	5794	965	375	1340	5.0
1–1½ mi.	3.9	5.6	52.6	4406	479	138	617	0.50
1½–2 mi.	5.5	1.6	7.3	410	25	4	29	0.0
2–3 mi.	15.7	0.4	4.1	451	59	9	68	0.06
Total	28.3	2.1	16.5	1520	209	65	271	0.99

and recoveries per square mile of study area. The authors do not feel that these data should be submitted to extensive statistical analysis. The numbers of recoveries made were too small to suggest their use for derivation of estimates of the natural mosquito populations in the experimental area as was carried out by Eyles and Cox [5] with *Anopheles quadrimaculatus* Say; or analysis of flight distribution patterns as was carried out by Russell *et al.*[6] with *Anopheles culicifacies* Giles; Gilmour *et al.* with blowflies [7]; or Jackson [8] with tsetse flies. The above authors found large standard errors in their data and encountered many difficulties in interpretation even though their numbers of specimens released, and numbers recovered were much larger than those in the present study; and their methods of sampling were much better standardized than was possible in the present study.

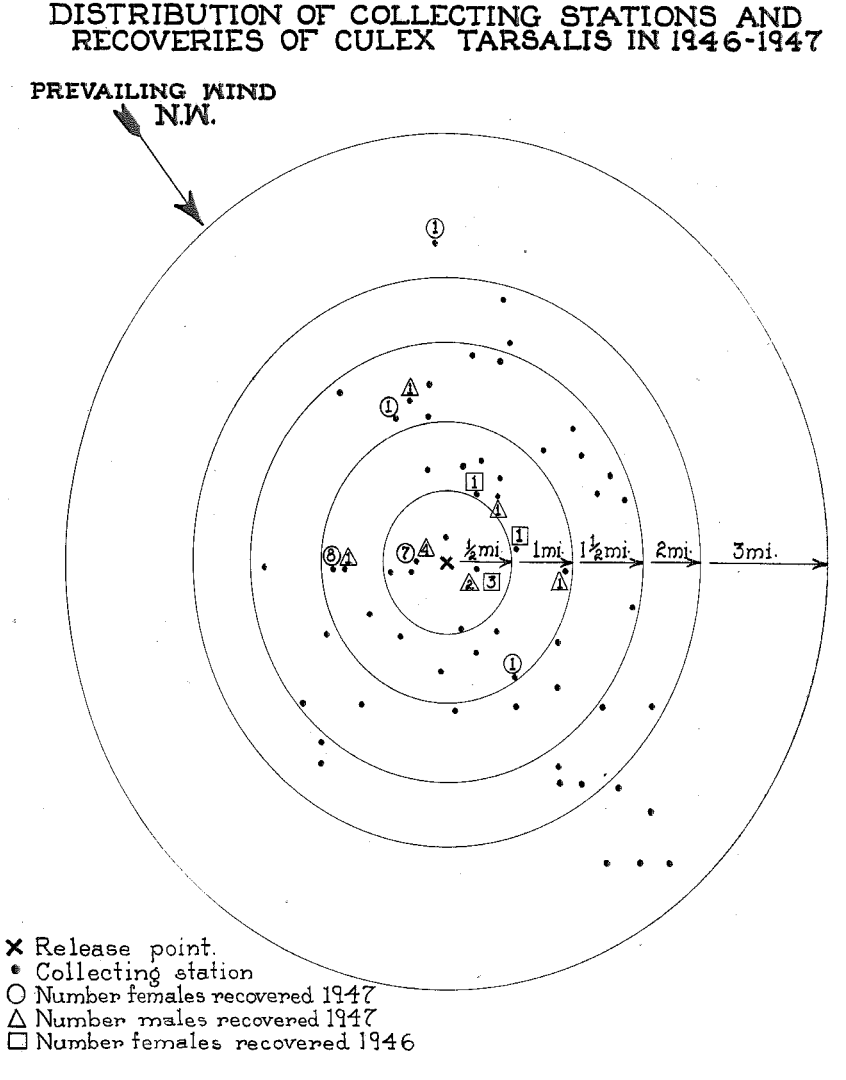
The present studies were not designed to determine maximum range of flight, but rather were designed to estimate what Russell *et al.*[6] have termed "the effective flight range." * The bulk of the recoveries were made within a mile of the release point. However, even with limited sampling beyond one mile, one recovery each of *Culex tarsalis* and *Culex quinquefasciatus* was made at 2½ miles distance from the release point. We cannot say that these species will not travel longer distances. It can be seen even from these relatively limited studies that an extensive breeding ground of *C. tarsalis, C. quinquefasciatus* and possibly *C. stigmatosoma* although located a mile or more outside of the boundaries of a mosquito control district or municipality could serve as an important source of infestation. Thus we can not consider infestations with these species solely as a backyard or neighborhood problem with regard to either their pest or disease vector activity. As a result of the findings of this survey, although the data are admittedly inadequate, we feel that as a tentative practical limit for control measures they should be carried out in a zone not less than one and one-half miles beyond the human or equine hosts to be protected. It could very well be that under different conditions of temperature, humidity, wind or topography this species would have a longer life or an inclination to travel longer distances. If this is demonstrated by future studies the above recommendations will have to be revised.

The fact that recoveries of *Anopheles pseudopunctipennis franciscanus,* and *Culiseta incidens* were made up to 0.9 and 0.6 mile respectively means that these

* The effective flight range of a species being the maximum distance from its breeding places that a species normally is able to fly in effective numbers. By effective numbers these authors meant in numbers sufficient to propagate a mosquito borne disease or to constitute a mosquito nuisance.

species will travel at least that far, and it may be that they will travel further. The information obtained on these was incidental to the main study, and they were included only because of the impracticability of separating the immature stages from those of *C. tarsalis* at the time of rearing for marking and release.

The new technique of marking mosquitoes with a fluorescent dye in the form of a dust, or as a solution in sugar water by feeding has definite advantages under some circumstances over liquid dyes used as sprays, or over metallic dusts. However, it must be emphasized that in certain areas at least, naturally occurring fluorescent materials may be found in or on mosquitoes. Such fluorescence may lead to false results and confusion unless the dye selected for use in an experiment is distinc-



tive in color. Thus, a large series of normal field-collected mosquitoes from any study area must be examined prior to selection of the dye, in order to rule out the possibility of a naturally occurring fluorescent material being mistaken for the dye used.

SUMMARY

The fluorescent dye rhodamine-B has been proven satisfactory for the marking of mosquitoes in flight range studies. Use of this dye and its recognition by ultra violet light after recapture leaves the specimens in suitable condition for other studies, including virus isolation or dissection for *Plasmodia*. Details are given of the methods of applying and recognizing the dye found most satisfactory for field studies.

In 1946 *Culex quinquefasciatus, Culex tarsalis* and *Culex stigmatosoma* were demonstrated to have a flight range of at least 2.5, 0.5 and 1.0 miles respectively.

In 1947 studies, observation of the flight range for *Culex tarsalis* was extended to 2.5 miles, and *Culiseta incidens* and *Anopheles pseudopunctipennis franciscanus* were found at distances of 0.6, and 0.9 mile respectively from the release point.

It is concluded that in Kern County, California, under the conditions of these experiments the effective flight range of *Culex tarsalis, Culex quinquefasciatus* and possibly *Culex stigmatosoma* is at least 1 mile. For protection against disease bearing or pest activities of these three species, mosquito control activities should be carried out for a radius of at least one and a half miles from any population it is necessary to protect.

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KING HONORS DR. BISHOPP

Dr. F. C. Bishopp of our Bureau of Entomology and Plant Quarantine was recently awarded His British Majesty's Medal for Service in the Cause of Freedom, in recognition of his valuable services to the Allied war effort in various fields of scientific research and development. Lord Inverchapel, the British Ambassador, conveyed his personal congratulations as well. The decoration will be tendered the Chief of Protocol, Department of State, who will hold it until Dr. Bishopp can legally receive it. Because of material shortage in the United Kingdom, it will be some months before the insignia becomes available, but the ribbon will be sent the Department of State in the near future. This is a signal honor for a USDA worker.—(From Vol. 8, No. 6. of USDA, a government publication for Agriculture employees.)